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Lowry, E. (2020). Could vitamin D reduce obesity-associated inflammation? Observational and Mendelian randomization study. *The American Journal of Clinical Nutrition*, 111(5), 1036–1047. <https://doi.org/10.1093/ajcn/nqaa056>

Published in:

The American Journal of Clinical Nutrition

Document Version:

Publisher's PDF, also known as Version of record

Queen's University Belfast - Research Portal:

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Could vitamin D reduce obesity-associated inflammation? Observational and Mendelian randomization study

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ABSTRACT

Background: Obesity is associated with inflammation but the role of vitamin D in this process is not clear.

Objectives: We aimed to assess the associations between serum 25-hydroxyvitamin D [25(OH)D], BMI, and 16 inflammatory biomarkers, and to assess the role of vitamin D as a potential mediator in the association between higher BMI and inflammation.

Methods: Northern Finland Birth Cohort 1966 (NFBC1966) 31-y data on 3586 individuals were analyzed to examine the observational associations between BMI, 25(OH)D, and 16 inflammatory biomarkers. Multivariable regression analyses and 2-sample regression-based Mendelian randomization (MR) mediation analysis were performed to assess any role of vitamin D in mediating a causal effect of BMI on inflammatory biomarkers [soluble intercellular adhesion molecule 1 (sICAM-1), high sensitivity C-reactive protein (hs-CRP), and α 1-acid glycoprotein (AGP)] for which observational associations were detected. For MR, genome-wide association study summary results ranging from 5163 to 806,834 individuals were used for biomarkers, 25(OH)D, and BMI. Findings were triangulated with a literature review of vitamin D supplementation trials.

Results: In NFBC1966, mean BMI (kg/m^2) was 24.8 (95% CI: 24.7, 25.0) and mean 25(OH)D was 50.3 nmol/L (95% CI: 49.8, 50.7 nmol/L). Inflammatory biomarkers correlated as 4 independent clusters: interleukins, adhesion molecules, acute-phase proteins, and chemokines. BMI was positively associated with 9 inflammatory biomarkers and inversely with 25(OH)D (false discovery rate < 0.05). 25(OH)D was inversely associated with sICAM-1, hs-CRP, and AGP, which were positively associated with BMI. The MR analyses showed causal association of BMI on these 3 inflammatory biomarkers. There was no observational or

MR evidence that circulating 25(OH)D concentrations mediated the association between BMI and these 3 inflammatory markers. Review of randomized controlled trials (RCTs) supported our findings showing no impact of vitamin D supplementation on inflammatory biomarkers.

Conclusions: The findings from our observational study and causal MR analyses, together with data from RCTs, do not support a beneficial role of vitamin D supplementation on obesity-related inflammation. *Am J Clin Nutr* 2020;111:1036–1047.

Keywords: vitamin D, BMI, obesity, Mendelian randomization, mediation, inflammation, 25(OH)D

Introduction

Obesity is a global public health problem reaching epidemic proportions (1), with estimated costs >\$91 billion/y. Moreover, obesity plays an important role in the development of chronic diseases including the major cardiometabolic diseases (2, 3) and certain forms of cancer. There is biological evidence supporting that inflammatory biomarkers (**Supplemental Table 1**) could mediate obesity-related pathological outcomes, and therefore inflammation could be a modifiable target (2, 3).

The hormonal form of vitamin D, calcitriol, has immunomodulatory properties acting via the vitamin D receptor (VDR) (4), making it a potential mediator in the association between obesity and inflammation (5). A review of 12 cross-sectional studies examining the association between vitamin D and inflammation has

highlighted a possible link between vitamin D and inflammatory biomarkers (**Supplemental Table 2**), albeit with some limitations (6). This is substantiated by our own investigation on the in vitro direct effects of calcitriol on inflammation-related pathways, the NF- κ B and mitogen-activated protein kinase (MAPK) pathways, which have been demonstrated in multiple cell types including isolated adipocytes (7, 8). Furthermore, vitamin D has been reported to suppress the synthesis of IL-8, IL-6, and monocyte chemoattractant protein 1 (MCP-1) (6, 7, 9). However, a recent systematic review and meta-analysis that examined randomized controlled trials (RCTs) looking at the effects of vitamin D supplementation on 12 inflammatory biomarkers has reported no beneficial effect of vitamin D supplementation for decreasing inflammation (10).

In obesity, vitamin D insufficiency and inflammation often co-occur (11–13), but it remains unclear whether low vitamin D concentrations could exacerbate the inflammatory condition associated with obesity. Evidence from Mendelian randomization (MR) studies supports a causal relation between higher BMI and lower vitamin D (11), as well as between higher BMI and inflammatory biomarkers [e.g., high sensitivity C-reactive protein (hs-CRP) (12) and α 1-acid glycoprotein (AGP) (13)]. However, it is not known whether the association between vitamin D and inflammatory biomarkers is causal. Moreover, there is only limited evidence for the efficacy of vitamin D supplementation in

reducing the risk of obesity-related pathological outcomes (14). RCTs with vitamin D supplementation in adults with overweight or obesity have reported contradictory or inconsistent results. To date, 13 completed (see **Supplemental Table 3**) and 55 ongoing RCTs are testing the above-mentioned association.

In the current study we first used conventional observational analyses to examine the cross-sectional associations between 1) BMI and 16 circulating inflammatory biomarkers, 2) serum 25(OH)D concentration and the same inflammatory biomarkers, and 3) the role of vitamin D as a potential mediator in the association of BMI and inflammatory biomarkers that were observationally related to both BMI and vitamin D. We then used MR mediation analyses to investigate whether vitamin D is mediating any causal effect of raised BMI on inflammatory biomarker concentrations that were observationally related to both BMI and vitamin D. We finally reviewed the current evidence on the impact of vitamin D supplementation on inflammation and metabolic health outcomes from RCTs of overweight/obese individuals.

Methods

Observational analyses

Data source.

We analyzed data from 3586 individuals using the Northern Finland Birth Cohort 1966 (NFBC1966) 31-y follow-up (**Supplemental Figure 1**). This population-based, longitudinal birth cohort study comprised offspring of pregnant women, residing in Northern Finland with expected delivery dates during 1966 (15). At 31 y of age (1997), the cohort members alive with a known address were sent a postal questionnaire (75% response, $n = 8767$). At the same time, those living in the original target area (Northern Finland), or in the capital (Helsinki) area were invited to a clinical examination, in which 71% ($n = 6033$) participated (16). The attendees gave written informed consent and the study was approved by the local ethical committee of the University of Oulu. The procedures follow the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Procedures.

Participants were invited to a clinical examination as described previously (17). Height and weight were measured to an accuracy of 0.1 cm and 0.1 kg, respectively, and converted to BMI (kg/m^2). LC-tandem MS was used to measure 25(OH)D₂ and 25(OH)D₃ concentrations, and total 25(OH)D [25(OH)D₂ + 25(OH)D₃] concentration was computed. The detailed assay procedure has previously been published, and the assay has been calibrated using National Institute of Standards and Technology standard reference material (18). Plasma inflammatory biomarker concentrations were analyzed by a multiplex array method using human cytokine/chemokine magnetic bead panel and nonmagnetic human CVD/cytokine panel (Cat# HCYTOMAG-60K-12 and SPR349; Merck Millipore) (19) (detailed in the **Supplemental Methods**). The 16 inflammatory biomarkers [IL-17, IL-1 α , IL-1 β , IL-4, IL-6, IL-8, interleukin-1 receptor antagonist (IL-1RA), interferon gamma-induced protein 10 (IP-10)/C-

This research was supported by the Academy of Finland grant numbers 24300796, 24302031, 285547 (EGEA); the Medical Research Council (MRC) UK (grant number G0601653); Medical Research Council Biotechnology and Biological Sciences Research Council *PRECISE* (Nutrition & Epigenome, The Joint Programming Initiative a Healthy Diet for a Healthy Life (JPI HDHL/EU-H2020)); the National Health and Medical Research Council, Australia (grant number APP1123603); Biocenter Oulu Doctoral Program, Juho Vainio Foundation, Orion Research Foundation sr, Emil Aaltonen Foundation, Finnish Cultural Foundation, Yrjö Jahansson Foundation, Päivikki and Sakari Sohlberg Foundation sr; the European Union's Horizon 2020 programmes, DynaHEALTH (grant number 633595), LifeCycle (grant number 733206), and iHealth-T2D (grant number 643774); and the Wellcome 4i Clinical PhD Program at Imperial College London.

No funding bodies had any role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors do not have the authority to make data public.

Supplemental Methods, Supplemental Figure 1, and Supplemental Tables 1–5 are available from the “Supplementary data” link in the online posting of the article and from the same link in the online table of contents at <https://academic.oup.com/ajcn/>.

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Abbreviations used: AGP, α 1-acid glycoprotein; FDR, false discovery rate; GWAS, genome-wide association study; hs-CRP, high sensitivity C-reactive protein; IL-1RA, interleukin-1 receptor antagonist; IP-10, interferon gamma-induced protein 10; IVW, inverse-variance weighted; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein 1; MR, Mendelian randomization; NFBC1966, Northern Finland Birth Cohort 1966; PAI-1, active plasminogen activator inhibitor-1; RCT, randomized controlled trial; sCD40L, soluble CD40 ligand; sICAM-1, soluble intercellular adhesion molecule 1; SNP, single nucleotide polymorphism; sVCAM-1, soluble vascular cell adhesion molecule 1; VDR, vitamin D receptor; WMD, weighted mean difference; 25(OH)D, 25-hydroxyvitamin D.

Received August 16, 2019. Accepted for publication March 2, 2020.

First published online March 31, 2020; doi: <https://doi.org/10.1093/ajcn/nqaa056>.

X-C motif chemokine 10 (CXCL10), MCP-1/CCL2, soluble CD40-ligand (sCD40L/CD154), TNF- α , soluble vascular cell adhesion molecule 1 (sVCAM-1), soluble intercellular adhesion molecule 1 (sICAM-1), active plasminogen activator inhibitor (active PAI-1), hs-CRP, and AGP] examined in the study are outlined in Supplemental Table 1. The inflammatory biomarker measurements were performed according to the manufacturer's instructions (detailed in the Supplemental Methods). hs-CRP (mg/L) concentration was measured by immunoenzymometric assay (Medix Biochemica) (20). AGP (mmol/L) was measured using high-throughput NMR spectroscopy (CV: 1.1%) (21).

Inclusion and exclusion criteria.

For the observational analyses we included those who attended the 31-y follow-up assessment and who had data on 25(OH)D, BMI, and inflammatory biomarkers ($n = 3586$). We excluded participants with imprecise or missing information, and with conditions susceptible to interfere via biological pathways (kidney and liver diseases) with the measure of vitamin D and/or the inflammatory markers. This included individuals with nonfasting blood samples, those on lipid-lowering medications, hs-CRP concentrations > 10 mg/L (sign of acute infection), self-reported fever during clinical assessment, or underweight (BMI < 18.5). In addition, we excluded women who were pregnant and those reporting use of oral contraceptive pills (they interfere with vitamin D metabolism and can also alter inflammatory process).

Covariates.

We included sex, season of blood sampling, smoking, alcohol consumption, physical activity, and socioeconomic position as covariates in the multivariable analyses (22) (detailed in the Supplemental Methods).

MR analyses

Data sources.

Genome-wide association study (GWAS) summary statistics for vitamin D were obtained from UK Biobank data in 337,199 individuals of European descent and downloaded from <http://www.nealelab.is/uk-biobank/> (23, 24). GWAS summary statistics for BMI were obtained from a GWAS meta-analysis of 806,834 individuals (25). The variant-inflammatory marker associations were extracted from the published GWAS studies of CRP in 204,402 individuals (26), of AGP in 24,925 individuals (27), and for sICAM-1 in 5163 individuals (28) (Supplementary file 2). Ethical approval had been obtained in the original studies.

Statistical analyses

Observational associations of BMI and serum 25(OH)D with inflammatory markers.

Descriptive statistics were generated for all explanatory variables and outcome measures. The differences between males and females were analyzed by chi-square test for categorical variables, independent-sample Student t test for normally distributed data, and Wilcoxon–Mann–Whitney U test for nonparametric data. BMI, 25(OH)D, and inflammatory

biomarkers were all converted to standardized scores (z -scores). Nonsupervised hierarchical clustering analysis was performed for all the inflammatory biomarkers included in our study, using a method adapted from Van den Ham et al. (29), and the detailed methodology is explained in Schipper et al. (30). Pearson correlation coefficients were used to assess the clustering patterns and heatmaps were used to represent the correlations of inflammatory biomarkers.

To assess the relation of BMI and serum 25(OH)D concentration with inflammatory biomarkers, we used adjusted linear regression models. We first examined BMI-inflammatory biomarker associations using 3 models; model 1 was unadjusted (β in Figure 1A), model 2 was adjusted for sex, and model 3 was adjusted for sex and potential covariates: smoking, alcohol, physical activity, and socioeconomic position. We next tested the association between BMI and serum 25(OH)D (β in Figure 1A). To examine 25(OH)D-inflammatory biomarker associations (β in Figure 1A), model 1 was adjusted for 25(OH)D batch, model 2 was further adjusted for sex and season of blood sampling, model 3 was additionally adjusted for smoking, alcohol, physical activity, and socioeconomic position, and model 4 included model 3 covariates and additional adjustment for BMI (β in Figure 1A, Supplemental Table 4). Regression coefficients from these associations can be interpreted as the change in 1 SD unit in inflammatory biomarker per 1 SD increase in BMI or 25(OH)D. P values were adjusted for multiple comparisons using the Benjamini–Hochberg (1995) false discovery rate (FDR < 0.05) approach (31). Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc.) and R version 3.3.1 (R Project for Statistical Computing).

Conventional mediation analysis of the association between BMI and inflammation by 25(OH)D.

We tested 25(OH)D as a potential mediator in the observational associations between BMI and the inflammatory markers using the widely used method of Baron and Kenny (32) and the Sobel test (33) for the inflammatory biomarkers (FDR < 0.05) that were associated with both BMI and 25(OH)D from the observational analyses above. Baron and Kenny proposed a 4-step approach in which several regression analyses are conducted, and significance of the coefficients is examined at each step. The Supplemental Methods give further details for the methodology of the conventional mediation analysis. As shown in Figure 1A, mediation analysis assumes causality between all 3 components; the exposure (BMI), outcome (inflammatory marker), and the mediator [25(OH)D]. The statistical tests and P values were 2-sided and statistical significance was set at $P < 0.05$.

MR analysis.

For those biomarkers that were associated with both BMI and 25(OH)D in the conventional observational analysis, we also performed MR to assess causality and mediation of any effect of BMI on the inflammatory biomarkers through 25(OH)D (Figure 1B and C). The Supplemental Methods give further details of the principles relating to MR and MR mediation analysis. Only genetic variants present for all GWAS summary

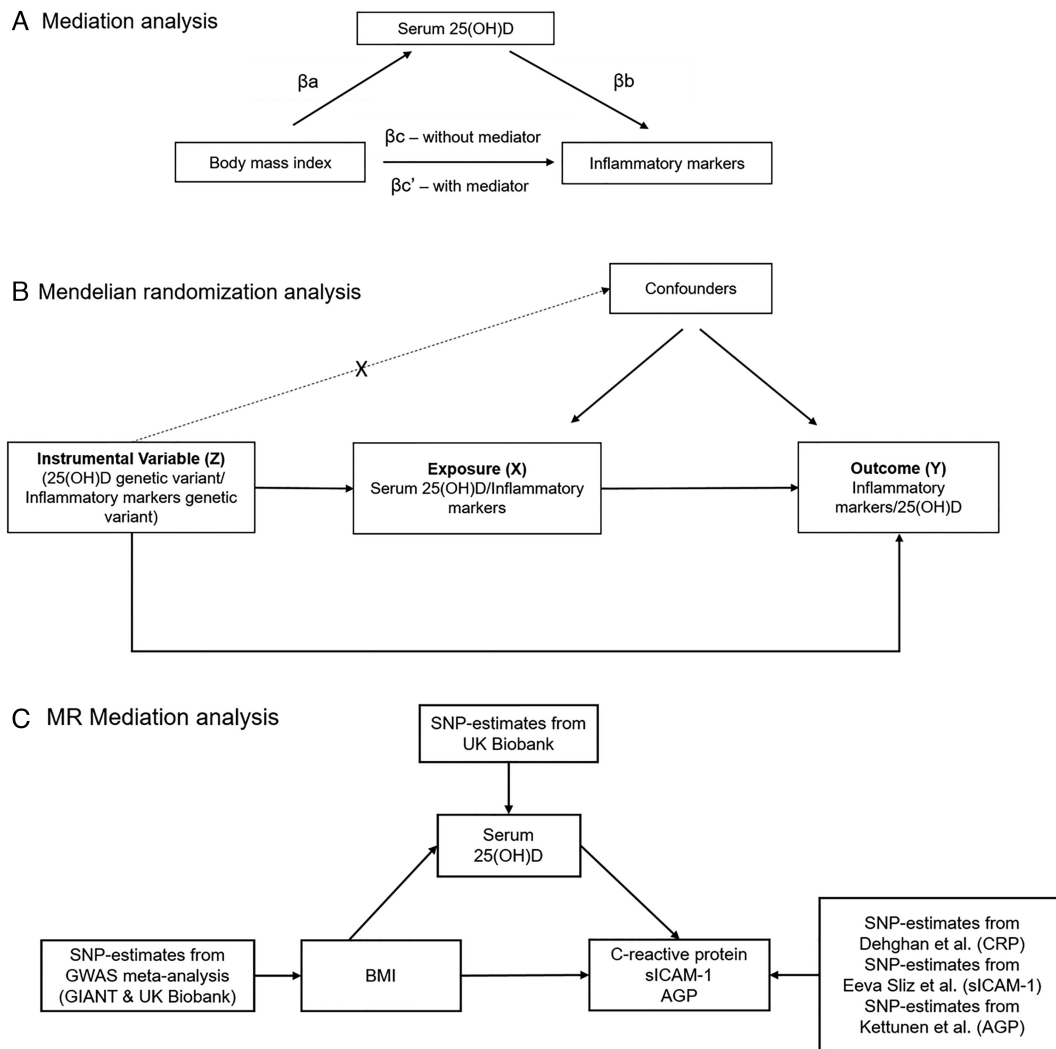


FIGURE 1 Graphical representation of proposed mediation through 25-hydroxyvitamin D [25(OH)D] in the association of BMI with inflammatory biomarkers: (A) mediation analysis; (B) Mendelian randomization (MR) analysis; and (C) MR mediation analysis. (A) β_a represents the regression coefficients for the association between BMI and 25(OH)D; β_b represents the regression coefficients for the association between 25(OH)D and inflammatory biomarkers; β_c , represents the total effect between BMI and inflammatory biomarkers, without the adjustment for mediator; and β_c' represents the direct effect between BMI and inflammatory biomarkers, taking into account adjustment for mediator. The regression models were adjusted for covariates (sex, socioeconomic position, smoking, alcohol consumption, 25(OH)D batch, and physical activity). (B) A schematic representation of an MR analysis used in examining the causal association between 25(OH)D and inflammatory biomarkers. (C) A schematic representation of the MR mediation analysis approach and the data sources used (23–28). AGP, α 1-acid glycoprotein; GWAS, genome-wide association study; sICAM-1, soluble intercellular adhesion molecule 1; SNP, single nucleotide polymorphism.

data were considered (Supplemental file 2). The following analyses were performed:

- 1) Total MR effects of BMI on 25(OH)D
- 2) Total MR effects of BMI on the inflammatory biomarkers
- 3) Total MR effects of 25(OH)D on the inflammatory biomarkers
- 4) Any MR evidence of 25(OH)D mediating the effect of BMI on the inflammatory biomarkers

For analyses considering total effects, instruments were selected as single nucleotide polymorphisms (SNPs) that associated with the exposure under consideration at genome-wide significance ($P < 5 \times 10^{-8}$) and were in pairwise linkage disequilibrium $r^2 < 0.001$. To select instruments for mediation

analysis, all SNPs related to either BMI or 25(OH)D at genome-wide significance were pooled and clumped to pairwise linkage disequilibrium $r^2 < 0.001$ based on the lowest P value for association with any trait. All clumping was performed using the TwoSampleMR package of R (34).

For analyses considering total effects, the random effects inverse-variance weighted (IVW) meta-analysis MR method was used for the main analysis, with the MR-Egger and weighted median MR methods that make distinct assumptions about the inclusion of pleiotropic variants used in sensitivity analyses (35). As the MR-Egger and weighted median methods were only used to support concordance with the main IVW findings, no statistical significance threshold was applied for these. The intercept of the MR-Egger regression offers a test for directional pleiotropy (36),

with $P < 0.05$ used as the threshold for evidence of this. For mediation analysis, summary data regression-based multivariable MR was performed, as previously described (37). Briefly, genetic instruments and associations for both BMI and 25(OH)D were included in the same MR model to investigate their direct effects on the inflammatory biomarkers after mutual adjustment. Any attenuation in the direct MR effect of BMI after adjusting for 25(OH)D, as compared with those observed in the MR measuring total effects of BMI, would support mediation through 25(OH)D. All MR analyses were performed on R version 4.2.

RCTs of vitamin D supplementation and AGP.

A systematic review published recently (38) has examined RCTs looking at the impact of vitamin D supplementation on 12 inflammatory biomarkers including CRP and sICAM-1, but not AGP, which was inversely associated with vitamin D in our study. To validate our findings from observational mediation and MR analyses, we performed a literature review of the impact of vitamin D supplementation on the acute-phase inflammatory biomarker AGP, using a similar protocol followed in the previous investigation (38). Here we have included all the search results identified using multiple databases including PubMed/MEDLINE, Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews, Web of Science, and Google Scholar until January 2020 from a literature search in relation to vitamin D and AGP (38).

RCTs of vitamin D supplementation in overweight and obese individuals.

We further reviewed the completed and ongoing RCTs to investigate the association between vitamin D supplementation and circulating inflammatory biomarkers and metabolic health outcomes of overweight and obese individuals. We performed this investigation to compare our study findings from a general adult population, which might provide additional information on the role of vitamin D supplementation in improving inflammation or health outcomes of overweight/obese individuals. To implement this, we examined the clinicaltrials.gov site “all studies” with option “condition or disease” with the terms “obesity and vitamin D” for reports published up to January 2020 in English. We further searched for the “completed trials” and further narrowed the search by choosing the option “completed trials with results.”

Results

Study characteristics and the clustering pattern of inflammatory biomarkers

The observational study included 3586 individuals (58% male) (Table 1). Mean BMI was 24.8 (95% CI: 24.7, 25.0) and mean serum 25(OH)D concentration was 50.3 nmol/L (95% CI: 49.8, 50.7 nmol/L). Except for hs-CRP and IL-17, all other plasma cytokine concentrations were higher in men than in women. There was no difference between complete (for all individuals all data available) and incomplete data analysis (i.e., maximum sample size for each variable analyzed, data not shown) ($P > 0.05$).

Figure 2 shows the correlation structure of the measured inflammatory biomarkers. In our analysis and in line with known biological function (31, 34), the inflammatory biomarkers clustered into 4 groups: “cluster 1” all ILs and TNF α ; “cluster 2” adhesion molecules, that is, sICAM-1 and sVCAM-1; “cluster 3” acute-phase proteins, that is, active PAI-1, CRP, and AGP; and “cluster 4” chemokines/CD154, that is, sCD40L/CD154, CXCL10/IP-10, and CCL2/MCP-1.

The observational associations of BMI and serum 25(OH)D with inflammatory markers

Figure 3 shows results from the multivariable regression analysis of BMI and serum 25(OH)D with 16 inflammatory biomarkers. In the fully adjusted model (model 3, FDR corrected, β_c in Figure 1A), BMI was positively associated with IL-6 and IL-1RA in cluster 1; sICAM-1 in cluster 2; active PAI-1, hs-CRP, and AGP in cluster 3; and with sCD40L/CD154, IP-10, and CCL2/MCP-1 in cluster 4. In contrast, BMI was inversely associated with sVCAM-1 ($\beta = -0.03$; 95% CI: $-0.07, -0.002$) in cluster 2. BMI was inversely associated with serum 25(OH)D concentration ($\beta_a = -0.05$; $P = 0.0005$; Table 2). For serum 25(OH)D (model 3, FDR corrected, β_b in Figure 1A), we observed inverse associations between 25(OH)D and IL-8 ($\beta = -0.07$; 95% CI: $-0.11, -0.04$), sICAM-1 ($\beta = -0.05$; 95% CI: $-0.08, -0.008$), hs-CRP ($\beta = -0.04$; 95% CI: $-0.08, -0.004$), and AGP ($\beta = -0.09$; 95% CI: $-0.13, -0.06$). Further adjustment of the association between 25(OH)D and these inflammatory biomarkers for BMI (Supplemental Table 4) showed no substantial change. We did not observe any interaction between 25(OH)D and BMI, fulfilling the criteria for mediation analyses.

Conventional mediation analysis of the association between BMI and inflammation by 25(OH)D

Observational mediation analysis results for the 3 inflammatory biomarkers sICAM-1, AGP, and hs-CRP are shown in Table 2. These 3 inflammatory biomarkers fulfilled the criteria for examining causal relations using mediation analyses as shown in Figure 1A; that is, the presence of an association between all 3 components: the exposure (BMI), outcome (inflammatory marker), and the mediator [25(OH)D]. In the mediation analyses, when 25(OH)D was added into the model, the association of BMI with AGP [$\beta_{c'} = 0.26$ (95% CI: 0.23, 0.29)] and sICAM-1 [$\beta_{c'} = 0.12$ (95% CI: 0.09, 0.15)] decreased slightly, albeit with nil results (Sobel P value = 0.08). In addition, the change in association of BMI with hs-CRP was negligible when adjusted for 25(OH)D (Sobel P value = 0.41).

MR analysis

The MR analyses supported the causal effects of BMI on the 3 inflammatory biomarkers (CRP, AGP, and sICAM-1) and vitamin D (Table 3). There was no MR evidence of a causal effect of vitamin D on concentrations of any of the 3 considered inflammatory biomarkers (Table 4). The results of the MR-Egger and weighted median sensitivity analyses were consistent with

TABLE 1 Descriptive statistics in the Northern Finland Birth Cohort 1966 study¹

	Total population	Males	Females	<i>P</i> value ²
Sample size (<i>n</i>)	3586	2073	1513	
Sex, % (<i>n</i>)				
Males	57.8 (2073)			
Females	42.2 (1513)			
Season of blood sampling, ³ % (<i>n</i>)				
High vitamin D months	64.8 (2323)	65.1 (1350)	64.3 (973)	0.62
Low vitamin D months	35.2 (1263)	34.9 (723)	35.7 (540)	
Socioeconomic position, % (<i>n</i>)				
I + II (professional)	24.5 (877)	27.5 (571)	20.2 (306)	<0.0001
III (skilled worker)	28.7 (1029)	18.1 (375)	43.2 (654)	
IV (unskilled worker)	27.1 (974)	35.9 (745)	15.2 (229)	
V (farmer)	3.8 (136)	4.7 (97)	2.6 (39)	
VI (other) ⁴	15.9 (570)	13.8 (285)	18.8 (285)	
Smoking, % (<i>n</i>)				
Nonsmoker	43.8 (1571)	40.2 (833)	48.8 (738)	<0.0001
Smoker	56.2 (2015)	59.8 (1240)	51.2 (775)	
Physical activity, ⁵ MET h/wk (95% CI)	14.96 (14.5, 15.4)	14.95 (14.3, 15.6)	14.97 (14.3, 15.6)	0.84
Alcohol intake, g/d (95% CI)	10.0 (9.4, 10.5)	13.7 (12.8, 14.6)	4.8 (4.4, 5.2)	<0.0001
BMI, kg/m ² (95% CI)	24.8 (24.7, 25.0)	25.2 (25.0, 25.3)	24.4 (24.2, 24.6)	<0.0001
Total serum 25(OH)D, nmol/L (95% CI)	50.3 (49.8, 50.7)	51.3 (50.6, 51.9)	48.9 (48.2, 49.6)	0.014
Inflammatory biomarkers (95% CI)				
IL-17, pg/mL	56.2 (49.4, 63.0)	56.1 (50.2, 62.0)	56.3 (42.3, 70.2)	0.166
IL-1 α , pg/mL	73.5 (63.4, 83.7)	89.0 (73.4, 104.7)	52.3 (41.6, 63.1)	<0.0001
IL-1 β , pg/mL	6.7 (5.1, 8.2)	7.8 (5.1, 10.5)	5.1 (4.2, 5.9)	0.0001
IL-6, pg/mL	19.0 (14.7, 23.2)	21.0 (17.1, 24.8)	16.2 (7.6, 24.8)	<0.0001
TNF- α , pg/mL	9.5 (8.4, 10.6)	10.8 (9.0, 12.6)	7.8 (7.0, 8.6)	<0.0001
IL-1RA, pg/mL	67.9 (52.5, 83.2)	78.1 (55.3, 100.9)	53.8 (35.2, 72.3)	<0.0001
IL-4, pg/mL	30.4 (25.8, 35.0)	35.7 (28.4, 43.0)	23.1 (18.7, 27.4)	<0.0001
IL-8, pg/mL	38.8 (33.7, 44.0)	39.9 (36.7, 43.1)	37.3 (25.9, 48.7)	<0.0001
IP-10, pg/mL	434.7 (422.4, 447.0)	448.7 (432.4, 465.0)	415.5 (396.9, 434.1)	<0.0001
MCP-1, pg/mL	319.6 (315.5, 323.8)	349.4 (343.8, 355.1)	278.9 (273.4, 284.3)	<0.0001
sCD40L, pg/mL	545.5 (525.0, 565.9)	588.9 (560.8, 616.9)	486.0 (456.7, 515.3)	<0.0001
sICAM, ng/mL	147.2 (145.3, 149.1)	156.6 (154.0, 159.3)	134.2 (131.5, 136.9)	<0.0001
sVCAM, ng/mL	1692.3 (1679.5, 1705.1)	1766.8 (1750.0, 1783.7)	1590.3 (1571.8, 1608.7)	<0.0001
Active PAI-1, ng/mL	22.2 (21.2, 23.1)	26.2 (24.8, 27.5)	16.7 (15.7, 17.7)	<0.0001
hs-CRP, mg/L	1.16 (1.11, 1.21)	1.15 (1.09, 1.22)	1.18 (1.10, 1.26)	0.097
α 1-Acid glycoprotein, mmol/L	1.36 (1.35, 1.37)	1.39 (1.38, 1.40)	1.32 (1.31, 1.33)	<0.0001

¹Data are presented as percentages (*n*) or means (95% CI) as appropriate. Active PAI-1, active plasminogen activator inhibitor 1; hs-CRP, high sensitivity C-reactive protein; IP-10, interferon gamma-induced protein 10; IL-1RA, interleukin-1 receptor antagonist; MCP-1, monocyte chemoattractant protein 1; sCD40L, soluble CD40 ligand; sICAM, soluble intercellular adhesion molecule; sVCAM, soluble vascular cell adhesion molecule; 25(OH)D, 25-hydroxyvitamin D.

²*P* value for heterogeneity between males and females is analyzed by chi-square test for categorical variables, Student *t* test for normally distributed variables, and Wilcoxon–Mann–Whitney *U* test for nonnormally distributed variables.

³Proportions of samples taken during high vitamin D months [summer (June 1 to August 30), autumn (September 1 to October 31)] and low vitamin D months [winter (November 1 to March 31) and spring (April 1 to May 31)].

⁴Includes students, pensioners, long-term unemployed, or not defined.

⁵MET is metabolic equivalent of task of physical activity.

those from the main IVW method. The MR–Egger intercept did not provide evidence for the presence of directional pleiotropy in any analysis.

In performing multivariable MR, there was no significant attenuation in the effect of genetically predicted BMI on these 3 inflammatory biomarkers after adjusting for genetically predicted vitamin D. There was no evidence of an effect of genetically predicted vitamin D on the biomarkers after adjusting for genetically predicted BMI (Table 5). These findings therefore do not provide evidence to support any role of vitamin D in mediating the effect of BMI on the 3 inflammatory biomarkers.

RCTs of vitamin D supplementation and AGP

Only 2 studies (39, 40) were identified where the impact of vitamin D supplementation on AGP had been assessed. Summary characteristics of the studies are presented in Supplemental Table 5. The included studies were published between 2015 and January 2020 from 2 different countries. One study reported a decrease in AGP concentrations compared with baseline in elderly women (39), whereas the other study reported no difference between the control and placebo group in pregnant women with vitamin D supplementation (40) (Supplemental Table 5).

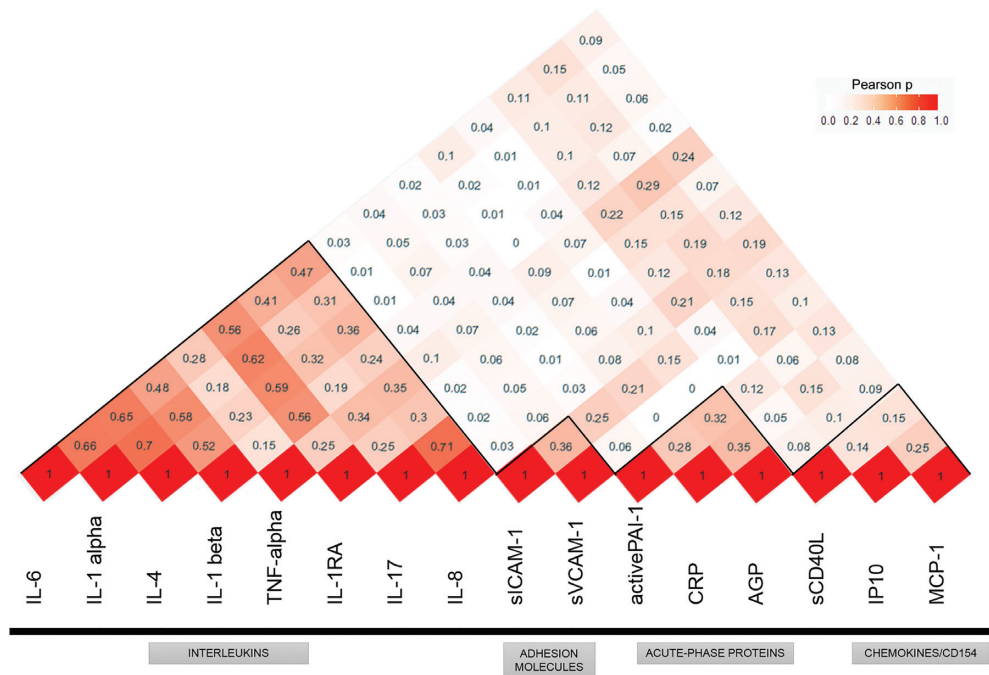


FIGURE 2 Correlation heat maps for inflammatory biomarkers using Pearson correlation analysis. Red and white colors represent a positive and negative correlation between the 2 inflammatory biomarker concentrations that meet at that cell, respectively. The darker and more saturated the color, the greater the magnitude of the correlation.

RCTs of vitamin D supplementation in overweight and obese individuals

From searches in the clinicaltrials.gov site we identified 13 completed and 55 ongoing RCTs that examine the association between vitamin D supplementation and circulating inflammatory biomarkers and metabolic health outcomes of overweight and obese individuals. In 13 completed RCTs the dose of vitamin D supplementation ranged from 400 IU/d to 150,000 IU/3 mo, duration of the trials was from 4 wk to 3 mo, and 1 study had vitamin D plus calcium supplementation. Four RCTs reported a small increase in circulating 25(OH)D concentrations in the overweight and obese individuals. However, subsequent improvement in the inflammatory status or health outcomes of overweight and obese individuals have not been reported (Supplemental Table 3).

Discussion

In the present study, we integrated conventional observational and MR approaches, including mediation analyses, to examine the role of 25(OH)D in the associations of BMI with circulating inflammatory biomarkers in the adult general population. We replicated the known positive associations of BMI with inflammatory biomarkers, and the inverse association of BMI and vitamin D. In addition, we have also shown that in observational analyses 25(OH)D, reflecting the nutritional vitamin D status, is inversely associated with 3 inflammatory biomarkers (sICAM-1, CRP, and AGP) related to higher BMI. This led us to examine the hypothesis that 25(OH)D was a potential mediator in the association between BMI and these

inflammatory markers because all 3 factors [BMI, 25(OH)D, and inflammatory markers] were observationally associated with each other in the expected directions. However, we did not find any evidence of such mediation when using conventional observational or MR approaches.

The 16 inflammatory biomarkers analyzed in the present study (Supplemental Table 1) correlated with each other forming 4 molecular clusters: ILs, adhesion molecules, acute-phase proteins, and chemokines. The clustering pattern of inflammatory biomarkers observed was in line with their biological response or organization during an inflammatory stimulus (41, 42). The inflammatory clusters we delineated were independent of each other and reported for the first time in our study to the best of our knowledge. The observed association between BMI and the inflammatory clusters might link higher BMI to a chronic state of inflammation (41, 42), which could be involved in the development of obesity-related pathological consequences. In addition, the results suggest that ≥ 4 different molecular and/or organ-specific pathways are present that link inflammation and cardiometabolic health outcomes.

Serum 25(OH)D concentration was inversely associated with 4 inflammatory biomarkers, IL-8, sICAM-1, CRP, and AGP, in 3 independent clusters (ILs, adhesion molecules, acute-phase proteins). The observed results were in line with an earlier experimental investigation, which reported downregulation of IL-8 secretion by calcitriol through interference with NF- κ B and MAPK signaling pathways (43–45). The negative association between 25(OH)D and sICAM-1 could indicate the role of calcitriol produced locally in endothelium, which results in downregulation of sICAM-1 expression as reported in a few, but not all, studies (38, 46, 47). Similarly, the association between 25(OH)D and

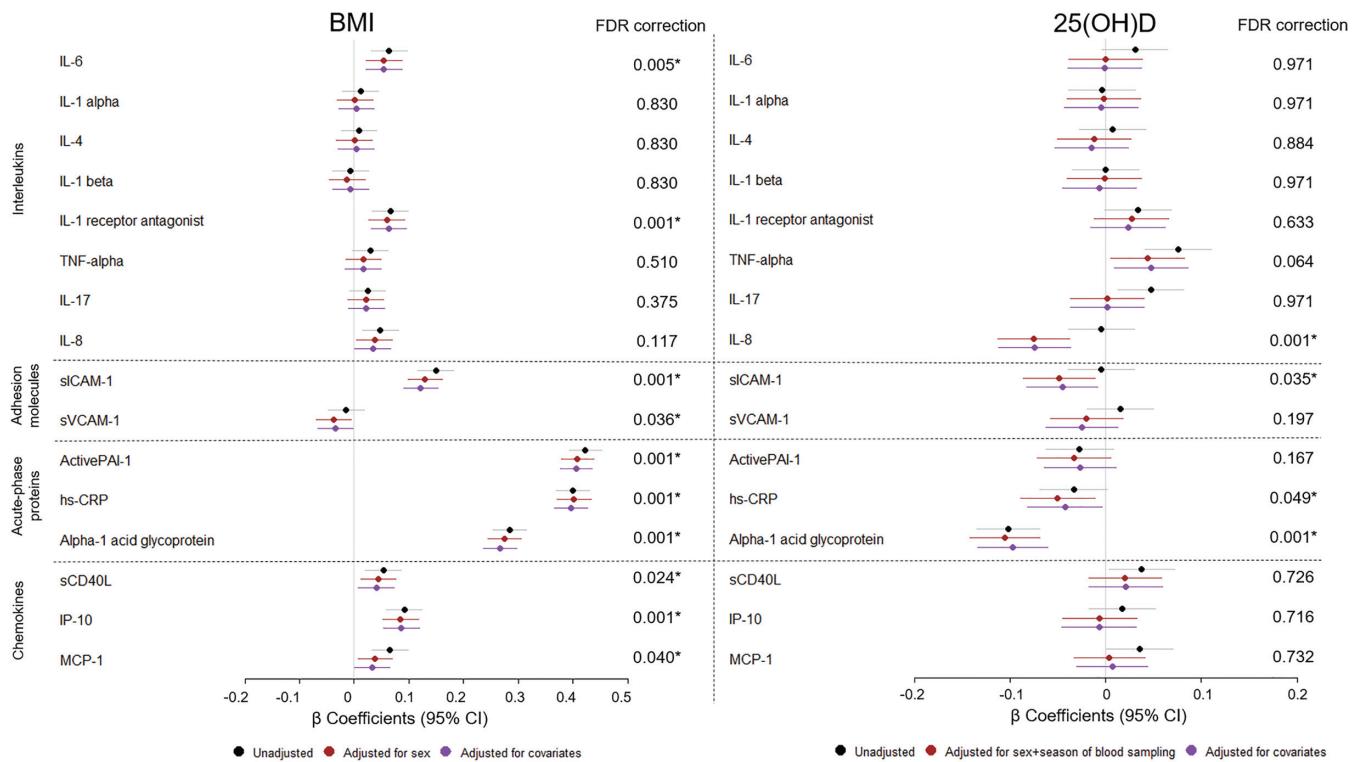


FIGURE 3 Multivariable regression analysis on the association of BMI and 25-hydroxyvitamin D [25(OH)D] (exposure) with 16 inflammatory biomarkers (outcome). The results are expressed as β coefficients change in inflammatory biomarkers (95% CI) per unit increase in BMI/25(OH)D. For BMI: model 1—unadjusted; model 2—adjusted for sex; model 3—adjusted for sex and covariates (smoking, physical activity, alcohol intake, socioeconomic position). For 25(OH)D: model 1—adjusted for vitamin D batch; model 2—adjusted for sex and season of blood sampling; model 3—model 2 + adjusted for covariates (smoking, physical activity, alcohol intake, socioeconomic position). ActivePAI-1, active plasminogen activator inhibitor 1; hs-CRP, high sensitivity C-reactive protein; IP-10, IFN γ -induced protein 10; MCP-1, monocyte chemoattractant protein 1; sCD40L, soluble CD40 ligand; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular cell adhesion molecule 1.

CRP remains controversial (38, 48). Furthermore, AGP in the circulation may arise during stressful conditions and 25(OH)D might play an inhibitory role in the regulation of AGP synthesis through VDR (49). These results support the anti-inflammatory function of calcitriol. In addition, the present study also demonstrated an independent inverse association of 25(OH)D with 3 inflammatory biomarkers even after adjusting for BMI (Supplemental Table 4). When compared with all the previous epidemiological investigations (Supplemental Table 2), our study has considered adjustment for potential covariates when reporting the associations of serum 25(OH)D with inflammatory biomarkers. In addition, serum 25(OH)D was inversely associated with BMI, which is in accordance with previous investigations (13, 50, 51).

In our observational analyses, BMI was positively associated with hs-CRP, AGP, and sICAM-1, and serum 25(OH)D was negatively associated with these inflammatory biomarkers. The observational associations of BMI and 25(OH)D with inflammatory biomarkers were in the expected directions, supporting a relation of higher BMI on higher inflammatory biomarkers via lowering of serum 25(OH)D concentration. However, observational mediation analysis, MR, and MR mediation analysis showed no evidence of a causal association between serum

25(OH)D concentration and the 3 inflammatory biomarkers (hs-CRP, sICAM-1, and AGP). The findings suggest that the association between 25(OH)D and inflammatory biomarkers from cross-sectional studies could be in part due to residual confounding (8).

In addition, a systematic review and meta-analysis that examined the RCTs to evaluate the impact of vitamin D supplementation on hs-CRP, and other inflammatory biomarkers including sICAM-1, has reported no impact on hs-CRP [weighted mean difference (WMD): -0.26 mg/L; 95% CI: $-0.75, 0.22$ mg/L; $n = 26$ arms; heterogeneity $P = 0.0042$; $I^2 = 54.2\%$], sICAM-1 (WMD: -0.79 pg/mL; 95% CI: $-1.33, 0.26$ pg/mL; $n = 4$ arms; heterogeneity $P < 0.001$; $I^2 = 62.1\%$), and 10 other biomarkers with vitamin D supplementation (38). Furthermore, the 2 studies identified to examine the impact of vitamin D supplementation on AGP (Supplemental Table 5) have also reported inconsistent results (39, 40). However, these results should be interpreted with caution because the RCTs included in the systematic review were performed on wider age groups, had small population samples, included vitamin D-deficient individuals at baseline, and were in populations with chronic diseases, resulting in heterogeneity in the interpretation of the results (38). RCTs with large sample sizes and longer follow-

TABLE 2 Results of the observational analysis of mediation through 25(OH)D in the relation between BMI and inflammatory biomarkers, soluble intercellular adhesion molecule-1, high sensitivity C-reactive protein, and α 1-acid glycoprotein (Figure 1A)¹

Inflammatory biomarker ²	Mediation analysis					
	Total BMI to inflammation (mediator unadjusted) ³	BMI to 25(OH)D ³	25(OH)D to inflammation ³	Direct BMI to inflammation (mediator adjusted) ³	% Mediation (indirect)	Sobel test (P value)
Soluble intercellular adhesion molecule 1	0.12 (0.09, 0.15)	-0.05 (-0.08, -0.02)	-0.04 (-0.07, -0.7 × 10 ⁻⁶)	0.12 (0.09, 0.15)	1.5	0.08
High sensitivity C-reactive protein	0.39 (0.37, 0.43)	-0.05 (-0.08, -0.02)	-0.02 (-0.05, -0.02)	0.39 (0.37, 0.43)	0.2	0.41
α 1-acid glycoprotein	0.27 (0.24, 0.30)	-0.05 (-0.08, -0.02)	-0.08 (-0.11, -0.04)	0.26 (0.23, 0.29)	1.5	0.08

¹Results are expressed as β coefficients (95% CI). 25(OH)D, 25-hydroxyvitamin D.

²BMI and 25(OH)D were associated with 3 inflammatory biomarkers in the regression analyses, thus fulfilling the criteria for mediation analyses as shown in Figure 1.

³The models were adjusted for covariates sex, season of blood sampling, 25(OH)D batch, socioeconomic position, smoking, alcohol consumption, and physical activity.

TABLE 3 Total effects from multivariable MR: BMI to inflammatory markers (CRP, sICAM-1, and AGP) and 25(OH)D (Figure 1C). MR estimates from the application of weighted median MR, IVW, and MR-Egger methodologies¹

Method	Estimate	SE	95% CI	P value
BMI-CRP				
Weighted median MR	0.390	0.025	0.342, 0.439	0.000
IVW	0.393	0.027	0.341, 0.445	0.000
MR-Egger	0.467	0.070	0.330, 0.604	0.000
(Intercept)	-0.001	0.001	-0.004, 0.001	0.252
BMI-sICAM-1				
Weighted median MR	0.330	0.106	0.122, 0.539	0.002
IVW	0.242	0.060	0.125, 0.360	0.000
MR-Egger	0.298	0.157	-0.010, 0.605	0.058
(Intercept)	-0.001	0.003	-0.006, 0.004	0.703
BMI-AGP				
Weighted median MR	0.346	0.054	0.240, 0.452	0.000
IVW	0.281	0.033	0.216, 0.346	0.000
MR-Egger	0.342	0.087	0.171, 0.513	0.000
(Intercept)	-0.001	0.001	-0.004, 0.002	0.449
BMI-25(OH)D				
Weighted median MR	-2.126	0.277	-2.668, -1.584	0.000
IVW	-2.516	0.209	-2.925, -2.107	0.000
MR-Egger	-1.532	0.547	-2.603, -0.461	0.005
(Intercept)	-0.017	0.009	-0.035, 0.000	0.051

¹AGP, α 1-acid glycoprotein; CRP, C-reactive protein; IVW, inverse-variance weighted; MR, Mendelian randomization; sICAM-1, soluble intercellular adhesion molecule 1; 25(OH)D, 25-hydroxyvitamin D.

up periods should be considered for future investigations to conclusively understand the role vitamin D supplementation has on inflammatory pathways.

It has been suggested that serum 25(OH)D status could modulate the inflammatory profile in individuals with overweight and obesity (52). However, BMI was reported to be causally associated with lower 25(OH)D, and vitamin D reduction in

TABLE 4 25(OH)D to inflammatory biomarkers (CRP, sICAM-1, and AGP) (Figure 1B). MR analysis results estimates from the application of weighted median MR, IVW, and MR-Egger methodologies¹

Method	Estimate ²	SE	95% CI	P value
25(OH)D-CRP				
Weighted median MR	0.002	0.001	-0.001, 0.004	0.167
IVW	-0.001	0.003	-0.007, 0.005	0.686
MR-Egger	0.000	0.004	-0.008, 0.009	0.913
(Intercept)	-0.002	0.004	-0.009, 0.005	0.614
25(OH)D-sICAM-1				
Weighted median MR	-0.003	0.005	-0.013, 0.008	0.589
IVW	-0.002	0.004	-0.009, 0.005	0.585
MR-Egger	0.000	0.006	-0.011, 0.011	0.945
(Intercept)	-0.002	0.005	-0.011, 0.007	0.691
25(OH)D-AGP				
Weighted median MR	0.004	0.003	-0.002, 0.010	0.154
IVW	-0.001	0.004	-0.008, 0.006	0.748
MR-Egger	0.007	0.005	-0.004, 0.017	0.223
(Intercept)	-0.009	0.005	-0.018, 0.000	0.054

¹AGP, α 1-acid glycoprotein; CRP, C-reactive protein; IVW, inverse-variance weighted; MR, Mendelian randomization; sICAM-1, soluble intercellular adhesion molecule 1; 25(OH)D, 25-hydroxyvitamin D.

²Estimates represent the estimated casual effect of 25(OH)D on inflammatory markers.

TABLE 5 Direct effects from multivariable MR: BMI to inflammatory markers [25(OH)D adjusted] and 25(OH)D to inflammatory biomarkers (BMI adjusted)¹

	Estimate	SE	95% CI	P value
Direct effects of BMI and 25(OH)D on CRP				
BMI	0.385	0.028	0.330, 0.715	$<2 \times 10^{-16}$
25(OH)D	-0.0006	0.002	-0.005, 0.003	0.744
Direct effects of BMI and 25(OH)D on sICAM-1				
BMI	0.234	0.059	0.118, 0.349	9.51×10^{-5}
25(OH)D	-0.003	0.004	-0.011, 0.005	0.371
Direct effects of BMI and 25(OH)D on AGP				
BMI	0.264	0.038	0.189, 0.338	9.04×10^{-12}
25(OH)D	-0.002	0.002	-0.006, 0.004	0.455

¹AGP, α 1-acid glycoprotein; CRP, C-reactive protein; MR, Mendelian randomization; sICAM-1, soluble intercellular adhesion molecule 1; 25(OH)D, 25-hydroxyvitamin D.

relation to higher BMI might contribute to increased BMI-related inflammation (13). In this case, vitamin D supplementation might be required in obese individuals to both replenish lowered 25(OH)D concentration and decrease the BMI-associated metabolic health outcomes (53). RCTs with vitamin D supplementation in adults with overweight or obesity have reported little to no evidence on the beneficial effects of supplementation in improving inflammation or the obesity-related metabolic health outcomes (Supplemental Table 3) (54–56). Our results are supported by a previous systematic review of RCTs that examined the association between vitamin D supplementation and inflammatory biomarkers and glycemic outcomes of overweight and obese adults (57). In addition, a recent systematic review and meta-analysis of completed RCTs that examined overweight and obese individuals with vitamin D supplementation has reported that the obese state decreased 25(OH)D concentration by -38.17 nmol/L (95% CI: -59.90 , -16.44 nmol/L) compared with the normal-weight group (58). It is noteworthy that 55 registered trials are still ongoing, and we recommend analyzing in greater details the impact of vitamin D supplementation on inflammation and obesity-related health outcomes. Furthermore, we have recently evaluated the association of the vitamin D status in older subjects born in 1945 from the Oulu region and found that low vitamin D status was associated with a high prevalence of metabolic syndrome (59). Recently, the vitamin D intervention trial in type 2 diabetes, consisting of 2423 participants supplemented with either 4000 IU vitamin D or placebo, failed to show a significant reduction in risk of diabetes (60). However, subgroup analyses in the type 2 diabetic subjects demonstrated that vitamin D supplementation had a lowering effect on the risk of diabetes in the study population with BMI <30 compared with subjects with BMI ≥ 30 (60). Therefore, in future RCTs, dosing and confounding in different BMI classes would need more careful considerations.

Strengths and limitations

To our knowledge this is the first study to investigate a comprehensive panel of inflammatory biomarkers in relation to 25(OH)D and BMI adjusting for multiple lifestyle covariates. In addition, this is the first study to use MR and MR mediation analyses to test if the association between vitamin D status and inflammatory

biomarkers is likely to be causal. The 2-sample MR approach (34) using the GWAS data sources does not require genetic associations with BMI, 25(OH)D, and AGP/CRP/sICAM-1 to be measured within the same population, which helps to overcome low power issues related to the availability of gene exposure and gene outcome data from the same study. Finally, we have tested our hypothesis using observational data from NFBC1966, publicly available GWAS summary statistics to examine the causal relation between 25(OH)D and inflammatory biomarkers, observational mediation, and MR mediation analysis to examine the role of vitamin D as a mediator, and followed the MR reporting checklist (61).

We acknowledge some limitations. Although BMI is an easily accessible measure of excessive body weight in the general population, it cannot differentiate between fat and lean body mass. Individuals with normal weight can still demonstrate harmful adiposity traits (62). Our study only considered BMI as a measure of adiposity and would not be sensitive to this. Furthermore, our findings were restricted to individuals of European descent. Further work, including validation in other populations, is required to replicate our findings across ethnicities. Concurrently, our intended follow-up study from NFBC1966 at 46 y might provide more information on validation of these findings between vitamin D, BMI, and inflammation at a different age group in the future.

Conclusions

The present study reports novel findings of clinical importance. We have reported: 1) the molecular clustering of 16 inflammatory biomarkers; 2) the complex association between BMI and the inflammatory biomarkers, suggesting that obesity might influence multiple inflammatory pathways differently; 3) the consistent inverse association of vitamin D with inflammatory biomarkers; 4) observational mediation analysis and 2-sample MR and MR mediation analysis to ascertain the causality between serum 25(OH)D and inflammatory biomarkers does not support the role of vitamin D as a causal mediator of BMI-associated inflammation although we show BMI's causal role on 3 tested inflammatory markers as a part of mediation analyses; and 5) further interpretation supported by the review of vitamin D supplementation RCTs also reported inconsistent results. Overall, this study does not support the hypothesis that

low vitamin D status co-occurring with obesity contributes to aggravation of the inflammatory status.

We thank the entire NFBC1966 study team, including the research staff, all others involved in the data collection and processing, and those involved in the oversight and management of the study. We acknowledge the late Professor Paula Rantakallio for the launch of the Northern Finland Birth Cohort 1966 and initial data collection, Sarianna Vaara for data collection, Markku Koiranen for data management, Tuula Ylitalo for administration, and Alicia Heath for the English language editing. The authors thank all the participants of the NFBC1966 study.

The authors' responsibilities were as follows—SP, EH, K-HH, SS, M-RJ: concept and design; M-RJ, K-HH, EH: data acquisition; SP, DG, NMDS: statistical analysis and interpretation of data; SP, DG, NMDS, SS, M-RJ: drafting of the manuscript; SP, DG, NMDS, EL, JJ, TK, SJM, AD, ES, DIC, MT, HV, SK-K, EH, K-HH, SS, M-RJ: critical revision of the manuscript; AD, DIC, ES, TK, SJM, K-HH, JJ, M-RJ: administrative, technical, or material support; and all authors: interpreted data, revised the manuscript, and read and approved the final manuscript. The authors report no conflicts of interest.

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