Lower vitamin D is associated with metabolic syndrome and insulin resistance in systemic lupus: data from an international inception cohort

[1]Christine Chew†, [2,3]John A Reynolds†, [4]Apinya Lertratanakul, [4]Peggy Wu, [5]Murray Urowitz, [5]Dafna D. Gladman, [6]Paul R Fortin, [7]Sang-Cheol Bae, [3]Caroline Gordon, [8]Ann E Clarke, [9]Sasha Bernatsky, [10]John G Hanly, [11]David Isenberg, [11]Anisur Rahman, [5]Jorge Sanchez-Guerrero, [12]Juanita Romero-Diaz, [13]Joan Merrill, [14]Daniel Wallace, [15]Ellen Ginzler, [16]Munther Khamashta, [17]Ola Nived, [17]Andreas Jönsen, [18]Kristjan Steinsson, [19]Susan Manzi, [20]Ken Kalunian, [21]Mary Anne Dooley, [22]Michelle Petri, [23]Cynthia Aranow, [24]Ronald van Vollenhoven, [25]Thomas Stoll, [26]Graciela S Alarcón, [27]S Sam Lim, [28]Guillermo Ruiz-Irastorza, [29]Christine A Peschken, [30]Anca D Askanase, [31]Diane L Kamen, [32]Murat İnanç, [4]Rosalind Ramsey-Goldman*, [33,34]Ian N Bruce*

†Christine Chew and John A. Reynolds contributed equally to this paper.
*Rosalind Ramsey-Goldman and Ian N Bruce contributed equally to this paper.

1 Lydia Becker Institute of Immunology and Inflammation, Manchester Collaborative Centre for Inflammation Research, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester Academic Health Science Centre, Manchester, UK.
2 Sandwell and West Birmingham NHS Trust, Birmingham, UK
3 Rheumatology Research Group, Institute of Inflammation and Ageing, University of Birmingham, Birmingham, UK
4 Northwestern University Feinberg School of Medicine, Chicago, IL, USA
5 Toronto Western Hospital Centre for Prognosis Studies in the Rheumatic Diseases Toronto, ON, CAN
6 Université Laval Faculté de médecine, Department of Rheumatology, Quebec, QC, CAN
7 Hanyang University Hospital for Rheumatic Diseases, Department of Rheumatology, Seongdong-gu, Seoul, KR
8 University of Calgary Cumming School of Medicine, Divisions of Clinical Immunology/Allergy and Clinical Epidemiology, Calgary, AB, CAN
9 McGill University Faculty of Medicine, Division of Rheumatology, Montreal, QC, CAN
10 Queen Elizabeth II Health Sciences Centre, Division of Rheumatology, Department of Medicine and Department of Pathology Halifax, NS, CAN
11 University College London Faculty of Medical Sciences, Division of Medicine, London, London, UK
12 Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Immunology and Rheumatology Tlalpan, DF, MX
13 Oklahoma Medical Research Foundation Arthritis and Clinical Immunology Research Program, Department of Clinical Pharmacology, Oklahoma City, OK, USA
14 Cedars-Sinai Medical Center, Los Angeles, CA, USA
15 SUNY Downstate Medical Center College of Medicine, Department of Medicine, Brooklyn, NY, USA
16 Rayne Institute, St Thomas' Hospital, King's College London School of Medicine, London, London, UK
17 Lunds University Faculty of Medicine, Department of Clinical Sciences Lund, Section of Rheumatology, Lund, SE

18 National University Hospital of Iceland, Department of Rheumatology, Reykjavik, Capital, IS

19 Allegheny Health Network, Lupus Center of Excellence, Pittsburgh, PA, USA

20 University of California San Diego School of Medicine, La Jolla, CA, USA

21 University of North Carolina at Chapel Hill, Division of Rheumatology and Immunology, Department of Medicine, Chapel Hill, NC, USA

22 Johns Hopkins University School of Medicine Center for Musculoskeletal Research, Department of Rheumatology, Baltimore, MD, USA

23 Northwell Health Feinstein Institutes for Medical Research, Manhasset, NY, USA

24 Amsterdam University Medical Centres, Duivendrecht, Noord-Holland, NL

25 Kantonsspital Schaffhausen, Department of Rheumatology, Schaffhausen, Schaffhausen, CH

26 The University of Alabama at Birmingham School of Medicine, Department of Medicine, Division of Clinical Immunology and Rheumatology, Birmingham, AL, USA

27 Emory University School of Medicine, Division of Rheumatology, Atlanta, GA, USA

28 Hospital Universitario Cruces, Autoimmune Diseases Units, Biocruces Bizkaia Health Research Institute, Barakaldo, País Vasco, ES

29 University of Manitoba, Winnipeg, MB, CAN

30 Columbia University Irving Medical Center, New York, NY, USA

31 Medical University of South Carolina, Charleston, SC, USA

32 Istanbul University Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Rheumatology, Istanbul, Istanbul, TR

33 Manchester University NHS Foundation Trust, NIHR Manchester Biomedical Research Centre, Manchester Academic Health Science Centre, Manchester, Greater Manchester, UK

34 Versus Arthritis Centre for Epidemiology, Division of Musculoskeletal and Dermatological Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, Oxford Road, Manchester, UK

Correspondence to: Ian Bruce, Versus Arthritis Centre for Epidemiology, Division of Musculoskeletal and Dermatological Sciences, Stopford Building, The University of Manchester, Oxford Road, Manchester, M13 9PT, UK. Email: ian.bruce@manchester.ac.uk

Abstract

Objectives: Vitamin D (25(OH)D) deficiency and metabolic syndrome (MetS) may both contribute to increased cardiovascular risk in systemic lupus erythematosus (SLE). We aimed to examine the association of demographic factors, SLE phenotype, therapy and vitamin D levels with MetS and insulin resistance.

Methods: The Systemic Lupus International Collaborating Clinics (SLICC) enrolled patients recently diagnosed with SLE (<15 months) from 33 centres across 11 countries from 2000. Clinical, laboratory and therapeutic data were collected. Vitamin D level was defined according to tertiles based on distribution across this cohort, which were set at T1 (10-36 nmol/L), T2 (37-60 nmol/L) and T3 (61-174 nmol/L). MetS was defined according to the 2009 consensus statement from the International Diabetes Federation. Insulin resistance was determined using the HOMA-IR model. Linear and logistic regressions were used to assess the association of variables with vitamin D levels.

Results: Of the 1847 patients, 1163 (63%) had vitamin D measured and 398 (34.2%) subjects were in the lowest 25(OH)D tertile. MetS was present in 286 of 860 (33%) patients whose status could be determined. Patients with lower 25(OH)D were more likely to have MetS and higher HOMA-IR. The MetS components, hypertension, hypertriglyceridemia and decreased HDL were all significantly associated with lower 25(OH)D. Increased average glucocorticoid exposure was associated with higher insulin resistance.

Conclusions: MetS and insulin resistance are associated with lower vitamin D in patients with SLE. Further studies could determine if vitamin D repletion confers better control of these cardiovascular risk factors and improve long-term outcomes in SLE.

Rheumatology key messages

- In patients newly diagnosed with SLE, lower 25(OH)D was associated with MetS and insulin resistance.
- Significant associations of MetS and lower 25(OH)D despite adjustment of patient factors indicates cardiovascular risk.
- Further trials exploring vitamin D supplementation are needed to optimise cardiometabolic outcomes in SLE.

INTRODUCTION

25-hydroxyvitamin D (25(OH)D) deficiency is associated with an increased prevalence of cardiovascular disease (CVD) in the general population (1, 2). Patients with systemic lupus erythematosus (SLE) have an excess cardiovascular risk, up to 50 times that seen in comparator populations (3-6). This increased incidence of CVD cannot be attributed to traditional cardiovascular risk factors alone (7), and therefore it is likely that other variables may have a contributing role.

Vitamin D is traditionally known for its primary function in calcium homeostasis, and has an emerging role in modulating immune responses and in autoimmune disease (8). Vitamin D receptor expression has been described in innate and adaptive immune cells (9, 10), and it has been shown that a vitamin D-deficient diet increased disease activity and CVD features in animal models of SLE (11). Indeed, vitamin D deficiency is more common in patients with SLE compared to age-matched controls (12-14). A previous study demonstrated a significant association between patients with low 25(OH)D and cardiovascular risk, which was no longer significant after adjusting for BMI (14). In the SLICC Inception Cohort our previous analysis involving 890 patients showed an association between lower vitamin D levels and an increased likelihood of having hypertension, hyperlipidaemia, higher C-reactive protein (CRP) and disease activity that remained significant despite adjusting for BMI (12).

While vitamin D deficiency has been associated with classic cardiovascular risk factors in SLE, much less is known about its relationship with metabolic syndrome (MetS) (15). A number of metabolic abnormalities constitute MetS; not all of which are assessed in routine cardiovascular screening tools. MetS is recognised to reflect an increased risk of atherosclerosis and type II diabetes mellitus and is also associated with increased insulin resistance (16). Compared to the general population, patients with SLE are more likely to fulfil MetS criteria (17-19). In addition to being more prevalent in SLE (38%), MetS was also independently associated with increased baseline renal disease, pre-existing organ damage, higher disease activity, older age and Hispanic or Black race/ethnicity (20).

Lower 25(OH)D levels have also previously been associated with increased insulin resistance in non-diabetic patients with SLE (13). The aim of our study was to extend the findings of our previous work (12) and to determine the association between vitamin D levels and MetS in a large international multi-centre SLE inception cohort.

METHODS

SLICC Inception Cohort

Between 1999 and 2011, 1847 patients were recruited into the inception cohort from 33 centres across North America, Europe and Asia. Patients included in this cross-sectional study were recruited within 15 months of confirming ≥4 SLE ACR 1997 classification criteria (20, 21). Disease activity was quantified by the SLE Disease Activity Index-2000 (SLEDAI-2K) (22). Laboratory tests (fasting or non-fasting) for clinical markers of disease activity and CVD risk factors, and to define MetS were performed at each site. All patients provided informed consent. This study was approved by the University Health Network Research Institute research ethics committee, Toronto, Canada and by the Institutional Research Ethics Boards of all participating centres in accordance with the Declaration of Helsinki's guidelines for research in humans.

Vitamin D measurement

Serum 25(OH)D was measured by LIAISON® Vitamin D TOTAL assay (310600, Diasorin, Toronto) through the University Health Network Laboratory Medicine Program. The intraassay coefficient of variation was 5.4% and the inter-assay coefficient of variation was 10.6%.

Traditional CVD risk factors and definition of MetS

Variables for traditional CVD risk factors were collected, including hypertension, body mass index (BMI), hypercholesterolemia, diabetes mellitus, smoking and post-menopausal status. Hypertension was defined as an elevated blood pressure of ≥140/90 mm Hg, or currently being prescribed anti-hypertensive medication. Diabetes mellitus was defined as fasting glucose ≥7.0 mmol/L or a documented diagnosis of diabetes. Insulin resistance was calculated using the homeostatic model assessment of insulin resistance (HOMA-IR) method (23) in patients who had a fasting sample for glucose and insulin measurement. Values of HOMA-IR above 1.0 denote increasing insulin resistance.

MetS was defined using the 2009 definition in the joint interim statement from the International Diabetes Federation Task Force on Epidemiology and Prevention and interested partners (15). This harmonising statement for a MetS diagnosis requires 3 or more of the following five criteria to be present: (1) elevated waist circumference (MetS WC) using gender and ethnicity-specific thresholds; (2) elevated serum triglyceride (MetS TG) of ≥ 1.7 mmol/l or being on lipid-lowering medication, (3) reduced HDL (MetS HDL) of <1.3 mmol/L in women and <1.0 mmol/L in men, (4) elevated BP (MetS BP) of ≥130/85 mm Hg or receiving anti-hypertensive medication, and (5) elevated fasting glucose (MetS Glu) of ≥5.6 mmol/L or being prescribed medication for hyperglycaemia. In patients with missing data on

specific MetS criteria; those who fulfilled 3 or more criteria from the data available could still be classified as MetS. For those in whom 3 or more criteria were not fulfilled from the data available they were still able to be classified as not having MetS. In other situations, patients remained unclassified.

Statistical analysis

Potential variables that may influence serum 25(OH)D and the prevalence of MetS in SLE were defined *a priori* including: age, gender, race/ethnicity (Caucasian, Hispanic, Asian, Black and other), location (Canada, USA, Mexico, Europe, South Korea), disease activity (SLEDAI-2K), cardiovascular risk factors, a MetS diagnosis and SLE-related medication (anti-malarials and glucocorticoids). To adjust for ultraviolet exposure, latitude was used as an approximation of the centre location (24), which were all located in the Northern hemisphere. Patients were classified as 'low latitude', 'middle latitude' and 'high latitude' based on their centre being located between 20° and 34°North (°N), 35° and 45°N and north of 45°N respectively. Continuous data were compared using Wilcoxon rank sum-test and categorical data using the χ^2 test. Due to varied definitions of cut-off values for 25(OH)D levels in the general population, our cohort was categorised into tertiles, defined as T1 (10-36 nmol/L), T2 (37-60 nmol/L) and T3 (61-174 nmol/L), which were used to determine the association between vitamin D levels and cardiovascular and SLE risk factors. Linear and logistic regression models were used to investigate associations between 25(OH)D treated as a continuous variable and predefined variables related to SLE disease phenotype and MetS, and traditional cardiovascular risk factor. For linear regression, some variables were log-transformed to satisfy assumptions of normality and 25(OH)D divided by a constant to produce interpretable beta-coefficients. Logistic regression was used to analyse categorical variables. Receiver operating characteristic (ROC) and area under the ROC curve (AUC) were used to assess the probability of patients having MetS and to assess the role of 25(OH)D in this model. Statistical analyses were performed using StataMP-64 v13.0 (StataCorps, USA).

RESULTS

Patients

Serum 25(OH)D was measured at enrolment in 1163 patients from a cohort of 1847 (63%). Patient characteristics are summarised in Table 1. The majority of the cohort were female (1034/1163, 88.9%) with a median (IQR) age of 33.1 (24.3, 43.6) years, disease duration was 21.7 (9.1, 39.9) weeks and 623/1163 (53.6%) were Caucasian. The group who did not have a sample to measure vitamin D status had similar demographics and disease activity but were different in ethnicity and country of residence compared to the remaining cohort,

and had a higher average daily oral glucocorticoid exposure, total cholesterol and serum triglyceride (Supplementary Table S1, available at *Rheumatology* online).

Vitamin D Status

The median (IQR) 25(OH)D level in the cohort of 1163 patients was 48 (30, 66) nmol/L, in which 398 (34.2%) were in the lowest tertile (T1: 10-36 nmol/L), 393 (33.8%) were in T2 (37-60 nmol/L) and 372 (32.0%) were in T3 (61-174 nmol/L) (Table 2). Patients in the lowest 25(OH)D tertile were younger compared to those in the higher tertiles (30 (23,40) (T1) vs. 34 (25, 45) (T2) and 35 (25, 46) years old (T3); p<0.001). Patients in the lowest 25(OH)D tertile were mostly non-Caucasian (65% vs. 35% Caucasian, p<0.001). Patients located in the 'low latitude' had higher median (IQR) 25(OH)D compared to those living in the 'middle latitude' and 'high latitude' areas (56 (40, 75) vs. 47 (29, 66) nmol/L and 47 (29, 64) nmol/L; p<0.001 for both comparisons) (Figure 1). Patients with active SLE disease (SLEDAI of ≥6) had lower median (IQR) 25(OH)D (45 (28-60) vs. 50 (32-70) nmol/L in patients with SLEDAI <6; p<0.001).

Lower vitamin D in patients with MetS

There were sufficient data to define the MetS diagnosis in 860/1163 patients (73.9%). At enrolment, 286/860 (33%) fulfilled the MetS classification criteria. MetS was more common in men (31.8% men vs. 23.7% women; p=0.04) and those with MetS were older (38.6 (15.2) vs. 33.0 (12.0) years; p<0.001). Patients diagnosed with MetS were more likely to be non-Caucasian (51% vs. 49% Caucasian, p=0.04). Patients diagnosed MetS had a higher the median (IQR) HOMA-IR was than those who did not have MetS (0.9 (0.6, 1.8) vs. 0.6 (0.3, 1.0), p<0.001). Patients with MetS were more likely to have lower median (IQR) 25(OH)D than those without MetS (43 (28, 59) vs. 49 (30, 70) nmol/L; p<0.0001).

Factors associated with lower vitamin D in SLE

In univariate linear regression models, lower 25(OH)D was associated with higher diastolic blood pressure, total cholesterol, serum triglyceride, fasting insulin and HOMA-IR, and low HDL levels (Table 3). Lower 25(OH)D was also associated with higher SLEDAI-2K scores, CRP, serum creatinine and glucocorticoid exposure. After adjusting for age, gender, latitude and ethnicity, lower 25(OH)D remained significantly associated with these outcomes, but not with serum creatinine and HDL.

In a logistic regression analysis, lower 25(OH)D was associated with MetS criteria of blood pressure, raised triglycerides and lower HDL (Table 4). After adjusting for age, latitude and ethnicity, these associations remained significant (variables contributing to individual MetS

criteria such as gender in MetS HDL and gender and ethnicity in MetS WC were not included in the adjusted regression models). The association between lower 25(OH)D and MetS also remained in adjusted models

We also found an association between lower 25(OH)D and an increased average daily oral glucocorticoid exposure, which remained significant after adjusting for disease activity and active renal disease (OR (95% CI): 0.93 (0.88, 0.98), p = 0.01). Average glucocorticoid exposure was also associated with increased insulin resistance (HOMA-IR), in the 396 patients who had fasting insulin and glucose samples.

We also repeated our logistic regression analyses on the subset who had no missing data to classify MetS (n=548). The associations between lower 25(OH)D and MetS remained significant (data on file). In addition, the association between lower 25(OH)D and MetS remained in adjusted models, which also included social factors such as current smoking status, known to be a risk factor in the context of MetS (data on file).

We then performed a ROC curve analysis to identify variables associated with the diagnosis of MetS in patients with SLE. Renal disease and average glucocorticoid exposure were risk factors associated with MetS in SLE, producing an area under the ROC curve (AUC) of 0.6488. Adding vitamin D levels to the model improved the AUC from 0.6488 to 0.6578 (p=0.20) (Figure 2).

DISCUSSION

In a large international cohort of SLE patients, we have found an association between lower 25(OH)D and MetS as well as elevated markers of insulin resistance. In our regression analyses, lower vitamin D remained associated with MetS after adjustment for other SLE related factors.

The reasons for a higher prevalence of MetS in patients in the lowest 25(OH)D tertile are not well understood. Patients with lower 25(OH)D were more likely to have increased disease activity, in agreement with some previous (25-27), but not all, cross-sectional studies (28, 29) and in part may reflect vitamin D being a negative acute phase reactant (30). Given that photosensitivity is a key feature of SLE, it is also likely that sun avoidance, the usage of high-factor sunblock and living further from the equator contributes to lower 25(OH)D levels. We therefore estimated sun exposure using the latitude of recruiting centre location, as the amount of ultraviolet in ambient sunlight varies greatly by latitude (24). The centre locations ranged between 20°N and over 45°N and significantly higher 25(OH)D was observed in

patients living closer to the equator despite the hypothesis that sun-seeking behaviour is less common in this part of the world due to the hotter climate (31).

Patients with lower 25(OH)D were more likely to have MetS and this association was significant after adjusting for age, latitude and BMI. Our results suggest that co-existing physiological abnormalities may contribute to long-term cardiovascular risk early on in SLE. In animal models of SLE, 25(OH)D deficiency is associated with endothelial dysfunction and impaired angiogenesis (11). Similarly, in SLE patients treated for vitamin D deficiency, improvement in endothelial function correlated with the increment of 25(OH)D and may be related to changes in myeloid angiogenic cell function (32). In agreement with trends seen in the general population and patients with SLE, we found significant associations between lower 25(OH)D and recognised cardiovascular risk factors including hypertension and low HDL (12, 14, 33, 34). We also found a significant association between lower 25(OH)D and hypertriglyceridemia in adjusted models. The mechanisms underlying these associations are not clear but may be linked to the negative feedback of 25(OH)D on the renin-angiotensin system (35, 36). In the 322/1163 patients prescribed anti-hypertensive medication, there was a loss of association between lower 25(OH)D and the presence of some but not all cardiovascular risk factors (data not shown), which may justify the concurrent treatment of 25(OH)D levels however further studies are required to validate this strategy.

Insulin resistance, measured by elevated fasting serum insulin and HOMA-IR, is prevalent in SLE population and has been linked to an increased risk of CVD (37, 38). Previous studies have demonstrated in non-diabetic patients with SLE, that elevated fasting insulin and HOMA-IR were both associated with lower 25(OH)D (13, 27). We confirmed this finding and showed that lower 25(OH)D was associated with hyperglycaemia, which persisted in adjusted models. These findings are not surprising given the multifactorial contribution to increased cardiovascular risk and insulin resistance, which may be an early link to the heightened risk of developing MetS (39). Our fasting insulin subset was smaller (n=396). This is because not all centres were able to perform fasting blood samples due to clinic scheduling. Nevertheless, our study represents the largest and most diverse cohort thus far assessing the association between 25(OH)D and markers of insulin resistance.

Insulin resistance is known to be associated with obesity and glucocorticoid use in SLE (13) and indeed, this was the case in our cohort. Hence, we adjusted for central obesity (MetS WC) and glucocorticoid exposure and found that the association between lower 25(OH)D and elevated fasting glucose and insulin persisted (data not shown). There was also an association between lower 25(OH)D and central obesity, which was significant only after

adjusting for ethnicity and region. This may be in part due to a subset of patients of Korean ethnicity (n=156) when compared to all other ethnicities, had a lower prevalence of central obesity (20.5% vs. 51.4%; p = 0.006) and median (IQR) BMI (20.9 (18.9, 22.9) vs. 24.5 (21.6, 28.5); p < 0.001), consistent with a previous analysis of the SLICC cohort (40). Interestingly, patients of Korean descent compared to all other ethnicities had lower median (IQR) 25(OH)D (28 (20, 39) vs. 51 (34, 69) nmol/L; p<0.001). We also found that a significantly higher proportion of patients in the Korean subset had raised triglycerides, low HDL and hyperglycaemia (data not shown). This suggested that an ethnic gradient may contribute to enhanced sensitivity to adverse effects of inflammation and an increased susceptibility to MetS (40).

There is evidence that glucocorticoids increase vitamin D catabolism by activating nuclear receptors, which triggers 25(OH)D breakdown through the expression of CYP3A4. Thus, it may be beneficial in such patients to increase vitamin D supplementation or minimise glucocorticoid exposure (41, 42). Our study found an association between lower 25(OH)D and increased glucocorticoid exposure, which differed from previous work reporting no correlation between these variables (43, 44). We also found that lower 25(OH)D was less likely in patients prescribed anti-malarial therapy, which has been seen in some (12, 28) but not all previous studies (43, 45). Previous work has linked the potential and protective effect of anti-malarial medication against MetS in patients with mild to active SLE (18, 40, 46). However, the relationship between anti-malarials and 25(OH)D levels in SLE is complex. As our study only included patients with a short exposure to medication in the context of active disease, the influence of longer-term exposure remains to be explored.

This is the largest study to date examining the associations between 25(OH)D levels and MetS in SLE; it also has the advantage of being an international cohort. The SLICC Inception Cohort recruited from 33 centres in 11 countries with diverse racial and ethnic backgrounds, therefore results will be generalizable across many settings. Our cohort included younger patients with a wide range of disease activity, enabling effective investigation into the association of 25(OH)D with MetS and extends our previous findings to MetS and markers of insulin resistance. We also used a definition of MetS, which recognises that patients without central obesity can also be included in this phenotype (15). Our population also contained detailed data on glucocorticoid exposure, which enabled detailed analysis of their associations with vitamin D levels.

Our study has several limitations worth considering. Of the total number of patients in the cohort, sample availability meant that 25(OH)D was not measured in 37.1%. It was not

possible to measure 25(OH)D levels from all patients due to blood samples not being available from some centres, for instance from patients in Mexico. The subset of patients without 25(OH)D data had similar demographics and disease activity to those who have had their levels measured, although the average glucocorticoid exposure was higher. However, our analyses within the cohort adjusted for these factors and so our associations are likely to remain valid. While 34.1% of patients reported taking vitamin D supplements, the dose taken was not available and therefore we were not able to accurately assess its effects in this cohort. Data were not available to confirm a MetS diagnosis in 26.1% of our patients with available 25(OH)D. Of the components required to fulfil a MetS diagnosis, HDL was the most commonly missing variable, as this test was not routinely performed in all centres. As stated in the results, using a complete case subset did not materially affect the results of our analysis. Also, whilst social determinants of health such as a more active lifestyle, a healthier diet etc may serve as confounders, such data was not available in our cohort, although adjusting for smoking status did not influence the associations observed. The use of MetS to predict CVD has not yet been validated in SLE, however we do know that MetS susceptibility can be determined early in the SLE disease course and inform risk stratification in individual patients (20). Finally, although the cross-sectional nature of this study prevents the determination of causal associations between MetS, markers of insulin resistance and lower 25(OH)D, our study provides important information required for prospective studies.

In patients with recently diagnosed SLE we report an association between lower vitamin D levels and both MetS and insulin resistance. This association may have a physiological basis linked to endothelial or vascular dysfunction. Increased susceptibility to MetS and cardiovascular risk factors in patients with low 25(OH)D may provide a mechanism to modify systemic inflammation in SLE. The significant association between increased glucocorticoid exposure and lower 25(OH)D also emphasises the importance of minimising glucocorticoid exposure in SLE. The potential role of vitamin D supplementation to improve cardiometabolic outcomes in SLE will require further trials and seems justified on the basis of our findings.

Acknowledgements

Contributors: The study was conceived by INB and RRG. Data analysis and interpretation and manuscript preparation was performed by CC, JAR and INB. All authors contributed to data collection, critically reviewed and edited the manuscript and approved the final version.

Disclosure statement: INB has received grant/research support from Genzyme/Sanofi, GSK, Roche and UCBS; received consulting fees from Eli Lilly, GSK, Merck Serono, UCB, Aurinia and ILTOO; and was a speaker for GSK, UCB and AstraZeneca. The other authors have declared no conflicts of interest.

Data availability statement: The data underlying this article cannot be shared publicly due to the privacy of individuals who have participated in the SLICC inception cohort. The data

will be shared on reasonable request to the corresponding author.

REFERENCES
1.   Kendrick J, Targher G, Smits G, et al. 25-Hydroxyvitamin D deficiency is independently associated with cardiovascular disease in the Third National Health and Nutrition Examination Survey. Atherosclerosis. 2009;205(1):255-60.
2.   Dobnig H, Pilz S, Scharnagl H, et al. Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin d levels with all-cause and cardiovascular mortality. Archives of internal medicine. 2008;168(12):1340-9.
3.   Urowitz MB, Gladman D, Ibanez D, et al. Accumulation of coronary artery disease risk factors over three years: data from an international inception cohort. Arthritis and rheumatism. 2008;59(2):176-80.
4.   Manzi S, Meilahn EN, Rairie JE, et al. Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: comparison with the Framingham Study. Am J Epidemiol. 1997;145(5):408-15.
5.   Bruce IN, Gladman DD, Urowitz MB. Premature atherosclerosis in systemic lupus erythematosus. Rheumatic diseases clinics of North America. 2000;26(2):257-78.
6.   Ward MM. Premature morbidity from cardiovascular and cerebrovascular diseases in women with systemic lupus erythematosus. Arthritis and rheumatism. 1999;42(2):338-46.
7.   Esdaile JM, Abrahamowicz M, Grodzicky T, et al. Traditional Framingham risk factors fail to fully account for accelerated atherosclerosis in systemic lupus erythematosus. Arthritis and rheumatism. 2001;44(10):2331-7.
8.   Adorini L, Penna G. Control of autoimmune diseases by the vitamin D endocrine system. Nature clinical practice Rheumatology. 2008;4(8):404-12.
9.   Sigmundsdottir H, Pan J, Debes GF, et al. DCs metabolize sunlight-induced vitamin D3 to 'program' T cell attraction to the epidermal chemokine CCL27. Nature immunology. 2007;8(3):285-93.
10.   Chandra P, Wolfenden LL, Ziegler TR, et al. Treatment of vitamin D deficiency with UV light in patients with malabsorption syndromes: a case series. Photodermatol Photoimmunol Photomed. 2007;23(5):179-85.
11.   Reynolds JA, Rosenberg AZ, Smith CK, et al. Brief Report: Vitamin D Deficiency Is Associated With Endothelial Dysfunction and Increases Type I Interferon Gene Expression in a Murine Model of Systemic Lupus Erythematosus. Arthritis & rheumatology. 2016;68(12):2929-35.
12.   Lertratanakul A, Wu P, Dyer A, et al. 25-hydroxyvitamin D and cardiovascular disease in patients with systemic lupus erythematosus: data from a large international inception cohort. Arthritis care & research. 2014;66(8):1167-76.
13.   Sabio JM, Vargas-Hitos JA, Martinez-Bordonado J, et al. Association between low 25-hydroxyvitamin D, insulin resistance and arterial stiffness in nondiabetic women with systemic lupus erythematosus. Lupus. 2015;24(2):155-63.
14.   Wu PW, Rhew EY, Dyer AR, et al. 25-hydroxyvitamin D and cardiovascular risk factors in women with systemic lupus erythematosus. Arthritis and rheumatism. 2009;61(10):1387-95.
15.   Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009;120(16):1640-5.
16.   Alberti KG, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med. 2006;23(5):469-80.
17.   Parker B, Ahmad Y, Shelmerdine J, et al. An analysis of the metabolic syndrome phenotype in systemic lupus erythematosus. Lupus. 2011;20(14):1459-65.

18.   Sabio JM, Zamora-Pasadas M, Jimenez-Jaimez J, et al. Metabolic syndrome in patients with systemic lupus erythematosus from Southern Spain. Lupus. 2008;17(9):849-59.
19.   Chung CP, Avalos I, Oeser A, et al. High prevalence of the metabolic syndrome in patients with systemic lupus erythematosus: association with disease characteristics and cardiovascular risk factors. Annals of the rheumatic diseases. 2007;66(2):208-14.
20.   Parker B, Urowitz MB, Gladman DD, et al. Impact of early disease factors on metabolic syndrome in systemic lupus erythematosus: data from an international inception cohort. Annals of the rheumatic diseases. 2015;74(8):1530-6.
21.   Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis and rheumatism. 1997;40(9):1725.
22.   Gladman DD, Ibanez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. The Journal of rheumatology. 2002;29(2):288-91.
23.   Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.
24.   Chang YM, Barrett JH, Bishop DT, et al. Sun exposure and melanoma risk at different latitudes: a pooled analysis of 5700 cases and 7216 controls. Int J Epidemiol. 2009;38(3):814-30.
25.   Petri M, Bello KJ, Fang H, et al. Vitamin D in systemic lupus erythematosus: modest association with disease activity and the urine protein-to-creatinine ratio. Arthritis and rheumatism. 2013;65(7):1865-71.
26.   Amital H, Szekanecz Z, Szucs G, et al. Serum concentrations of 25-OH vitamin D in patients with systemic lupus erythematosus (SLE) are inversely related to disease activity: is it time to routinely supplement patients with SLE with vitamin D? Annals of the rheumatic diseases. 2010;69(6):1155-7.
27.   Reynolds JA, Haque S, Berry JL, et al. 25-Hydroxyvitamin D deficiency is associated with increased aortic stiffness in patients with systemic lupus erythematosus. Rheumatology. 2012;51(3):544-51.
28.   Ruiz-Irastorza G, Egurbide MV, Olivares N, et al. Vitamin D deficiency in systemic lupus erythematosus: prevalence, predictors and clinical consequences. Rheumatology. 2008;47(6):920-3.
29.   Kim HA, Sung JM, Jeon JY, et al. Vitamin D may not be a good marker of disease activity in Korean patients with systemic lupus erythematosus. Rheumatology international. 2011;31(9):1189-94.
30.   Waldron JL, Ashby HL, Cornes MP, et al. Vitamin D: a negative acute phase reactant. Journal of clinical pathology. 2013;66(7):620-2.
31.   Mithal A, Wahl DA, Bonjour JP, et al. Global vitamin D status and determinants of hypovitaminosis D. Osteoporos Int. 2009;20(11):1807-20.
32.   Reynolds JA, Haque S, Williamson K, et al. Vitamin D improves endothelial dysfunction and restores myeloid angiogenic cell function via reduced CXCL-10 expression in systemic lupus erythematosus. Sci Rep. 2016;6:22341.
33.   Martins D, Wolf M, Pan D, et al. Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin D in the United States: data from the Third National Health and Nutrition Examination Survey. Archives of internal medicine. 2007;167(11):1159-65.
34.   Kim DH, Sabour S, Sagar UN, et al. Prevalence of hypovitaminosis D in cardiovascular diseases (from the National Health and Nutrition Examination Survey 2001 to 2004). The American journal of cardiology. 2008;102(11):1540-4.
35.   Akin F, Ayca B, Kose N, et al. Serum vitamin D levels are independently associated with severity of coronary artery disease. J Investig Med. 2012;60(6):869-73.
36.   Tomaschitz A, Pilz S, Ritz E, et al. Independent association between 1,25-dihydroxyvitamin D, 25-hydroxyvitamin D and the renin-angiotensin system: The Ludwigshafen Risk and Cardiovascular Health (LURIC) study. Clin Chim Acta. 2010;411(17-18):1354-60.

37.   El Magadmi M, Ahmad Y, Turkie W, et al. Hyperinsulinemia, insulin resistance, and circulating oxidized low density lipoprotein in women with systemic lupus erythematosus. The Journal of rheumatology. 2006;33(1):50-6.

38.   Posadas-Romero C, Torres-Tamayo M, Zamora-Gonzalez J, et al. High insulin levels and increased low-density lipoprotein oxidizability in pediatric patients with systemic lupus erythematosus. Arthritis and rheumatism. 2004;50(1):160-5.

39.   Parker B, Bruce I. SLE and metabolic syndrome. Lupus. 2013;22(12):1259-66.

40.   Parker B, Urowitz MB, Gladman DD, et al. Clinical associations of the metabolic syndrome in systemic lupus erythematosus: data from an international inception cohort. Annals of the rheumatic diseases. 2013;72(8):1308-14.

41.   Zhou C, Assem M, Tay JC, et al. Steroid and xenobiotic receptor and vitamin D receptor crosstalk mediates CYP24 expression and drug-induced osteomalacia. The Journal of clinical investigation. 2006;116(6):1703-12.

42.   Holick MF. Vitamin D deficiency. The New England journal of medicine. 2007;357(3):266-81.

43.   Schoindre Y, Jallouli M, Tanguy ML, et al. Lower vitamin D levels are associated with higher systemic lupus erythematosus activity, but not predictive of disease flare-up. Lupus Sci Med. 2014;1(1):e000027.

44.   Yap KS, Northcott M, Hoi AB, et al. Association of low vitamin D with high disease activity in an Australian systemic lupus erythematosus cohort. Lupus Sci Med. 2015;2(1):e000064.

45.   Huisman AM, White KP, Algra A, et al. Vitamin D levels in women with systemic lupus erythematosus and fibromyalgia. The Journal of rheumatology. 2001;28(11):2535-9.

46.   Bellomio V, Spindler A, Lucero E, et al. Metabolic syndrome in Argentinean patients with systemic lupus erythematosus. Lupus. 2009;18(11):1019-25.

Figure 1: 25(OH)D levels (mean (IQR)) and sun exposure based on latitude (^{0}N) of patients (***p<0.0001).

Figure 2: Receiving operating characteristics (ROC) curve analysis for identifying variables predicting the diagnosis of MetS in patients with SLE. Renal disease and average glucocorticoid exposure were risk factors associated with MetS in SLE producing an area under the ROC curve (AUC) of 0.6488 (Model). Adding vitamin D to the model improved the AUC to 0.6578 (Model + 25(OH)D).

Table 1: Demographic and baseline disease characterisation of patients the SLICC population.

Characteristics (n≥90% cohort unless stated otherwise)	n (%)/median (IQR)
Age (years)	33.1 (24.3, 43.6)
Gender	
Female	1034 (88.9)
Male	129 (11.1)
Ethnicity	
Caucasian	623 (53.6)
Hispanic	47 (4.0)
Asian	243 (20.9)
Black	202 (17.4)
Other	48 (4.1)
Country	
Canada	346 (29.8)
USA	312 (26.8)
Mexico	10 (0.9)
Europe	339 (29.1)
Asia	156 (13.4)
Latitude (°N)	
20 – 34°N	116
34 – 45°N	625
North of 45°N	422
25(OH)D (nmol/L)	
Serum 25(OH)D levels (nmol/L)	48 (30, 66)
CVD risk factors	
Systolic BP (mm Hg)	120 (110, 130)
Diastolic BP (mm Hg)	75 (70, 80)
BMI (kg/m^2)	23.7 (21.1, 27.7)
Waist circumference (cm), n=987	80 (73, 90)
Smoking status	173 (14.9)
Post-menopausal[a], n=1034	104 (10.1)
Total cholesterol (mmol/L)	4.6 (3.9, 5.5)
Serum triglyceride (mmol/L), n=987	1.3 (1.0, 2.0)
HDL-cholesterol (mmol/L), n=634	1.3 (1.0, 1.7)
LDL-cholesterol (mmol/L), n=531	2.5 (2.0, 3.1)
Fasting glucose (mmol/L), n=985	4.7 (4.3, 5.3)
Measures based on Fasting Insulin	
Fasting insulin (uIU/ml), n=413	3.0 (1.9, 5.4)
HOMA-IR, n=396	0.7 (0.4, 1.3)
SLE-related characteristics	
Disease duration (wks)	21.7 (9.1, 39.9)
SLEDAI-2K score	4 (2, 8)
Anti-dsDNA positive	450 (42.0)

Low C3/C4[b]	440 (40.6)
CRP (mg/L), n=868	3.0 (1.9, 6.3)
Creatinine (umol/L), n=746	69.0 (61.0, 79.6)
Active renal disease[c]	231 (19.9)
Receiving oral glucocorticoids	801 (68.9)
Average glucocorticoid dose (mg/day)	10 (0, 25)
Anti-malarials	828 (71.3)
Immunosuppressants	452 (38.9)
Cardiovascular-related medications (n, %)	
Anti-hypertensive medication	322 (27.7)
Lipid-lowering medication	112 (9.6)

25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; BP, blood pressure; CRP, C-reactive protein; CVD, cardiovascular disease; HOMA-IR, HDL, high-density lipoprotein; homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein; SLEDAI-2K, Systemic Lupus Erythemathosus Disease Activity Index 2000; SLICC, Systemic Lupus International Collaborating Clinics; T, 25(OH)D tertiles. [a]Percentage of women.[b]Decrease in CH50, C3 and C4 below the lower limit of normal for testing laboratory. [c]Active nephritis or renal item on SLEDAI-2K (haematuria, proteinuria, pyuria or casts).

Table 2: Association between 25(OH)D tertiles and cardiovascular risk factors and SLE disease at baseline

CVD risk factors n (%)/median (IQR)	Vitamin D tertile (range)			P value
	T1 (n=398) 10-36 nmol/L	T2 (n=393) 37-60 nmol/L	T3 (n=372) 61-174 nmol/L	
Age (years)	30.2 (23.0, 39.7)	34.3 (24.9, 44.7)	34.7 (25.1, 45.6)	<0.001
BMI, kg/m^2	23.1 (20.2, 27.4)	24.5 (21.5, 28.4)	23.9 (21.6, 27.5)	0.22
Waist circumference, cm	79.0 (71.7, 90.0)	82.0 (74.0, 91.2)	80.0 (73.0, 89.0)	0.32
CVD risk factors				
Hypertension	161/391 (41.2)	149/382 (39.0)	110/363 (30.3)	0.005
Systolic BP (mm Hg)	120 (110, 130)	120 (110, 130)	120 (109, 126)	0.04
Diastolic BP (mm Hg)	74 (70, 80)	76 (70, 82)	72 (68, 80)	0.005
Anti-hypertensive medication	126/398 (31.7)	117/393 (29.8)	79/372 (21.2)	0.003
Total cholesterol (mmol/L)	4.7 (3.9, 6.0)	4.6 (3.9, 5.5)	4.4 (3.8, 5.1)	<0.001
Serum triglyceride (mmol/L)	1.6 (1.1, 2.2)	1.4 (1.0, 2.0)	1.2 (0.9, 1.6)	<0.001
HDL (mmol/L)	1.3 (1.0, 1.7)	1.3 (1.0, 1.7)	1.4 (1.1, 1.7)	0.76
Lipid-lowering medication	60 (15.1)	28 (7.1)	24 (6.5)	<0.001
Fasting glucose (mmol/L)	4.8 (4.3, 5.4)	4.8 (4.3, 5.4)	4.7 (4.1, 5.2)	0.33
Fasting insulin (uIU/ml)	3.8 (2.0, 6.9)	3.4 (1.9, 5.1)	2.4 (1.8, 3.3)	<0.001
HOMA-IR	0.8 (0.4, 1.7)	0.7 (0.4, 1.1)	0.5 (0.4, 0.7)	<0.001
MetS Criteria				
MetS BP	183/392 (46.7)	192/383 (50.1)	140/364 (38.5)	0.005
MetS TG	182/354 (51.4)	135/326 (41.4)	84/319 (26.3)	<0.001
MetS HDL	156/240 (65.0)	114/220 (51.8)	89/209 (42.5)	<0.001
MetS Glu	71/346 (20.5)	70/326 (21.5)	55/321 (17.1)	0.35
MetS WC	158/356 (44.4)	172/332 (51.8)	131/299 (43.8)	0.07
Metabolic syndrome	115/303 (38.0)	103/280 (36.8)	68/277 (24.6)	<0.001

SLE-related characteristics				
SLEDAI-2K	4 (2, 8)	4 (2, 8)	4 (1, 6)	<0.001
Positive dsDNA	166/355 (46.8)	149/367 (40.6)	135/350 (38.6)	0.07
Low complement	171/360 (47.5)	148/373 (39.7)	121/351 (34.5)	0.002
CRP (mg/L)	3.9 (2.0, 7.0)	3.2 (2.0, 6.0)	2.9 (1.0, 6.6)	0.23
Creatinine (umol/L)	62 (59, 77)	70 (62, 80)	71 (62, 80)	0.09
Glucocorticoids				
Glucocorticoids	303 (76.1)	278 (70.7)	220 (59.1)	<0.001
Average GC dose (mg/day)	15 (4, 30)	13 (0, 27)	6 (0, 18)	<0.001
Anti-malarial therapy	264/397 (66.5)	274/393 (69.7)	290/371 (78.2)	0.001

Comparisons between 25(OH)D tertiles of continuous variables were carried out using one-way analysis of variance (ANOVA) and categorical variables by chi-squared test. BMI, body mass index; CVD, cardiovascular; GC, glucocorticoids; HDL, high-density lipoprotein; HOMA-IR, homeostatic model of assessment of insulin resistance. Defined according to the metabolic S (MetS) criteria (15): MetS BP, elevated blood pressure ≥130/85 or receiving anti-hypertensive medication; MetS HDL, reduced HDL <1.3 mmol/L in women and <1.0 mmol/L in men; MetS Glu, fasting glucose ≥5.6 mmol/L or being prescribed medication for hyperglycaemia; MetS TG, elevated serum triglyceride of ≥ 1.7 mmol/l or being on lipid-lowering medication; MetS WC, elevated waist circumference using gender and ethnicity-specific thresholds

Table 3: Association between 25(OH)D and cardiovascular risk and SLE factors at baseline

CV risk factors	Unadjusted			†Adjusted model 1			††Adjusted model 2		
	β	95% CI	P value	β	95% CI	P value	β	95% CI	P value
Waist	-0.01	-0.35, 0.33	0.96	-0.18	-0.51, 0.16	0.30	-0.17	-0.36, 0.03	0.10
Systolic BP	-0.29	-0.67, 0.10	0.14	-0.56	-0.93, -0.20	0.003	-0.57	-0.93, -0.21	0.002
Diastolic BP	-0.26	-0.51, -0.02	0.04	-0.32	-0.57, -0.07	0.01	-0.32	-0.57, -0.07	0.01
Total cholesterol	-0.10	-0.13, 0.06	<0.001	-0.10	-0.14, -0.07	<0.001	-0.10	-0.14, -0.07	<0.001
*Triglyceride**	-0.05	-0.06, -0.04	<0.001	-0.05	-0.07, -0.04	<0.001	-0.05	-0.07, -0.04	<0.001
*HDL cholesterol**	0.01	0.002, 0.03	0.02	0.01	-0.001, 0.02	0.08	0.01	-0.001, 0.03	0.08
Fasting glucose	-0.02	-0.06, 0.03	0.47	-0.04	-0.08, 0.01	0.08	-0.04	-0.09, 0.01	0.09
*Fasting insulin**	-0.06	-0.10, -0.03	<0.001	-0.05	-0.09, -0.02	0.002	-0.05	-0.09, -0.02	0.002
*HOMA-IR**	-0.07	-0.12, -0.03	<0.001	-0.06	-0.10, -0.02	0.003	-0.06	-0.10, -0.02	0.002
SLE factors	β	95% CI	P value	β	95% CI	P value	β	95% CI	P value
*SLEDAI-2K***	-0.11	-0.15, -0.07	<0.001	-0.10	-0.15, -0.06	<0.001	-0.11	-0.15, -0.06	<0.001
*CRP**	-0.05	-0.09, -0.02	0.002	-0.06	-0.10, -0.03	0.001	-0.07	-0.10, -0.03	<0.001
*Creatinine**	0.01	0.001, 0.02	0.03	0.003	-0.004, 0.01	0.36	0.003	-0.004, 0.01	0.34
*Average GC**	-0.06	-0.08, -0.04	<0.001	-0.06	-0.08, -0.04	<0.001	-0.06	-0.08, -0.03	<0.001

β, β-coefficient; BP, blood pressure; CI, confidence intervals; CRP, C-reactive protein; CV, cardiovascular; GC, glucocorticoid; HOMA-IR, homeostatic model of assessment of insulin resistance
† Adjusted model 1: controlled for age, gender, average latitude and ethnicity
‡ Adjusted model 2: as in adjusted model 1†, and including body mass index
*Log transformed variables
**Ordered logistic regression (SLEDAI-2K expressed in tertiles)

Table 4: Association between 25(OH)D and individual binary cardiovascular risk criteria contributing to MetS

CV risk factors required for a MetS diagnosis	Unadjusted			Adjusted model 1 († unless specified)			Adjusted model 2 (†† unless specified)		
	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Hypertension	0.94	0.90, 0.99	0.02	0.92	0.88, 0.97	0.003	0.92	0.87, 0.97	0.003
Hypertriglyceridemia	0.85	0.80, 0.89	<0.001	0.84	0.79, 0.89	<0.001	0.83	0.79, 0.89	<0.001
HDL (low/high)*	0.86	0.81, 0.92	<0.001	0.88	0.82, 0.94	<0.001	0.88	0.82, 0.94	<0.001
Hyperglycaemia	0.95	0.89, 1.02	0.147	0.92	0.86, 0.99	0.02	0.92	0.85, 0.99	0.02
Waist circumference (low/high)**	1.00	0.95, 1.05	0.969	0.97	0.92, 1.02	0.289	0.94	0.88, 1.01	0.08
Metabolic syndrome**	0.90	0.85, 0.96	0.001	0.86	0.81, 0.91	<0.001	0.85	0.79, 0.91	<0.001

CI, confidence intervals; CV, cardiovascular; OR, odds ratio

†   Adjusted model 1: controlled for age, gender, average latitude and ethnicity

†† Adjusted model 2: as in adjusted model 1†, and including body mass index and current smoking status

*   Adjusted model 1 and 2 without gender

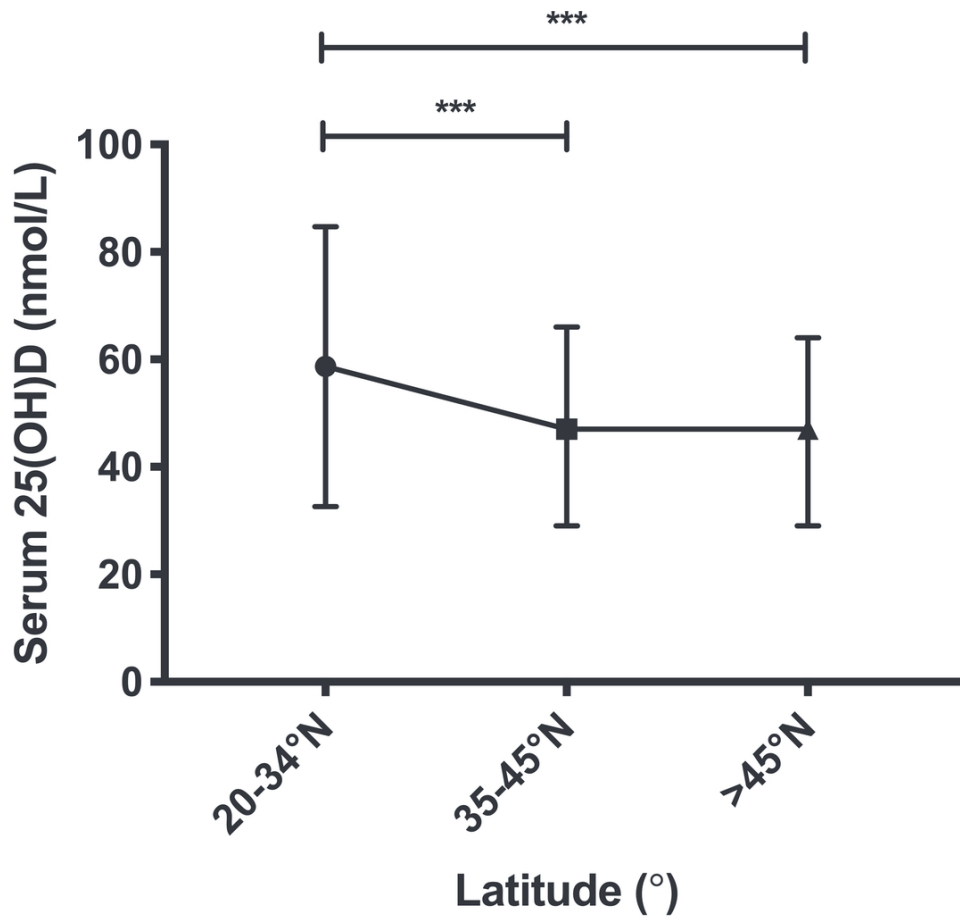** Adjusted model 1 and 2 without gender and ethnicity
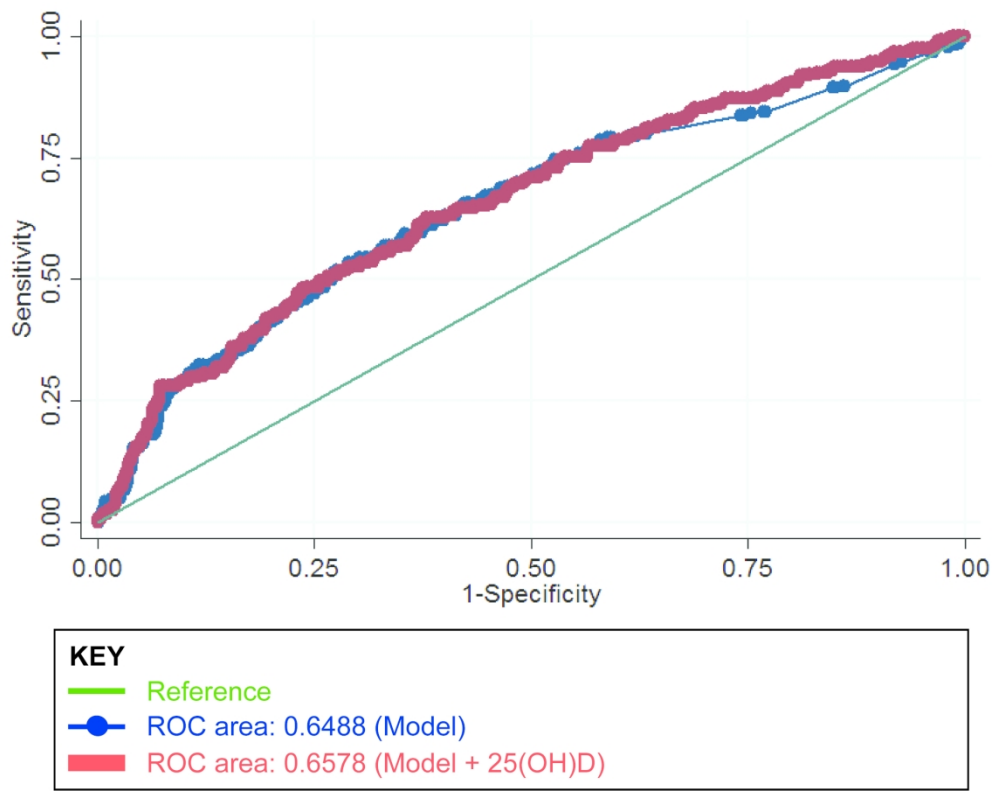
Figure 1

94x90mm (300 x 300 DPI)

KEY
— Reference
●— ROC area: 0.6488 (Model)
▬ ROC area: 0.6578 (Model + 25(OH)D)

Figure 2.