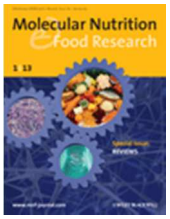

Weekday sunlight exposure, but not vitamin D intake, influences the association between vitamin D receptor genotype and circulating concentration 25-hydroxyvitamin D in a pan-European population: the Food4Me study

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Title

Weekday sunlight exposure, but not vitamin D intake, influences the association between vitamin D receptor genotype and circulating concentration 25-hydroxyvitamin D in a pan-European population: the Food4Me study

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Abbreviations: 25-hydroxyvitamin vitamin D (25(OH)D), Body mass index (BMI), Food frequency questionnaire (FFQ), Healthy eating index (HEI), Mediterranean diet (MD); Moderate and vigorous physical activity (MVPA); Physical activity level (PAL), Personalized Nutrition (PN), Randomized controlled trial (RCT), Sedentary behavior (SB), Waist circumference (WC)

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Abstract

Scope

Little is known about diet- and environment-gene interactions on 25-hydroxyvitamin D (25(OH)D concentration. This cross-sectional study aimed to investigate i) predictors of 25(OH)D concentration and relationships with vitamin D genotypes and ii) whether dietary vitamin D intake and sunlight exposure modified these relationships.

Methods and results

Participants from the Food4Me study (n=1312; age 18-79) were genotyped for vitamin D receptor (*VDR*) and vitamin D binding protein at baseline and a genetic risk score was calculated. Dried blood spot samples were assayed for 25(OH)D concentration and dietary and lifestyle information collected. Circulating 25(OH)D concentration was lower with increasing genetic risk score, lower in females than males, higher in supplement users than non-users and higher in summer than winter. Carriage of the minor *VDR* allele was associated with lower 25(OH)D concentration in participants with the least sunlight exposure. Vitamin D genotype did not influence the relationship between vitamin D intake and 25(OH)D concentration.

Conclusion

Age, sex, dietary vitamin D intake, country, sunlight exposure, season and vitamin D genetic risk score were associated with circulating 25(OH)D concentration in a pan-European population. The relationship between *VDR* genotype and 25(OH)D concentration may be influenced by weekday sunlight exposure but not dietary vitamin D intake.

INTRODUCTION

Vitamin D deficiency, defined by the Institute of Medicine as a circulating concentration of 25-hydroxyvitamin D (25(OH)D) <30 nmol/L (1), is highly prevalent (2). In addition to increased incidence of skeletal health outcomes such as rickets (3) and osteoporosis (4), research suggests that inadequate vitamin D intake is linked with greater risk of cardiovascular disease (5), obesity (6), diabetes (7) and cancer (8).

Determinants of 25(OH)D concentrations include skin exposure to sunlight and dietary vitamin D intake. Vitamin D synthesis occurs in the skin during exposure to ultraviolet (UVB) radiation (290–320 nm), which converts 7-dehydrocholesterol to vitamin D (9). Dietary sources include vitamin D2, derived from fungi, and vitamin D, derived from fish and other animal sources (9). Vitamin D undergoes a series of enzymatic conversions in the liver to form 25(OH)D, which is the accepted blood-based biomarker for vitamin D status (10).

Although estimates differ, approximately 25% of the variability in 25(OH)D concentrations is attributable to dietary and environmental influences (11), whereas twin and family studies have shown heritability of vitamin D status to be as high as 80% (11, 12). A recent genome-wide association study (13) and a systematic review (14) identified that variation in the vitamin D binding protein gene, *GC*, and the vitamin D receptor gene, *VDR*, modulated circulating concentrations of 25(OH)D. These findings have been replicated in a subsequent cross-sectional study in 201 healthy Danish adults, where polymorphisms in *GC* and *GC* risk score were associated with lower total 25(OH)D concentration (15).

While previous studies have evaluated the relationship between 25(OH)D concentrations, vitamin D genotypes and sociodemographic characteristics (16-20), few studies have

comprehensively evaluated interactions between genotype and dietary and sunlight exposure on circulating vitamin D concentrations.

The present study uses baseline data from the Food4Me study, a pan-European randomized controlled trial, designed to investigate the effect of personalized nutrition (PN) advice on changes in diet and physical activity after a 6-month intervention. Our study had two aims to i) identify predictors of circulating 25(OH)D concentration in healthy European adults and investigate relationships with specific vitamin D genotypes and ii) to determine whether these relationships are modified by dietary vitamin D intake and sunlight exposure.

METHODS

Study population

This analysis used baseline data from the Food4Me study (21), which was a 6-month, 4-arm, internet-based, randomized controlled trial (RCT) conducted in 7 European countries (22). A total of 1607 participants (age range 18 to 79) were randomized into the study and recruited between August 2012 and August 2013 from the following centers: University College Dublin (Ireland), Maastricht University (The Netherlands), University of Navarra (Spain), Harokopio University (Greece), University of Reading (United Kingdom, UK), National Food and Nutrition Institute (Poland) and Technical University of Munich (Germany). The Research Ethics Committees at each University or Research Center delivering the intervention granted ethical approval for the study. The Food4Me trial was registered as a RCT (NCT01530139) at Clinicaltrials.gov. All participants expressing an interest in the study were asked to sign online consent forms at two stages in the screening

process. These consent forms were automatically directed to the local study investigators to be counter-signed and archived (22).

Blood sample collection

Participants collected finger-prick blood samples using a pack provided by Vitas Ltd, Oslo, Norway and DSM, Kaiseraugst, Switzerland. Pre-treated filter cards were packed in an airtight aluminium bag (Whatman Foil Bags, item no. 10534321, Whatman Inc., Sanford, ME) with a drying agent (Sorb-it, item no. 10548234, Süd-Chemie, Germany) and stored at room temperature until use. To aid collection, participants had access to an online video with instructions and frequently asked questions in their local language. Participants were asked to fill two dried blood spot (DBS) cards (500 µL of blood in total), each requiring five spots, and were instructed to dry the cards at room temperature for at least 2 hours, but not longer than four hours, before returning by post to the corresponding recruiting center. Centers then shipped the DBS cards to DSM (DSM Nutritional Products Ltd. Switzerland) for measurements of 25(OH)D3 and 25(OH)D2. No results are presented for 25(OH)D2 due to all values being below the lower limit of quantification (<25 nmol/L) and detection (8 nmol/L) of the analytical method and thus all results for 25(OH)D presented in the manuscript refer to 25(OH)D3. Further details of the blood spot preparation and analysis, including method validation and performance parameters, are provided elsewhere (23).

Genotyping

Participants collected buccal cell samples at baseline using Isohelix SK-1 DNA buccal swabs and Isohelix dried-capsules and posted samples to each recruiting center for shipment to

LCG Genomics (Hertfordshire, United Kingdom). LCG Genomics extracted DNA and genotyped specific loci using KASP™ genotyping assays to provide bi-allelic scoring of single nucleotide polymorphisms (SNPs) of the genes encoding the vitamin D receptor (*VDR*) *rs1544410* and *rs2228570* and the vitamin D binding protein (*GC*) *rs2282679, rs4588* and *rs7041*. These 5 SNPs were selected based on findings from Genome Wide Association Studies (13).

Dietary intake, sunlight exposure and confounders

Participants completed an online food frequency questionnaire (FFQ), which was developed and validated for the Food4Me Study (24, 25), and included 157 food items consumed frequently in each of the 7 recruitment countries. Usual intakes of total vitamin D from foods and supplements at baseline were computed using a food composition database based on McCance & Widdowson's "The composition of foods" (26) and a dichotomous variable created to indicate if an individual was taking a vitamin D supplement.

Weekend and weekday sunlight exposure was estimated by asking participants if they spent "Less than 20 min", "Between 20 and 45 min", "Between 45 min and 1 hour", "Between 1 and 2 hours" or "More than 2 hours" outside during daylight hours on a typical week day and on a weekend day during the sunny months of the year (i.e. April to September) (27). Responses were collapsed into "Less than 20 min", "Between 20 min and 2 hour" and "More than 2 hours". Season was defined as spring (March to May), summer (June to August), autumn (September to November) and winter (December to February) for the purpose of descriptive statistics. Season was operationalized as a function of seasonal variation estimated by sin (SampleYear×2×pi) and cos (SampleYear×2×pi) for inclusion as a covariate in linear regression models. Full details are described elsewhere (23).

Physical activity level (PAL, ratio between total energy expenditure and basal metabolic rate (BMR)), moderate and vigorous PA (MVPA) and time spent in sedentary behaviors (SB) were directly estimated from triaxial accelerometers (TracmorD, Philips Consumer Lifestyle, The Netherlands). Participants self-reported occupations, which were grouped according to the European commission list of occupations (28, 29). To facilitate data analysis according to geographic location, participant centers were grouped according to the United Nations Composition of geographical regions, geographical sub-regions, and selected economic and other groupings (30). Thus, Ireland and United Kingdom were grouped as “British Isles”, Germany and The Netherlands as “Western Europe”, Poland as “Eastern Europe”, and Spain and Greece as “Southern Europe”. Body weight (kg) and height (m) were self-measured and self-reported with the aid of information sheets and online video instructions in their own language. Body mass index (BMI; kg/m^2) was estimated from body weight and height and anthropometric measurements showed a high degree of reliability (31). Further details on these variables are provided elsewhere (22).

Statistical analyses

Participants with complete data for vitamin D-related genotype, diet, vitamin D status from DBS and covariates at baseline were included in the present analyses. Data were analyzed using Stata (version 14; StataCorp, College Station, TX, USA). Variables were tested for skewness and kurtosis, and if not normally distributed, were log transformed. Thus circulating 25(OH)D concentrations, vitamin D intake from food and from food and supplements, PAL and MVPA were log transformed prior to analyses. A total genetic risk score was created by summing the minor alleles for *GC rs2282679 (CC), GC rs4588 (AA), GC*

rs7041 (TT), VDR rs2228570 (TT), VDR rs1544410 (AA), which were coded as 0, 1 and 2. Individuals were then grouped according to whether they carried 0-2, 3 to 5 or 6 or more minor alleles. Additive allele coding (coded as 0, 1 and 2 for no copies, one copy and two copies of the minor allele respectively) was used throughout, with the exception of tests for interactions, where the dominant model (coded as 0 and 1 for no copies and one or two copies respectively) was used to increase statistical power. To address our first aim, multiple linear regression tested for differences in 25(OH)D concentration (dependent variable) according to sociodemographic characteristics (independent variables) and logistic and multiple linear regression were used to test for significant differences in baseline sociodemographic characteristics (dependent variable) between genotypes (independent variable) for categorical and continuous variables, respectively. Analyses were adjusted for age, sex, BMI, ethnicity, country, season, vitamin D intake (food only) and vitamin D supplementation. Models were adjusted for season by inclusion of two estimates of seasonal variation as described earlier and detailed elsewhere (23). Physical activity outcomes were further adjusted for the length of time that the accelerometers were worn (32). To investigate our second aim, interactions between vitamin D-related genotype and diet or sunlight exposure on baseline 25(OH)D concentrations were tested by including an interaction term in the model. To estimate any moderating effects on this interaction, alcohol intake (operationalized as meeting alcohol guidelines of less than 24g of alcohol/day or not), smoking habits (current smoker or not current smoker) and occupation (professional or managerial, intermediate, manual or routine, student and retired as defined by EU classifications of occupations (28)) were included in the model as a sensitivity analysis. In addition, to investigate any effect of linkage disequilibrium on the total risk score, linkage disequilibrium (LD) between all SNPs was calculated. If LD was observed then a revised total

genetic risk score was derived excluding any relevant SNPs. Data were considered statistically significant at $P<0.05$.

RESULTS

Of the 1607 participants who were randomized into the study, 1312 individuals had complete data for concentrations of 25(OH)D, *GC* and *VDR* genotype, dietary intakes and covariates (**Figure 1**). Mean 25(OH)D concentration was 60.6 (SD 26.4) nmol/L. A total of 22% of participants took vitamin D supplements. Mean vitamin D3 intake from foods only was 159 (SD 100) IU/d and 327 (SD 1675) IU/d from foods and supplements. The mean proportion of individuals spending more than 2 hours in the sun on a weekday was 12% and 40% during the weekend (**Supplemental Table 1**). The majority of individuals were tested during the spring months (56%), with 28% tested in winter, 9% in summer and 7% in autumn. No significant deviation from the Hardy-Weinberg Equilibrium was observed for any genotype (Table 2).

Associations with 25(OH) vitamin D concentration

Concentrations of 25(OH)D were higher with increasing age ($P=0.002$), PAL ($P<0.001$), MVPA ($P=0.002$), total vitamin D intake ($P<0.001$) and vitamin D intake from food only ($P=0.010$) and lower with increasing time spent sedentary ($P=0.011$; **Table 1**). Concentrations of 25(OH)D were also lower in females than males ($P<0.001$), highest in Western Europe ($P=0.001$) compared with the British Isles, and higher in supplement users than non-supplement users ($P<0.001$). In addition, concentrations of 25(OH)D were higher when

measured in spring, summer and autumn compared with winter (*P*<0.001) and with increasing time spent in the sunlight during the weekend (P-trend=0.007) and weekday (P-trend=0.006).

Concentrations of 25(OH)D were lower with each additional copy of the minor allele of *GC rs2282679*, *GC rs4588* and *GC rs7041* (*P*<0.001) but did not vary with *VDR* genotype (**Table 2**). When assessed according to a total genetic risk score, concentrations of 25(OH)D were lower with increasing number of minor alleles (*P*-trend<0.001; **Figure 2**). Baseline socio-demographic characteristics of participants according to VDR and GC genotype are presented in Supplemental Table 1 and **Supplemental Table 2**.

Dietary vitamin D and sunlight exposure interactions

The relationship between *VDR rs2228570* genotype and 25(OH)D concentration was modulated by time spent in the sunlight during the week (*P*-interaction=0.009; **Figure 3**). Individuals with no copies of the *VDR rs2228570* minor allele (*TT*) and reporting less than 20 minutes of sunlight exposure per day had 14.6 nmol/L lower concentrations of 25(OH)D than participants reporting more than 2 hours of sunlight exposure (*P*-trend=0.036). Individuals with one or more copies of the minor allele had 5.7 nmol/L higher concentrations (*P*-trend=0.06; Figure 3). When total sunlight exposure (weekdays plus weekend days) was considered, the interaction with *VDR rs2228570* remained significant but evidence for the interaction was weaker (*P*=0.045) (See **Supplemental Figure 1**). No significant interactions were observed between genotype and dietary vitamin D intake from foods and supplements alone or combined on vitamin D status.

Sensitivity analyses

Inclusion of alcohol consumption, smoking and occupation as covariates in statistical models did not affect the pattern of results significantly. LD was observed between *GC rs2282679* and *GC rs4588* (r^2=0.96) and *GC rs7041* (r^2=0.69) and between *GC rs4588* and *GC rs7041* (r^2=0.72). VDR SNPs showed no LD. Removal of two *GC* SNPs (*GC rs4588* and *GC rs7041*) in the generation of the total risk score attenuated slightly the observed relationship between total risk score and 25(OH)D concentration but did not change the pattern of significant results (*P*-trend=0.005).

DISCUSSION

Main findings

Our main findings were that 25(OH)D concentration was associated with both modifiable (e.g. vitamin D intake, physical activity and sunlight exposure) and non-modifiable (e.g. age, sex, country and season) predictors, and that concentrations were lower in carriers of the minor alleles of vitamin D risk genotypes. Moreover, the relationship between time spent in the sunlight during the week and 25(OH)D was modulated by the *VDR rs2228570* genotype in a dose-wise manner. Larger studies are warranted to confirm these findings.

Comparison with other studies

For the first time in a pan-European setting, we observed that concentrations of 25(OH)D were higher in individuals who were more physically active, which is likely to be a proxy for time spent outdoors, and in participants with higher vitamin D intakes from food or supplements, which confirms findings from previous single country studies (13, 15, 33). Our

findings are in agreement with the latest National Diet and Nutrition Survey (NDNS) in the UK, where 25(OH)D concentration increased with age in adults up to 70 years of age (34). However, over the age of 70 years, 25(OH)D concentrations decrease due to impaired skin photosynthesis and renal conversion to the active form (2). We observed that circulating 25(OH)D concentrations were lower in females than males, which confirms previous findings among adults (35) and children (36). A proposed explanation for this is that women are more diligent in applying sunscreen and in covering skin for social reasons and that men spend more time outdoors during physical activity and in manual occupations.

Our study complements findings from a genome-wide association study designed to identify common genetic predictors of vitamin D insufficiency (13). Participants in the highest quartile of genotype score (combining *GC*, *DHCR7* and *CYP2R1* variants were at increased risk of having 25(OH)D concentrations lower than 50 nmol/L (OR: 1.92, 95% CI: 1.70–2.16, $P<0.001$) compared with participants in the lowest quartile (13). Potential mechanisms for this genotype-related lower vitamin D status include impaired synthesis of 25(OH)D due to lower activity of the enzyme 7-dehydrocholesterol reductase by *DHCR7* variants (13) and an impairment of *CYP2R1* activity, which may be a microsomal enzyme responsible for 25-hydroxylation of vitamin D in the liver (37). In addition, *GC* encodes DBP, a protein synthesized in the liver that binds and transports vitamin D and its metabolites (38), and studies have suggested that alterations in DBP could influence proportions of free, circulating 25(OH)D, thereby being a rate limiting factor in the production of the vitamin D metabolite 1,25(OH)2D (13, 17).

For the first time, we observed that weekday sunlight exposure modulated the relationship between vitamin D-related genes and circulating vitamin D concentrations. We observed that greater weekday sunlight exposure mitigated the detrimental effect of carriage of the

minor allele of *VDR rs2228570 (TT)* on 25(OH)D concentration. We anticipated that total sun exposure (weekday + weekend) would be the best overall measure of exposure. However, when considering total sun exposure, we found that the interaction with *VDR* genotype on circulating 25(OH)D concentration remained significant but evidence for the interaction was weaker for total sun exposure compared with when only weekday exposure was considered. Our data do not allow us to determine the reason for this unexpected finding but we hypothesise that it may be related to the differences in the precision of measurement of sun exposure on weekdays *v*. weekend days. For most people, assessing time spent outdoors may be easier (and, therefore made with greater precision) during weekdays than at weekends because of the more regular activity patterns (for both work and leisure) during weekdays. Given the role of *VDR rs2228570* genotype in enhancing risk of vitamin D deficiency (12, 13), the present study demonstrates the importance of sunlight exposure (both adventitious and recreational) in obviating the genotype-based risk. Previous research has shown that the vitamin D intake required to maintain circulating 25(OH)D concentrations in late winter above 25 nmol/L in older Irish adults was 492, 352, and 288 IU/d for individuals who avoided sunlight exposure, those who had some exposure, and those who enjoyed exposure, respectively (39). Cumulatively, this evidence emphasizes the importance of considering both genotype and sunlight exposure when developing policies to ensure vitamin D adequacy (40). The molecular mechanism responsible for this interaction is not known. As a first step, it would be helpful to confirm our findings in larger, independent, studies with a wider range of sunlight exposures. If the interaction is confirmed, this would provide a robust foundation for future investigations of the underlying mechanism. Inter-personal differences in efficiency of endogenous vitamin D synthesis, as well as clothing and sunscreen use and inter- and intra-country variations in

latitude, altitude, cloud cover and air pollution, mean that setting population-wide recommendations for sunlight exposure is difficult (41). However, PN has been highlighted as an important feature of current and future nutrition research (42, 43) and current findings should stimulate research to address these challenges within the context of PN. Our evidence concerning interactions between vitamin D-related genotype and vitamin D sources (diet and sunlight exposure) on vitamin D concentration may help in designing future PN interventions to improve vitamin D status in Europeans.

Strengths and limitations

The present study had a number of strengths. Our participants were drawn from 7 European countries, facilitating the comparison of vitamin D concentrations between European countries. Vitamin D status was determined using a relatively novel approach in which 25(OH)D concentrations were measured in dried blood spots self-collected by the study participants. Although performance characteristics did not meet those of current gold standard liquid chromatography in plasma, they were found to be suitable for status-level determination under field conditions (23) and this approach offers a novel and likely cost-effective method for collecting blood samples remotely for 25(OH)D quantification.

A limitation of our study is that data were self-measured and self-reported via the Internet, which may have introduced measurement error. Nonetheless, the accuracy of internet-based, self-reported anthropometric data is high (44), which this has been confirmed in our study (31). Dietary intakes were estimated by a FFQ which is subject to misreporting error (45) but this was minimized by prior validation against a 4-day weighed food record (25). Furthermore, our measure of sunlight exposure was limited to an indirect measurement that may be subject to self-reporting bias (27, 46), however, similar measures of indirect

sunlight exposure have been used successfully previously (47, 48). Small sample size limited our power to investigate the effect of individual gene variants in the present study. Moreover, 97% of our study participants were Caucasians and thus further research in wider ethnicity groups is required to generalize our findings to other population groups. Our sample is a self-selected group of individuals, who may be more health-conscious than the general population. However, characterization of our participants suggests that they were similar to most European adults and would benefit from improved diet and physical activity (49). For technical reasons, we were unable to evaluate the role of 25(OH)D2, which may have influenced estimates of total vitamin D concentration (50), but research suggests that genetic variation in the vitamin D binding protein influences responsiveness to 25(OH)D only (51). Future studies should consider the complex interplay between lifestyle factors and other genetic variants, such as *rs12785878*, located near the 7-dehydrocholesterol reductase *DHCR7* gene, *rs10741657*, located near the *CYP2R1* gene and variations in *CYP24A1*, which encodes the kidney 24-hydroxylase enzyme (13) on vitamin D status. In addition, information on factors influencing individual level sun exposure including weather, skin type and clothing habits should be collected. Finally, although the present analysis is in a large pan-European populations, we may have been under-powered to detect some significant interactions.

Implications of findings

We demonstrate the importance of dietary and environmental influences on circulating vitamin D concentration in a pan-European setting. Our findings also show that the relationship between genes related to vitamin D metabolism and vitamin D status is modified by sunlight exposure. These findings have important implications for the design of

PN interventions targeted towards vitamin D and for the development of vitamin D-related policy on sunlight exposure. However, larger studies are needed to understand the clinical relevance of these findings for the health of European (and other) adults.

Conclusions

Our findings identify several cross-sectional dietary (vitamin D intake and supplement use) socio-demographic (age and sex) and environmental (country, season, sunlight exposure, physical activity and vitamin D axis genes) predictors of circulating 25(OH)D concentration in a pan-European population. We observed that carriage of the minor allele for *VDR rs2228570* genotype was associated with lower circulating 25(OH)D concentrations and that this relationship may be modulated by weekday sunlight exposure.

Author contributions

YM, IT, CAD, ERG, LB, JAL, JAM, WHS, PW, HD, MG, TH and JCM contributed to the research design. JCM was the Food4Me Proof of Principle study leader. CCM, ERG, LB and JCM contributed to the developing the Standardized Operating Procedures. CCM, CPL, GM, ALM, RF and JCM conducted the intervention. CCM and WHS contributed to physical activity measurements. UH, MB, IB, FFR, KG and JB contributed towards the vitamin D measurements. KML and CCM wrote the paper and performed the statistical analysis and are joint first authors. All authors contributed to a critical review of the manuscript during the writing process. All authors approved the final version to be published.

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Conflict of interest statement

UH, MB, IB, FFR and PW are employed by DSM Nutritional Products. No other authors had a personal or financial conflict of interest.

REFERENCES

1. Institute of Medicine Food and Nutrition Board. Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC, 2010.
2. Holick MF. Vitamin D Deficiency. New Engl J Med 2007;357(3):266-81. doi: doi:10.1056/NEJMra070553.
3. Munns C, Zacharin MR, Rodda CP, Batch JA, Morley R, Cranswick NE, Craig ME, Cutfield WS, Hofman PL, Taylor BJ, et al. Prevention and treatment of infant and childhood vitamin D deficiency in Australia and New Zealand: a consensus statement. The Medical Journal of Australia 2006;185(5):268-72.
4. Sunyecz JA. The use of calcium and vitamin D in the management of osteoporosis. Ther Clin Risk Manag 2008;4(4):827-36.
5. Norman PE, Powell JT. Vitamin D and Cardiovascular Disease. Circul Res 2014;114(2):379-93. doi: 10.1161/circresaha.113.301241.
6. Pereira-Santos M, Costa PRF, Assis AMO, Santos CAST, Santos DB. Obesity and vitamin D deficiency: a systematic review and meta-analysis. Obesity Reviews 2015;16(4):341-9. doi: 10.1111/obr.12239.
7. Stivelman E, Retnakaran R. Role of Vitamin D in the Pathophysiology and Treatment of Type 2 Diabetes. Current Diabetes Reviews 2012;8(1):42-7. doi: 10.2174/157339912798829179.
8. Chowdhury R, Kunutsor S, Vitezova A, Oliver-Williams C, Chowdhury S, Kiefte-de-Jong JC, Khan H, Baena CP, Prabhakaran D, Hoshen MB, et al. Vitamin D and risk of cause specific death: systematic review and meta-analysis of observational cohort and randomised intervention studies. BMJ 2014;348. doi: 10.1136/bmj.g1903.
9. Jones G, Strugnell SA, DeLuca HF. Current Understanding of the Molecular Actions of Vitamin D. Physiol Rev 1998;78(4):1193-231.
10. Yetley EA. Assessing the vitamin D status of the US population. The American Journal of Clinical Nutrition 2008;88(2):558S-64S.
11. Shea MK, Benjamin EJ, Dupuis J, Massaro JM, Jacques PF, D'Agostino RB, Ordovas JM, O'Donnell CJ, Dawson-Hughes B, Vasan RS, et al. Genetic and non-genetic correlates of vitamins K and D. Eur J Clin Nutr 2009;63(4):458-64. doi: 10.1038/sj.ejcn.1602959.
12. Hunter D, De Lange M, Snieder H, MacGregor AJ, Swaminathan R, Thakker RV, Spector TD. Genetic Contribution to Bone Metabolism, Calcium Excretion, and Vitamin D and Parathyroid Hormone Regulation. J Bone Miner Res 2001;16(2):371-8. doi: 10.1359/jbmr.2001.16.2.371.
13. Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, Kiel D, Streeten EA, Ohlsson C, Koller DL, et al. Common genetic determinants of vitamin D insufficiency: a

genome-wide association study. Lancet 2010;376(9736):180-8. doi: 10.1016/S0140-6736(10)60588-0.

14. McGrath JJ, Saha S, Burne THJ, Eyles DW. A systematic review of the association between common single nucleotide polymorphisms and 25-hydroxyvitamin D concentrations. The Journal of Steroid Biochemistry and Molecular Biology 2010;121(1–2):471-7. doi: http://dx.doi.org/10.1016/j.jsbmb.2010.03.073.

15. Nissen J, Rasmussen LB, Ravn-Haren G, Andersen EW, Hansen B, Andersen R, Mejborn H, Madsen KH, Vogel U. Common Variants in CYP2R1 and GC Genes Predict Vitamin D Concentrations in Healthy Danish Children and Adults. PLoS ONE 2014;9(2):e89907. doi: 10.1371/journal.pone.0089907.

16. Engelman CD, Fingerlin TE, Langefeld CD, Hicks PJ, Rich SS, Wagenknecht LE, Bowden DW, Norris JM. Genetic and Environmental Determinants of 25-Hydroxyvitamin D and 1,25-Dihydroxyvitamin D Levels in Hispanic and African Americans. The Journal of Clinical Endocrinology & Metabolism 2008;93(9):3381-8. doi: doi:10.1210/jc.2007-2702.

17. Sinotte M, Diorio C, Bérubé S, Pollak M, Brisson J. Genetic polymorphisms of the vitamin D binding protein and plasma concentrations of 25-hydroxyvitamin D in premenopausal women. The American Journal of Clinical Nutrition 2009;89(2):634-40. doi: 10.3945/ajcn.2008.26445.

18. Janssens W, Bouillon R, Claes B, Carremans C, Lehouck A, Buysschaert I, Coolen J, Mathieu C, Decramer M, Lambrechts D. Vitamin D deficiency is highly prevalent in COPD and correlates with variants in the vitamin D-binding gene. Thorax 2010;65(3):215-20. doi: 10.1136/thx.2009.120659.

19. Ahn J, Yu K, Stolzenberg-Solomon R, Simon KC, McCullough ML, Gallicchio L, Jacobs EJ, Ascherio A, Helzlsouer K, Jacobs KB, et al. Genome-wide association study of circulating vitamin D levels. Hum Mol Genet 2010;19(13):2739-45. doi: 10.1093/hmg/ddq155.

20. Robien K, Butler LM, Wang R, Beckman KB, Walek D, Koh W-P, Yuan J-M. Genetic and environmental predictors of serum 25-hydroxyvitamin D concentrations among middle-aged and elderly Chinese in Singapore. Br J Nutr 2013;109(03):493-502. doi: doi:10.1017/S0007114512001675.

21. Food4Me. Internet: http://www.food4me.org/ (accessed 12th February 2016).

22. Celis-Morales C, Livingstone KM, Marsaux CFM, Forster H, O'Donovan CB, Woolhead C, Macready AL, Fallaize R, Navas-Carretero S, San-Cristobal R, et al. Design and baseline characteristics of the Food4Me study: a web-based randomised controlled trial of personalised nutrition in seven European countries. Genes Nutr 2015;10(1):450. doi: 10.1007/s12263-014-0450-2.

23. Hoeller U, Baur M, Roos FF, Brennan L, Daniel H, Fallaize R, Forster H, Gibney ER, Gibney M, Godlewska M, et al. Application of dried blood spots to determine vitamin D status in a large nutritional study with unsupervised sampling: the Food4Me project. Br J Nutr 2016;115(02):202-11. doi: doi:10.1017/S0007114515004298.

24. Forster H FR, Gallagher C, O'Donovan CB, Woolhead C, Walsh MC, Macready AL, Lovegrove JA, Mathers JC, Gibney MJ, Brennan L, Gibney ER. Online Dietary Intake Estimation: The Food4Me Food Frequency Questionnaire. J Med Internet Res 2014;16(6):e150.

25. Fallaize R, Forster H, Macready AL, Walsh MC, Mathers JC, Brennan L, Gibney ER, Gibney MJ, Lovegrove JA. Online Dietary Intake Estimation: Reproducibility and Validity of the Food4Me Food Frequency Questionnaire Against a 4-Day Weighed Food Record. J Med Internet Res 2014;16(8):e190. doi: 10.2196/jmir.3355.

26. Food Standards Agency. McCance and Widdowson's The Composition of Foods. Sixth summary edition ed. Cambridge: Royal Society of Chemistry, 2002.

27. Diffey BL. An overview analysis of the time people spend outdoors. Br J Dermatol 2011;164(4):848-54. doi: 10.1111/j.1365-2133.2010.10165.x.

28. European Commission. 1 April 2015. Internet: https://ec.europa.eu/esco/web/guest/hierarchybrowser/-/browser/Occupation (accessed 1st April 2015).
29. European Commission. 27 March 2015. Internet: http://ec.europa.eu/eurostat/web/products-datasets/-/earn_ses_agt28 (accessed 27th March 2015).
30. United Nations. Internet: http://millenniumindicators.un.org/unsd/methods/m49/m49regin.htm (accessed 4th April 2016).
31. Celis-Morales C, Livingstone K, Woolhead C, Forster H, O'Donovan C, Macready A, Fallaize R, Marsaux CM, Tsirigoti L, Efstathopoulou E, et al. How reliable is internet-based self-reported identity, socio-demographic and obesity measures in European adults? Genes Nutr 2015;10(5):1-10. doi: 10.1007/s12263-015-0476-0.
32. Marsaux CF, Celis-Morales C, Livingstone KM, Fallaize R, Kolossa S, Hallmann J, San-Cristobal R, Navas-Carretero S, O'Donovan CB, Woolhead C, et al. Changes in Physical Activity Following a Genetic-Based Internet-Delivered Personalized Intervention: Randomized Controlled Trial (Food4Me). J Med Internet Res 2016;18(2):e30. doi: 10.2196/jmir.5198.
33. Daly RM, Gagnon C, Lu ZX, Magliano DJ, Dunstan DW, Sikaris KA, Zimmet PZ, Ebeling PR, Shaw JE. Prevalence of vitamin D deficiency and its determinants in Australian adults aged 25 years and older: a national, population-based study. Clin Endocrinol (Oxf) 2012;77(1):26-35. doi: 10.1111/j.1365-2265.2011.04320.x.
34. Public Health England. National Diet and Nutrition Survey: results from Years 1 to 4 (combined) of the rolling programme for 2008 and 2009 to 2011 and 2012. 2014.
35. Xu C, Perera RAPM, Chan Y-H, Fang VJ, Ng S, Ip DKM, Kam AM-S, Leung GM, Peiris JSM, Cowling BJ. Determinants of serum 25-hydroxyvitamin D in Hong Kong. Br J Nutr 2015;114(01):144-51. doi: doi:10.1017/S0007114515001683.
36. Soininen S, Eloranta A-M, Lindi V, Venäläinen T, Zaproudina N, Mahonen A, Lakka TA. Determinants of serum 25-hydroxyvitamin D concentration in Finnish children: the Physical Activity and Nutrition in Children (PANIC) study. Br J Nutr 2016;115(06):1080-91. doi: doi:10.1017/S0007114515005292.
37. Cheng JB, Levine MA, Bell NH, Mangelsdorf DJ, Russell DW. Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase. Proc Natl Acad Sci U S A 2004;101(20):7711-5. doi: 10.1073/pnas.0402490101.
38. Speeckaert M, Huang G, Delanghe JR, Taes YEC. Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism. Clin Chim Acta 2006;372(1–2):33-42. doi: http://dx.doi.org/10.1016/j.cca.2006.03.011.
39. Cashman KD, Hill TR, Lucey AJ, Taylor N, Seamans KM, Muldowney S, FitzGerald AP, Flynn A, Barnes MS, Horigan G, et al. Estimation of the dietary requirement for vitamin D in healthy adults. The American Journal of Clinical Nutrition 2008;88(6):1535-42. doi: 10.3945/ajcn.2008.26594.
40. Baggerly CA, Cuomo RE, French CB, Garland CF, Gorham ED, Grant WB, Heaney RP, Holick MF, Hollis BW, McDonnell SL, et al. Sunlight and Vitamin D: Necessary for Public Health. J Am Coll Nutr 2015;34(4):359-65. doi: 10.1080/07315724.2015.1039866.
41. Scientific Advisory Committee on Nutrition. Draft Vitamin D and Health report. 2015.
42. Interagency Committee on Human Nutrition Research. 11 April 2016. Internet: https://fnic.nal.usda.gov/sites/fnic.nal.usda.gov/files/uploads/2016-03-30-%20ICHNR%20NNRR%20%282%29.pdf (accessed 9th March 2016).
43. Hackman RM, Aggarwal BB, Applebaum RS, deVere White RW, Dubick MA, Heber D, Ito T, Johnson GH, Keen CL, Winters BL, et al. Forecasting Nutrition Research in 2020. J Am Coll Nutr 2014;33(4):340-6. doi: 10.1080/07315724.2014.943113.

44. Pursey K, Burrows LT, Stanwell P, Collins EC. How Accurate is Web-Based Self-Reported Height, Weight, and Body Mass Index in Young Adults? J Med Internet Res 2014;16(1):e4. doi: 10.2196/jmir.2909.
45. Macdiarmid J, Blundell J. Assessing dietary intake: Who, what and why of under-reporting. Nutr Res Rev 1998;11(02):231-53. doi: doi:10.1079/NRR19980017.
46. Diffey B. A Behavioral Model for Estimating Population Exposure to Solar Ultraviolet Radiation†. Photochem Photobiol 2008;84(2):371-5. doi: 10.1111/j.1751-1097.2007.00271.x.
47. Humayun Q, Iqbal R, Azam I, Khan AH, Siddiqui AR, Baig-Ansari N. Development and validation of sunlight exposure measurement questionnaire (SEM-Q) for use in adult population residing in Pakistan. BMC Public Health 2012;12(1):1-8. doi: 10.1186/1471-2458-12-421.
48. O'Riordan DL, Glanz K, Gies P, Elliott T. A Pilot Study of the Validity of Self-reported Ultraviolet Radiation Exposure and Sun Protection Practices Among Lifeguards, Parents and Children. Photochem Photobiol 2008;84(3):774-8. doi: 10.1111/j.1751-1097.2007.00262.x.
49. Livingstone KM, Celis-Morales C, Navas-Carretero S, San-Cristobal R, O'Donovan CB, Forster H, Woolhead C, Marsaux CFM, Macready AL, Fallaize R, et al. Profile of European adults interested in internet-based personalized nutrition: The Food4Me Study. Eur J Nutr 2015.
50. Cashman KD, Kinsella M, McNulty BA, Walton J, Gibney MJ, Flynn A, Kiely M. Dietary vitamin D2 – a potentially underestimated contributor to vitamin D nutritional status of adults? Br J Nutr 2014;112(02):193-202. doi: doi:10.1017/S0007114514000725.
51. Nimitphong H, Saetung S, Chanprasertyotin S, Chailurkit L-o, Ongphiphadhanakul B. Changes in circulating 25-hydroxyvitamin D according to vitamin D binding protein genotypes after vitamin D(3) or D(2) supplementation. Nutrition Journal 2013;12:39-. doi: 10.1186/1475-2891-12-39.

Table 1 Associations between circulating 25(OH) vitamin D concentrations, socio-demographic and lifestyle characteristics

	25 OH- Vitamin D	P value
Age (year)	0.004 (0.002, 0.005)	0.002
Female (ref male)	-0.176 (-0.224, -0.127)	<0.001
Caucasian (ref other)	0.074 (-0.060, 0.207)	0.28
Country (ref British Isles)		
Western Europe	0.272 (0.208, 0.336)	<0.001
Southern Europe	0.190 (0.123, 0.258)	<0.001
Eastern Europe	0.185 (0.108, 0.261)	<0.001
Physical activity[3]		
PAL	0.537 (0.281, 0.792)	<0.001
MVPA (MET/min/week)	0.047 (0.017, 0.077)	0.002
Sedentary behavior	-0.001 (-0.001, -0.001)	0.011
Vitamin D		
Supplement user (ref non-user)	0.242 (0.184, 0.300)	<0.001
Total Vitamin D intake (IU/d)	0.091 (0.056, 0.126)	<0.001
Vitamin D intake (food; IU/d)	0.055 (0.013, 0.096)	0.010
Weekday sunlight exposure (ref < 20 min/d)		
20 min to 2 h/d	0.038 (-0.028, 0.104)	0.225
> 2 h/d	0.133 (-0.042, 0.224)	0.004
Weekend sunlight exposure (ref < 20 min/d)		
20 min to 2 h/d	0.780 (-0.045, 0.201)	0.21
> 2 h/d	0.136 (0.009, 0.262)	0.036
Season (ref winter)		
Spring	0.155 (0.100, 0.210)	<0.001
Summer	0.263 (0.170, 0.356)	<0.001
Autumn	0.306 (0.197, 0.414)	<0.001

1, Values represent log-transformed (ln) coefficients (95% CI); PAL, physical activity level; MVPA, Moderate and vigorous physical activity; PA, physical activity.

2, Multiple linear regression was used to test for significant differences between groups. Analyses were adjusted for age, sex, ethnicity, season, country, BMI (where appropriate), vitamin D intake from foods (where appropriate) and vitamin D supplementation.

3, PA measures were available in 1161 participants only

25

Table 2 Associations between vitamin D-related genotype and circulating 25(OH) vitamin D concentrations

SNP	Minor allele frequency	Hardy Weinberg equilibrium (P value)	M/m	Gt	N	25 OH- vitamin D (nmol/L, 95% CI)[1]	P[2]
VDR rs1544410	40.2	0.811	G/A	GG	467	54.9 (52.8, 57.0)	0.82
				AG	635	54.9 (53.1, 56.7)	
				AA	210	54.4 (51.4, 57.6)	
VDR rs2228570	38.8	0.654	C/T	CC	487	54.9 (52.9, 57.0)	0.74
				CT	631	55.0 (53.2, 56.8)	
				TT	194	54.0 (50.9, 57.3)	
GC rs2282679	27.2	0.259	A/C	AA	704	58.1 (56.3, 59.9)	<0.001
				CA	503	51.7 (49.8, 53.6)	
				CC	105	49.1 (45.3, 53.1)	
GC rs4588	28.6	0.912	C/A	CC	670	58.9 (57.1, 60.8)	<0.001
				CA	534	51.1 (49.3, 52.9)	
				AA	108	49.6 (45.9, 53.6)	
GC rs7041	43.0	0.824	G/T	GG	429	59.5 (57.2, 61.9)	<0.001
				TG	639	53.4 (51.7, 55.2)	

TT	244	50.7 (48.1, 53.4)

1, Values represent adjusted means and 95% CI. SNP, single nucleotide polymorphism; Gt, genotype

2, Multiple linear regression was used to test for significant differences between genotype groups. Analyses were adjusted for age, sex, ethnicity, season, country, BMI, vitamin D intake from foods and vitamin D supplementation.

FIGURE LEGENDS

Figure 1 Consort diagram of participants randomized into the Food4Me Proof of Principle Study. * Total number of participants reporting one or more exclusion criteria. SNP, single nucleotide polymorphism

Figure 2 Concentration of 25(OH) vitamin D according to vitamin D genetic risk score. Minor alleles for *GC rs2282679, GC rs4588, GC rs7041, VDR rs2228570, VDR rs1544410* were coded as 0, 1 and 2 and summed to generate the risk score and grouped into 0-2, 3 to 5 or 6 or more minor alleles.

Values represent adjusted means and SE. Analyses were adjusted for age, sex, ethnicity, season, country, BMI, vitamin D intake from foods and vitamin D supplementation.

Figure 3 Impact of time spent in sunlight during a weekday on the relationship between Vitamin D receptor (VDR) gene SNP *VDR rs2228570 (TT)* and 25(OH) vitamin D concentrations.

Values represent adjusted means and SE. Analyses were adjusted for age, sex, ethnicity, season, country, BMI, vitamin D intake from foods and vitamin D supplementation. SNP, single nucleotide polymorphism

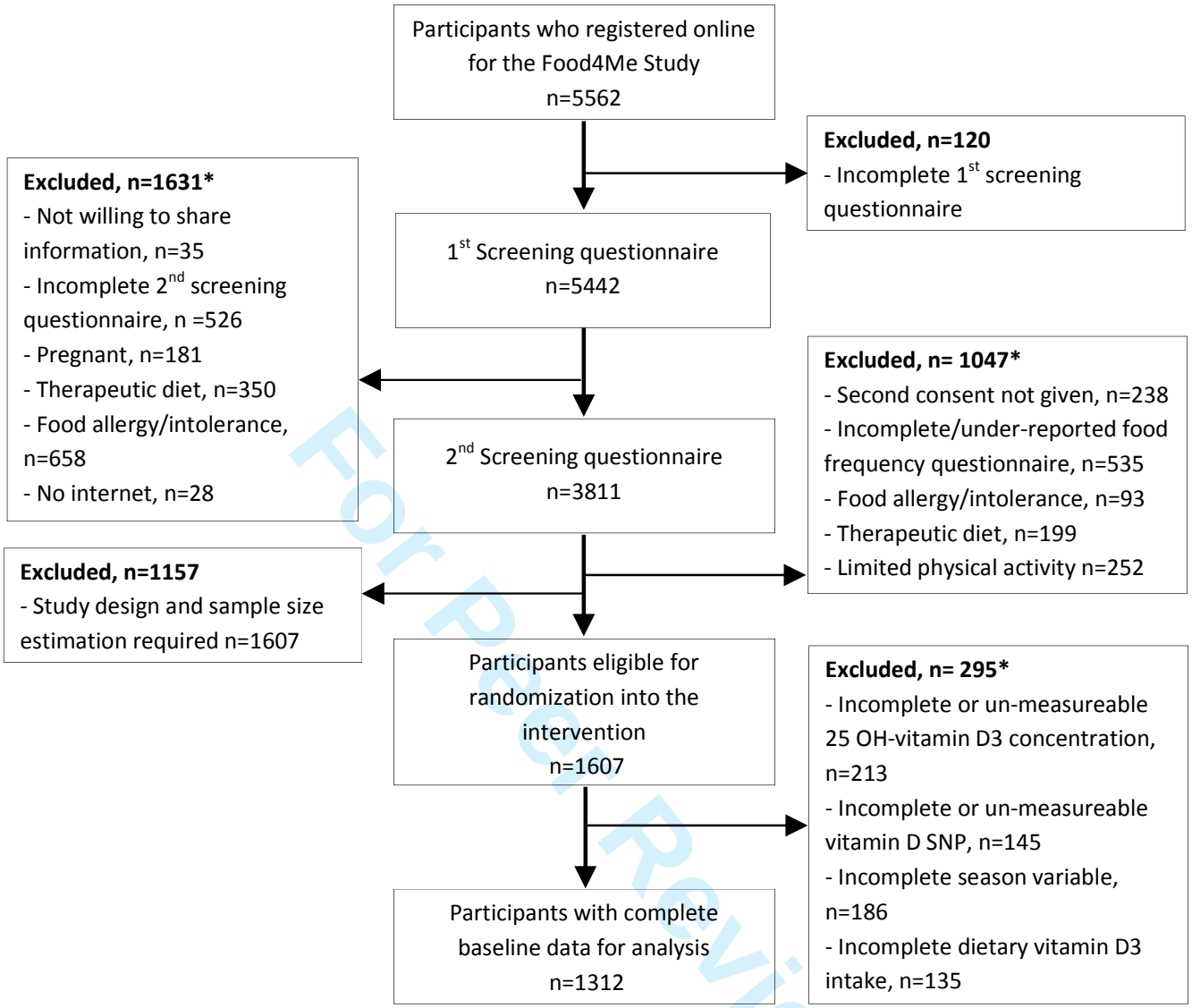

Figure 1

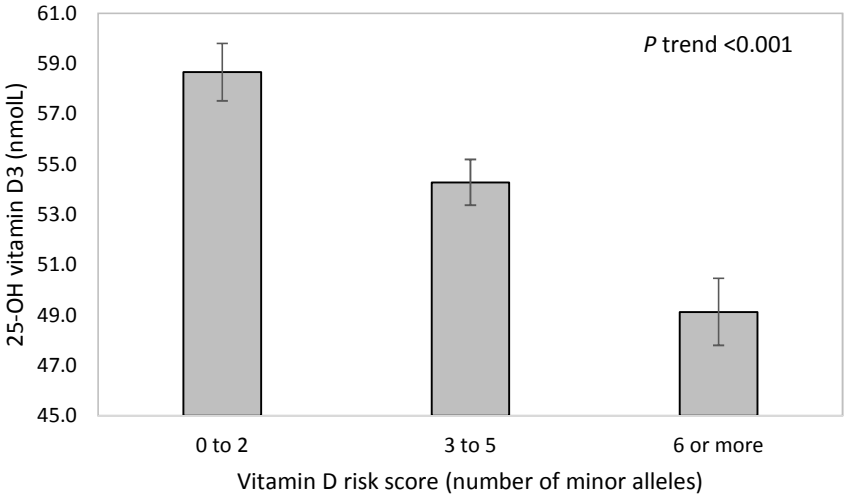

Figure 2

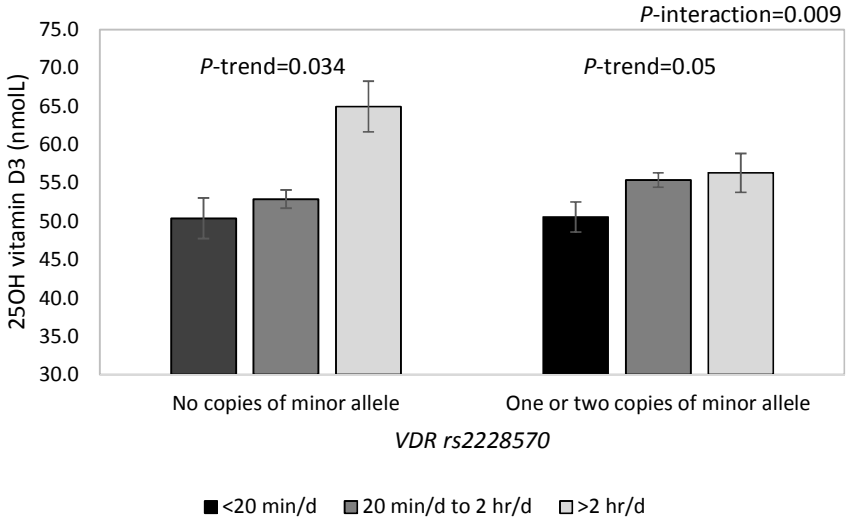

Figure 3