Remote testing of vitamin D levels across the UK MS population – a case control study

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Abstract

Objective: The association between vitamin D deficiency and multiple sclerosis (MS) is well described. We set out to use remote sampling to ascertain vitamin D status and vitamin D supplementation in a cross-sectional study of people with MS across the UK.

Methods: People with MS and matched controls were recruited from across the UK. 1768 people with MS enrolled in the study; remote sampling kits were distributed to a subgroup. Dried blood spots (DBS) were used to assess serum 25(OH)D in people with MS and controls.

Results: 1768 MS participants completed the questionnaire; 388 MS participants and 309 controls provided biological samples. Serum 25(OH)D was higher in MS than controls (median 71nmol/L vs 49nmol/L). A higher proportion of MS participants than controls supplemented (72% vs 26%, $p<0.001$); people with MS supplemented at higher vD doses than controls (median 1600 vs 600 IU/day, $p<0.001$). People with MS who did not supplement had lower serum 25(OH)D levels than non-supplementing controls (median 38 nmol/L vs 44 nmol/L). Participants engaged well with remote sampling.

Conclusions: The UK MS population have higher serum 25(OH)D than controls, mainly as a result of vitamin D supplementation. Remote sampling is a feasible way of carrying out large studies.

Introduction

MS susceptibility is a complex trait influenced by genetic and environmental factors. Established environmental risk factors include EBV seropositivity, smoking, and childhood obesity [1–3]. Low serum 25-hydroxyvitamin D (25(OH)D) levels in adulthood, or even soon after birth, are associated with greater risk of developing MS [4-6]. Vitamin D is primarily derived from the UV light-dependent conversion of 7-dehydrocholesterol to cholecalciferol in skin. Serum 25(OH)D is formed by the hepatic 25-hydroxylation of cholecalciferol, which is further hydroxylated in the kidney to generate the biologically active compound (1,25 hydroxyvitamin D). 25(OH)D is most commonly used as a measure of vitamin D status due to its long half-life, relative stability and direct biological relationship to 1,25 hydroxyvitamin D [7].

Vitamin D is an attractive target for potential intervention in MS as it represents an easily modifiable factor. However, data is conflicting regarding the role of vitamin D in driving inflammation and/or progression in people with established MS. Clinical trials of vitamin D supplementation in MS have failed to provide robust evidence of benefit [8]. Several recent meta-analyses looking at clinical trials of vitamin D for the treatment of MS have demonstrated at best modest reductions in annualised relapse rates (ARR) and/or brain lesion activity but no impact on disability [9–11] .

There are thought to be multiple factors influencing vitamin D status in MS populations [12]. Current population guidelines recommend an intake of at least 400IU/day vitamin D for all [13]. There is a lack of consensus and evidence on whether people with MS should be advised to supplement with vitamin D over and above the advice given to the general population. Single centre studies examining vitamin D supplementation behaviours are subject to bias due to practices of individual neurologists; collecting supplementing information without the wider lifestyle context or serum vitamin D levels significantly limits interpretation.

Remote sampling using dried blood spots provides a means of testing biomarkers across an entire population without the need for in-person visits, which is of rapidly increasing relevance in the current COVID-19 pandemic. We set out to examine the feasibility of a large-scale research project performed entirely remotely, including remote sampling using dried blood spots. We used remotely deployed questionnaires backed up with biological sampling to examine the behaviours and lifestyle factors that influence vitamin D and assess their contribution to the serum vitamin D status across the UK MS population.

Methods

Study recruitment

The primary method of recruitment was via the UK MS Register [15]. 14,991 individuals with MS were invited to participate; 1722 people with MS provided informed consent and completed a baseline questionnaire over 6 weeks using the online platform; an additional 25 participants (postal participants) directly contacted the study site (Figure 1). Individuals were additionally recruited via regional MS networks. Questionnaires with sampling kits were distributed to three MS clinics across the UK - Edinburgh, Lanarkshire and London. 68 sampling kits were handed out to potential participants. Each MS participant who was given a sampling pack was asked to recruit an unrelated friend as a matched control. They were asked to select someone of the same gender, within 5 years of age and living within a 50-mile radius (but not in the same house) as themselves.

Ethical permissions

The UK MS Register has ethical approval via South West Bristol REC (16/SW/0194). This study had additional ethical permissions via London Stanmore REC (18/LO/1455).

Stratified random sampling

Stratified random sampling was used to select 575 UK MS register participants to receive kits. Participants were grouped (stratified) based on geographical location (100km x 100km square), MS type (RRMS, SPMS, PPMS) and disability (low disability classified as EDSS <6, high disability EDSS ≥6). Random sampling within groups was then performed.

Questionnaire data

A host of demographic and MS-specific data were collected including geographical location, gender, age, BMI, smoking status, MS type, EDSS, MSIS and date of diagnosis. Where available, Expanded Disability Status Scores (EDSS) derived from a web-based application were used as a proxy for disability levels [16], and estimates of disease physical and psychological impact via the Multiple Sclerosis Impact Scale (MSIS-29). Data on vitamin D supplementation was collected including supplement use, frequency, and dose at

the time of questionnaire completion. Participants completing the online form were invited to upload an image of their supplement to validate supplement dose. Information of diet type and consumption of oily fish, and assessment of time spent on outdoor activities and UV sun protection averaged over the past 3 months was also collected. To ensure complete capture of sun protection factor containing products in addition to sunblock (moisturiser, foundation, mineral powder etc.), participants were asked about both 'cosmetic sunblock' and 'sunblock' usage.

Sampling kits

Each sampling pack contained two sampling kits, one for the MS participant and one for their matched control. Each sampling kit contained a fully equipped dried blood spot (DBS) sampling system to collect a blood sample for vitamin D analysis, and a buccal swab for genetic material. A questionnaire was included for controls, and for those MS participants where data was not entered via the online system. Sampling packs were sent out February-July 2019. Samples were received back at the study site February-September 2019.

25(OH)Vitamin D analysis

Serum vitamin D concentrations were measured from DBS [27]. Upon receipt samples were stored at -80°C and underwent analysis in four batches. Liquid chromatography tandem mass spectrometry was used to determine total 25(OH)D [17,18]. Two DBS were analysed per participant; results were excluded if duplicate analysis differed by ≥15%, if only one viable DBS was available, or if DBS were deemed to be of poor quality, i.e, spots too small, not fully soaked through or multiple overlapping spots.

Vitamin D levels and MS in UK Biobank

We then set out to verify our findings using an independent sample set derived from UK Biobank (UKBB) [19]. Questionnaire and biomarker data from participants' baseline visit (2006-2010) were used. Each individual with MS at the time of UK Biobank registration (n=1978) was randomly matched to four controls (n=7912), stratified by age, gender, and ethnicity (white vs non-white). Data including baseline serum 25(OH)D levels, vitamin D supplementation (yes/no; no dose information available), oily fish consumption, time spent outdoors and UV sun protection usage were analysed.

Statistical Analysis

Statistical analyses for MS register data were performed using SPSS v26 and R (v.1.2.5001). Geographical mapping was performed using ArcGIS 10.5. Analysis of UK Biobank data was carried out using R (version 3.6.1). Relationships between categorical variables were analysed using the chi-squared test of association; non-normally distributed continuous variables were analysed using the Mann-Whitney U test and the Kruskal Wallis test was used to compare 3+ groups. Simple linear regression was used to examine the relationship between demographic, solar and lifestyle behaviour that may affect dose of vitamin D and serum 25(OH)D levels.

Data availability

Individual level data used in this study is available via the UK MS Register by application from any suitably qualified investigator to the UK MS Register steering committee.

Results

Questionnaire data

1768 participants with MS provided questionnaire data. This group consisted of 1722 individuals recruited via the UK MS Register, 25 postal participants and 21 participants from local MS clinics who returned packs. This group had a wide geographical distribution across the UK (Supplementary Figure 1a). Their demographics were consistent with that expected across an MS population; 75% female and predominantly relapsing remitting MS (RRMS) (Table 1).

Biological sampling and matched controls

600 sampling kits were posted out to participants. Of 100 kits sent to network sites, 68 were distributed to potential participants. Sampling packs were sent out to participants from across the United Kingdom including the Shetland Islands, Orkney Islands, Outer Hebrides, Isle of Man and Channel Islands (Supplementary Figure 1b). 388 sample kits (58%) were completed and returned. 326/388 returned kits (84%) included a matched control. 17 MS and 17 control participants had DBS samples excluded or not received, and 7 controls did not complete a questionnaire (4 of whom provided a DBS). Thus 388 MS cases (371 with DBS), 309 control DBS, and 305 control questionnaires were included in the analysis (Figure 1).

The demographics of the group from whom biological samples were obtained reflected stratified sampling across MS type and disability levels (Table 1), with approximately 35% RRMS, 31% SPMS, 24% PPMS. EDSS scores were available for 107 participants in this group; the median EDSS was 6.5 (IQR 3) (Table 1). Controls appeared well-matched (Table 1), with no significant difference in sex or age distribution. Controls had a slightly higher BMI than participants with MS (median BMI 25 in MS vs 26 in controls; p=0.02), and there was no difference in the proportion of current smokers in the two groups.

Vitamin D supplementation between MS and controls

72% (276/386) of the MS participants from the biological sampling group reported taking vitamin D supplements compared to 26% (79/305) of controls (p<0.001; Table 2). This did not appear to be restricted to the UK MS Register population: 63% (12/19) MS participants recruited through clinics supplemented compared to just 10% (2/21) of their matched controls. There was no difference in reported rates of vitamin D supplementation across gender, MS type, disability level or score on MSIS (Table 2). Where dose data were available, MS participants (n=238) reported a higher median vitamin D supplement dose than controls (n=63) (1600 vs. 600 IU/day; p<0.001) (Figure 2a).

Vitamin D supplementation in MS

Exploratory analysis of all MS questionnaire data (nMS=1768) demonstrated that both participants with RRMS and PPMS reported taking a higher vitamin D supplement dose than participants with SPMS (median dose 2000 IU/day for both RRMS and PPMS vs 1600 IU/day for SPMS; p=0.007) (Supplementary Table 1). Linear regression demonstrated that vitamin D supplement dose decreased with increasing years since diagnosis, although age did not appreciably affect dose (Supplementary Table 2).

Lifestyle factors influencing serum vitamin D levels

More MS participants identified as either vegetarian or vegan (11% vs 4% controls), p=0.003. There was no difference in oily fish consumption (Supplementary Table 3). MS participants were more likely to report rarely spending time on outdoor activities (44% vs 14% controls), p<0.001 (Supplementary Table 3), which was strongly associated with disability levels. 71% (47/66) of participants with high EDSS (≥6) rarely participated in outdoor activities compared to 17% (7/41) of participants with low EDSS (<6) (p<0.001) (data not shown). MS participants were less likely than controls to wear sunblock (31 vs 13% "never" wear sunblock, p<0.001) (Supplementary Table 3). Females, both cases and controls, were more likely to wear cosmetic sunblock than males (24% females vs 2% males reported wearing it weekly or

more, p<0.001) (Supplementary Table 3). There was no significant difference between MS and control females with respect to cosmetic sunblock usage, p=0.09 (Supplementary Table 3).

Serum 25(OH)D levels

Median serum 25(OH)D levels were higher in MS participants than controls: 71 vs 49nmol/L, p<0.001 (Figure 2b). MS participants were more likely to have adequate serum levels (defined as >50nmol/L) (75% MS vs 47% controls) (Table 3). There were no differences in serum vitamin D levels by gender, MS type or disability level (data not shown). Subgroup analyses stratified by supplementing status demonstrated that MS participants who did not supplement (n=92) had lower median serum 25(OH)D levels compared to non-supplementing controls (n=194) (38 vs 44nmol/L, p=0.06). Conversely, supplementing MS participants had higher 25(OH)D levels than supplementing controls (82 vs 68nmol/L, p<0.001) (Table 2; Figure 2c).

Solar contribution to serum 25(OH)D levels was studied using a linear regression model, which confirmed the assumption that, in the entire non-supplementing population (i.e. MS and control), latitude and time spent outdoors were significant contributors to serum 25(OH)D level (R^2 =0.22, p<0.001). There was a negative association between latitude and serum 25(OH)D and positive association with time spent outdoors. Season of sampling and use of sunblock did not affect serum levels (Supplementary Table 4).

In the non-supplementing MS population increasing age had a negative association with serum 25(OH)D in a multivariable model. BMI was not associated with serum 25(OH)D levels. In the supplementing MS population there was a positive association between increasing vitamin D dose and serum 25(OH)D levels, but age, BMI or solar contributions were not associated with serum levels (Supplementary table 4).

Vitamin D levels in UK Biobank

People with MS in UKBB had lower median serum 25(OH)D levels than matched controls (44 vs 47 nmol/L, p<0.001). There was no difference between supplementing participants with MS vs supplementing controls (median serum 25(OH)D level 57 vs 58nmol/L). Non-supplementing people with MS had lower median serum 25-(OH)D levels than either group (42nmol/L) (Supplementary Table 5). A lower proportion of people with MS took vitamin D supplementation at UKBB enrolment than in our

current study, however they were still more likely to do so than the matched controls (14% vs 6%, p<0.001) (Supplementary table 5).

Discussion

In this case-control study we found a striking difference in vitamin D supplementation between people with MS and controls. 72% participants with MS report taking vitamin D supplements compared to just 26% of controls. Not only were MS participants more likely to take vitamin D supplements, but they also took them at higher doses, such that people with MS in the UK now have overall higher serum 25(OH)D levels than controls. When stratified by supplementation habits we found that non-supplementing people with MS had lower levels of serum 25(OH)D. These findings carry implications for any future vitamin D supplementation trial - double blind, placebo-controlled supplementation trials need to take current behavioural patterns into account, and a "treat to target" trial utilising remote sampling is likely the most feasible study design for any large-scale study.

This study is novel in its use of remote sampling technology. Given the current COVID-19 pandemic, the use of remote technologies to enable clinical trials to continue is highly relevant; we demonstrate that this is feasible in MS. The wide coverage we were able to achieve using remote sampling is particularly important when studying an environmentally sensitive endpoint such as serum 25(OH)D. The recruitment of a large pool of participants allowed us to stratify and select participants for biological sampling which represented all stages of MS with a range of disability. The use of straightforward sampling techniques carried out by participants at home allowed us to enrol all members of the MS community regardless of care centre, location or disability level. The relatively low rate of responses to the initial questionnaire likely reflects that this was the first UK MS Register-hosted study where participants were asked to de-anonymise themselves for research purposes, and where biological sampling was required. The rate of return of usable sample packs (58%) is in keeping with other studies requiring sample return.

This study is not without limitations. As recruitment primarily took place through a voluntary MS Register, it could be argued that this high rate of supplementation resulted from a recruitment bias with an *a priori* interested population. People were aware from the information sheet that the purpose of the study was to establish vitamin D levels across the UK MS population. However, no overt reference was

made to either an underlying hypothesis linking vitamin D deficiency to MS or recommended intakes. It could also be argued that the population taking part in the UK MS Register represent a more engaged and educated group with respect to vitamin D supplementation and MS. Furthermore, the recruitment of a subset of individuals directly from MS clinics across the UK enabled us to estimate bias related to method of recruitment. Similarly high rates of supplementation were found in MS participants recruited via both means.

Participant recruitment of age and sex-matched controls may have induced bias related to overmatching, however the exclusion of household controls mitigates this to some degree. Whilst similarities may remain around socioeconomic status and other lifestyle factors, we see that the impact of differential vitamin D status far outweighs this.

Whilst the UKBB population demonstrated a lower rate of vitamin D supplementation amongst people with MS compared to our current study, vitamin D supplementation was still significantly higher than in controls. The reason(s) for the discrepancy between vitamin D usage between the current study and the UK Biobank population is unclear, but at least some of this difference may be attributed to the changes in vitamin D usage over the last 10 years. UKBB baseline data was collected 10-14 years ago, and attitudes towards vitamin D supplementation in the UK have changed significantly over this time [20].

Due to the cross-sectional nature of this observational case-control study we are unable to make inferences with regard to vitamin D status and disease progression. However, the potential to re-recruit via the same online platform for follow-up remains. The use of self-reported behaviours is a further limitation, however, the dose-response to vitamin D supplementation and validation using photographs of supplements overcome this to some degree. The UK MS Register population is predominantly White British [15] and this study needs to be replicated in an ethnically diverse population. Finally, whilst the return rate of biological samples was high for a survey-based study, it remains significantly lower than in direct sampling studies, and this must be considered in future remote sampling studies.

In conclusion we have characterised the behaviours influencing vitamin D and carried out a detailed analysis of the vitamin D status across the UK MS population. People with MS are more likely to supplement with vitamin D and at higher doses than matched controls. After supplementation behaviours, outdoor activity had the most significant impact on serum 25(OH)D levels. The solar

contribution to vitamin D levels was evidenced through both positive association with time spent outdoors and a negative association with increasing latitude. This study underlines the importance of considering participant lifestyle, behavioural and baseline vitamin D status when considering the design of interventional trials using vitamin D in MS.

Figure legends

Figure 1: Flow chart of the study recruitment and resulting study population.
388 MS participants and 309 matched controls returned sampling kits. In some cases, data or biological material was not available for all data points, resulting in 388 MS with data and 371 MS with DBS and 305 controls with data and 309 controls with DBS.

Figure 2: Vitamin D supplementation dose and serum 25(OH)D levels in MS and control participants. (a) Distribution of dose (IU/day) of vitamin D amongst those MS (n=238) and control (n=63) that take supplements. (b) Serum 25(OH)D levels (nmol/L) of MS (n=321) and control (n=261) participants. (c) Serum 25-(OH)D levels of MS and control split by Vitamin D supplementation status.

Table 1. Participant demographics

Table 2. Vitamin D supplementation behaviour and serum 25-(OH)D levels in the biological sampling group

Table 3. Vitamin D status of MS and matched controls based on 25-(OH)D levels

Supplementary Figure 1: Distribution of study participants across the UK. (a) The distribution of the 1768 study participants who provided questionnaire data. (b) The distribution of the MS participants selected to receive biological sampling kits.

Supplementary Table 1. Vitamin D supplementation behaviour and serum 25(OH)D levels in MS cases

Supplementary Table 2. Multivariable analysis of factors influencing vitamin D dose of participants

Supplementary Table 3. Lifestyle factors and behaviours known to influence serum vitamin D in those who provided biological samples

Supplementary Table 4. Multivariable analysis of variables influencing vitamin D serum 25(OH)D levels

Supplementary Table 5. Demographic details of those included in the UK Biobank study

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The authors have no conflicts of interest directly relevant to this study to declare.

Author contributions:

RD conceived the study with input from GG. NV and RD designed the study with input from MJ, BJ and RM. MJ and BJ designed and performed the statistical analysis. NV, SC, NM, RM played a role in acquiring and analysing data. NV drafted the manuscript with input from all coauthors.

References

1. Ascherio A, Munger KL, Lennette ET, Spiegelman D, Hernán MA, Olek MJ, et al. Epstein-Barr virus antibodies and risk of multiple sclerosis: a prospective study. JAMA. 2001;286: 3083–3088.

2. Jacobs BM, Noyce AJ, Giovannoni G, Dobson R. BMI and low vitamin D are causal factors for multiple sclerosis: A Mendelian Randomization study. Neurol Neuroimmunol Neuroinflamm. 2020;7. doi:10.1212/NXI.0000000000000662

3. Hedström AK, Olsson T, Alfredsson L. Smoking is a major preventable risk factor for multiple sclerosis. Mult Scler. 2016;22: 1021–1026.

4. Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA. 2006;296: 2832–2838.

5. Duan S, Lv Z, Fan X, Wang L, Han F, Wang H, et al. Vitamin D status and the risk of multiple sclerosis: a systematic review and meta-analysis. Neurosci Lett. 2014;570: 108–113.

6. Nielsen NM, Munger KL, Koch-Henriksen N, Hougaard DM, Magyari M, Jørgensen KT, et al. Neonatal vitamin D status and risk of multiple sclerosis: A population-based case-control study. Neurology. 2017;88: 44–51.

7. Holick MF. Vitamin D status: measurement, interpretation, and clinical application. Ann Epidemiol. 2009;19: 73–78.

8. Jagannath VA, Filippini G, Di Pietrantonj C, Asokan GV, Robak EW, Whamond L, et al. Vitamin D for the management of multiple sclerosis. Cochrane Database Syst Rev. 2018;9: CD008422.

9. McLaughlin L, Clarke L, Khalilidehkordi E, Butzkueven H, Taylor B, Broadley SA. Vitamin D for the treatment of multiple sclerosis: a meta-analysis. J Neurol. 2018;265: 2893–2905.

10. Doosti-Irani A, Tamtaji OR, Mansournia MA, Ghayour-Mobarhan M, Ferns G, Daneshvar Kakhaki R, et al. The effects of vitamin D supplementation on expanded disability status scale in people with multiple sclerosis: A critical, systematic review and metaanalysis of randomized controlled trials. Clin Neurol Neurosurg. 2019;187: 105564.

11. Martínez-Lapiscina EH, Mahatanan R, Lee C-H, Charoenpong P, Hong J-P. Associations of serum

25(OH) vitamin D levels with clinical and radiological outcomes in multiple sclerosis, a systematic review and meta-analysis. J Neurol Sci. 2020;411: 116668.

12. Kusumadewi W, Imran D, Witjaksono F, Pakasi TA, Rusmana AI, Pangeran D, et al. Low vitamin D-25(OH) level in Indonesian multiple sclerosis and neuromyelitis optic patients. Mult Scler Relat Disord. 2018;25: 329–333.

13. Dobson R, Cock HR, Brex P, Giovannoni G. Vitamin D supplementation. Pract Neurol. 2018;18: 35–42.

14. Multiple Sclerosis Top 10 | James Lind Alliance. [cited 20 Feb 2020]. Available: http://www.jla.nihr.ac.uk/priority-setting-partnerships/multiple-sclerosis/top-10-priorities/

15. Middleton RM, Rodgers WJ, Chataway J, Schmierer K, Rog D, Galea I, et al. Validating the portal population of the United Kingdom Multiple Sclerosis Register. Mult Scler Relat Disord. 2018;24: 3–10.

16. Leddy S, Hadavi S, McCarren A, Giovannoni G, Dobson R. Validating a novel web-based method to capture disease progression outcomes in multiple sclerosis. J Neurol. 2013 Oct;260(10):2505-10.

17. Eyles D, Anderson C, Ko P, Jones A, Thomas A, Burne T et al. A Sensitive LC/MS/MS Assay of 25OH Vitamin D3 and 25OH Vitamin D2 in Dried Blood Spots. Clin Chim Acta 2009 May;403(1-2):145-51.

18. Heath AK, Williamson EJ, Ebeling PR, Kvaskoff D, Eyles DW, English DR. Measurements of 25-hydroxyvitamin D concentrations in archived dried blood spots are reliable and accurately reflect those in plasma. J Clin Endocrinol Metab. 2014;99: 3319–3324.

19. UK Biobank. [cited 20 Feb 2020]. Available: http://www.ukbiobank.ac.uk/

20. Crowe FL, Jolly K, MacArthur C, Manaseki-Holland S, Gittoes N, Hewison M, et al. Trends in the incidence of testing for vitamin D deficiency in primary care in the UK: a retrospective analysis of The Health Improvement Network (THIN), 2005-2015. BMJ Open. 2019;9: e028355.

21. Shanbhag S, Nayak A, Narayan R, Nayak UY. Anti-aging and Sunscreens: Paradigm Shift in Cosmetics. Adv Pharm Bull. 2019;9: 348–359.

22. Passeron T, Bouillon R, Callender V, Cestari T, Diepgen TL, Green AC, et al. Sunscreen

photoprotection and vitamin D status. Br J Dermatol. 2019;181: 916–931.

23. Neale RE, Khan SR, Lucas RM, Waterhouse M, Whiteman DC, Olsen CM. The effect of sunscreen on vitamin D: a review. Br J Dermatol. 2019;181: 907–915.

24. Hempel S, Graham GD, Fu N, Estrada E, Chen AY, Miake-Lye I, et al. A systematic review of modifiable risk factors in the progression of multiple sclerosis. Mult Scler. 2017;23: 525–533.

25. Oliveira SR, Simão ANC, Alfieri DF, Flauzino T, Kallaur AP, Mezzaroba L, et al. Vitamin D deficiency is associated with disability and disease progression in multiple sclerosis patients independently of oxidative and nitrosative stress. J Neurol Sci. 2017;381: 213–219.

26. Smolders J, Menheere P, Kessels A, Damoiseaux J, Hupperts R. Association of vitamin D metabolite levels with relapse rate and disability in multiple sclerosis. Multiple Sclerosis Journal. 2008. pp. 1220–1224. doi:10.1177/1352458508094399.

27. Shea R and Berg J. Self-administration of vitamin D supplements in the general public may be associated with high 25-hydroxyvitamin D concentrations. Annals of Clinical Biochemistry 2017;54(3) 355-361.

Table 1. Participant demographics

	All participants	Biological sampling group[b]		
	MS (n=1768)	MS (n=388)	Control (n=305)	p-value
female, n (%)	1329 (75)	292 (75)	229 (75)	1
male, n (%)	439 (25)	96 (25)	76 (25)	
age, median (IQR)	53 (15)	56 (14)	55 (16)	0.37
BMI, median kg/m^2 (IQR)	25; 6	25; 6	26; 6	0.02
current smokers, n (%)	70; 5	16; 5	19; 6	0.56
MS type, n;%				
RRMS	976; 55	137; 35		
SPMS	459; 26	120; 31		
PPMS	203; 12	93; 24		
Other	130; 7	38; 10		
EDSS				
median; IQR (n)	6.0; 4	6.5; 3		
low EDSS (<6): n; %	247; 49	41; 38		
high EDSS (≥6); n; %	259; 51	66; 62		

[a]data was missing for the following; BMI 1374 participants, current smoking status 310 participants, EDSS 1262 participants. [b]data was missing for the following: Age, 5 MS and 7 control; BMI, 271 MS and 7 control; current smoking status 69 MS; EDSS 281 MS.

Table 2. Vitamin D supplementation behaviour and serum 25-(OH)D levels in the biological sampling group

	Supplementing behaviour				Serum 25(OH)D levels, median nmol/L (IQR); n[c]			
	Taking supplement n[a] (%)	p-value	Dose IU/day (IQR);n[b]	p-value	No supplement	p-value	Yes supplement	p-value
Disease Status								
MS (n=388)	276 (72)	<0.001	1600 (3200);238	<0.001	38 (35); 92	0.06	82 (47); 229	<0.001
Control (n=305)	79 (26)		600 (800);63		44 (21); 194		68 (34); 67	
MS split by sex								
female	209 (72)	0.67	2000 (3084);181	0.52	38 (36); 70	0.51	82 (46); 168	0.65
male	67 (70)		1000 (4200);57		38 (33); 22		82 (49); 61	
MS type								
RRMS	99 (73)	0.91	2000 (4000);86	0.11	46 (28); 31	0.10	81 (48); 83	0.11
SPMS	88 (73)		1000 (3200);74		32 (27); 26		79 (48); 69	
PPMS	66 (71)		1428 (4000);59		40 (42); 21		88 (51); 58	
MS Disability								
low EDSS (<6)	29 (71)	0.78	2000 (4100);25	0.08	47 (32); 11	0.13	82 (68); 24	0.76
high EDSS (≥6)	45 (68)		1000 (3343);36		28 (44); 16		82 (63); 38	
MSIS[d]								
physical -Low impact	58 (74)	0.70	2857 (4000);51	0.04	46 (64); 16	0.69	88 (51); 46	0.36
physical – High impact	57 (77)		1000 (3593);44		38 (50); 16		80 (51); 46	
psychological -Low impact	61 (75)	0.58	1800 (3750);57	0.77	46 (48); 17	0.76	83 (45); 51	0.66
psychological-High impact	55 (71)		1800 (4200);46		40 (41); 17		84 (48); 43	

[a]data was missing for the following: MS 2 participants, female MS 2 participants, MS type 2 participants, EDSS 281 participants, MSIS 236 participants; [b]of the total n that provided supplementation data this n had a dose available; [c]of the total n that provided supplementation data this n had serum 25(OH)D levels available; [d]MSIS-29 scores were divided into quartiles and comparisons were made between lowest quartile (low impact) and highest quartile (high impact)

Table 3. Vitamin D status of MS and matched controls based on 25(OH)D levels

Serum 25(OH)D nmol/L	Interpretation	MS (n=322) n (%)	Control (n=264) n (%)	p-value
<15	Severe deficiency	4 (1)	2 (1)	
15-30	Deficiency	29 (9)	30 (11)	p<0.001
30.1-50	Insufficiency	48 (15)	108 (41)	
>50	Adequate	241 (75)	124 (47)	

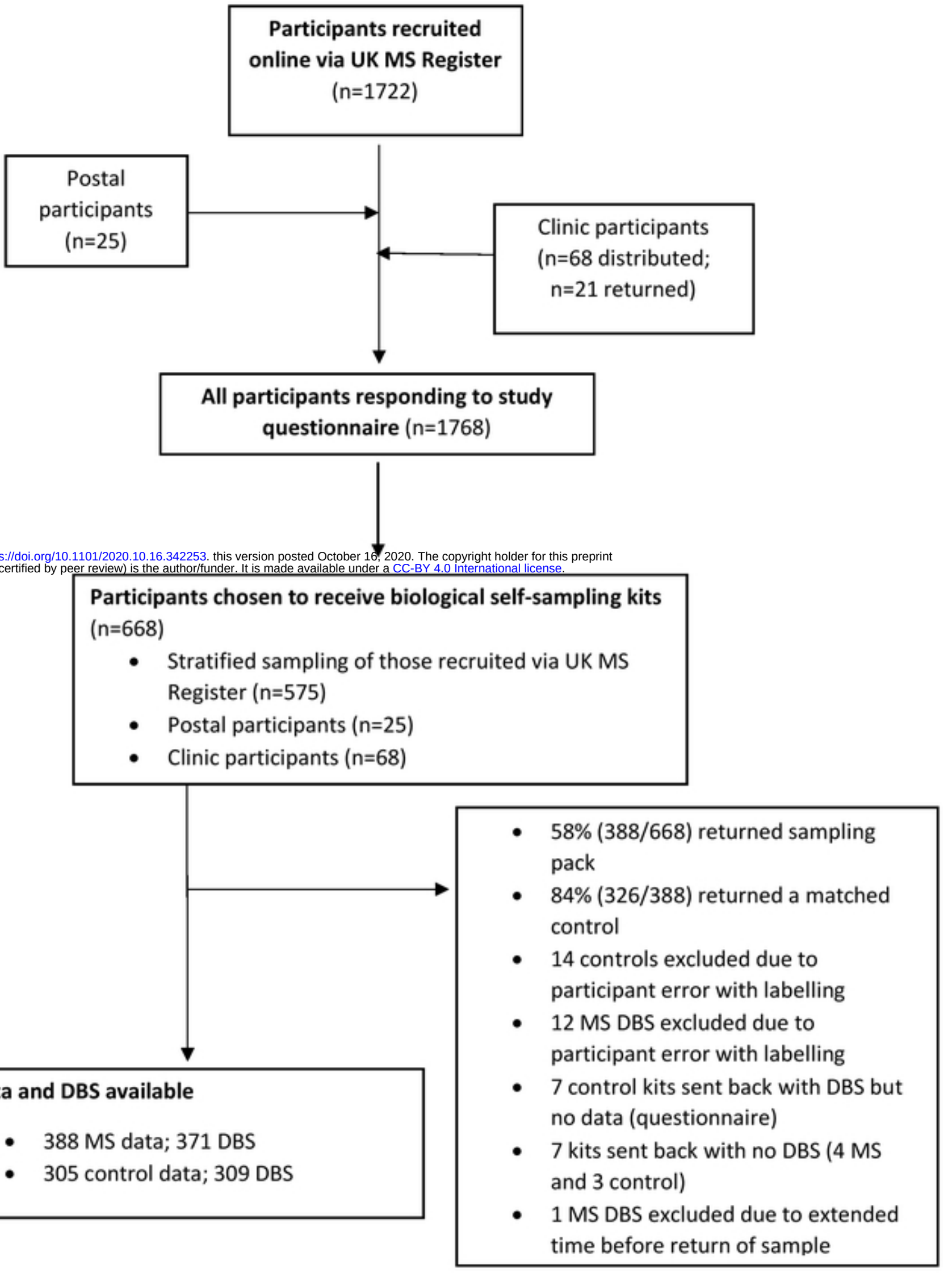

Figure 1

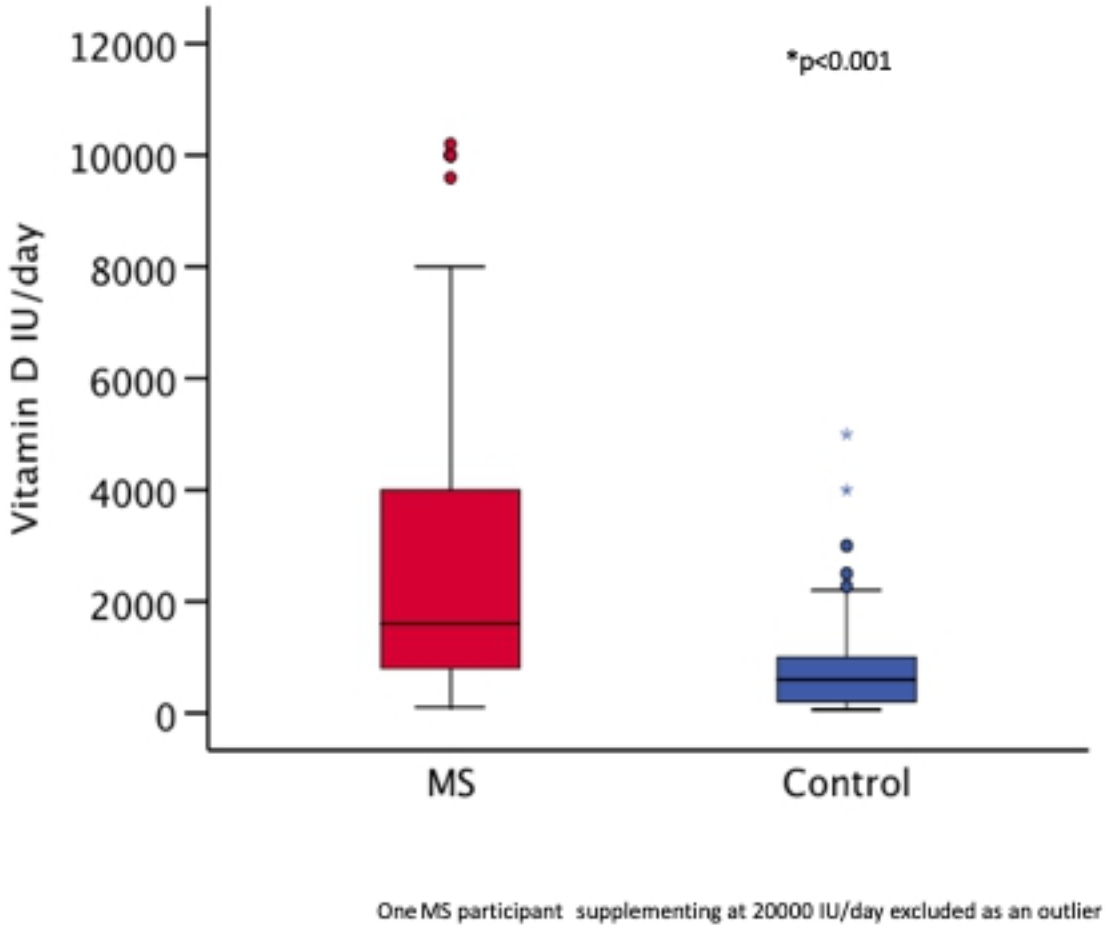
12000
10000
8000
6000
4000
2000
0
Vitamin D IU/day
*p<0.001
MS
Control
One MS participant supplementing at 20000 IU/day excluded as an outlier

Figure 2a

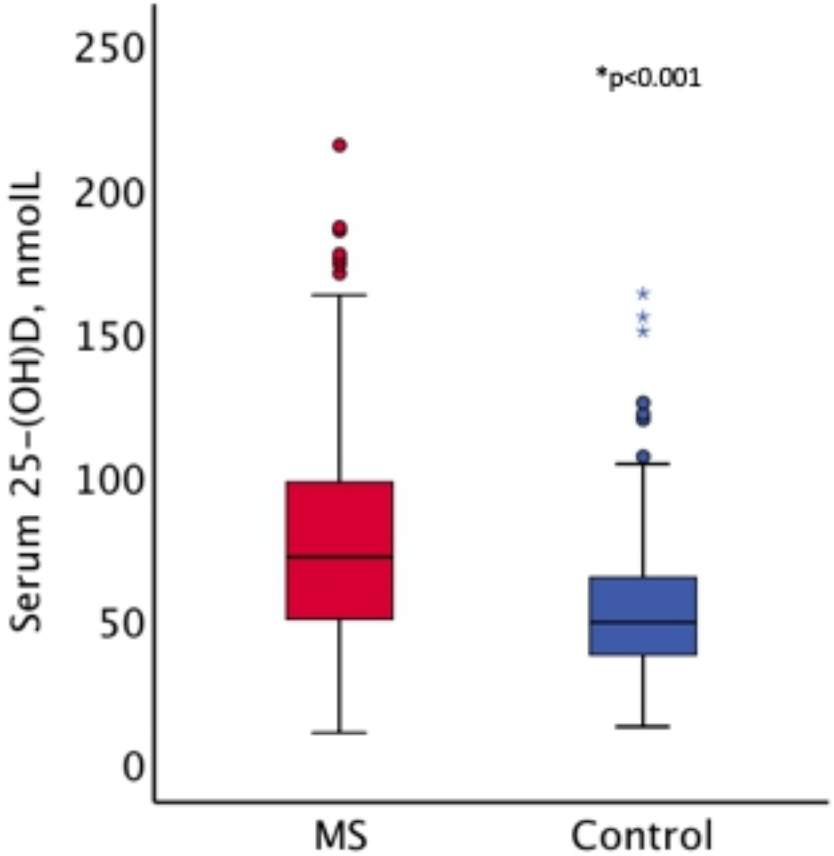

Figure 2b

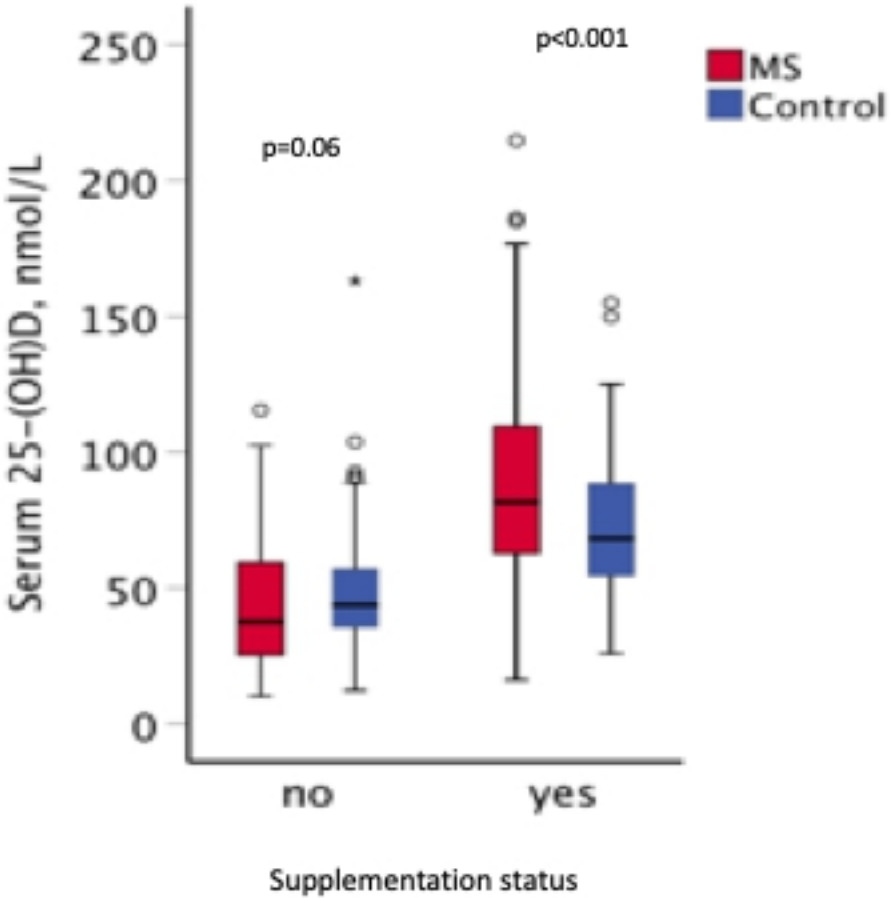
250
200
150
100
50
0
Serum 25-(OH)D, nmol/L
p=0.06
p<0.001
MS
Control
no
yes
Supplementation status

Figure 2c