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# The relative contributions of obesity, vitamin D, leptin and adiponectin to multiple sclerosis risk: a Mendelian randomization mediation analysis

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## Abstract

**Background:** Obesity is associated with increased risk of multiple sclerosis (MS); however, the underlying mechanisms remain unclear.

**Objective:** To determine the extent to which decreased vitamin D bioavailability and altered levels of adiponectin and leptin mediate the association between obesity and MS.

**Methods:** We performed Mendelian randomization (MR) analyses to estimate the effects on MS of body mass index (BMI), 25-hydroxyvitamin D (25OHD), adiponectin and leptin levels in a cohort of 14,802 MS cases and 26,703 controls. We then estimated the proportion of the effect of obesity on MS explained by these potential mediators.

**Results:** Genetic predisposition to higher BMI was associated with increased MS risk (OR=1.3340 per SD, 95% CI 1.0916-1.637), while higher 25OHD levels reduced odds of MS (OR=0.72 per SD, 95% CI 0.60-0.87). In contrast, we observed no effect of adiponectin or leptin. In MR mediation analysis, 5.24% of the association between BMI and MS was attributed to obesity lowering 25OHD levels (95% CI 0.34% to 30.531.0%).

**Conclusions:** This study found that a minority of the increased risk of MS conferred by obesity is mediated by lowered vitamin D levels, while leptin and adiponectin had no effect. Consequently, vitamin D supplementation would only modestly reverse the effect of obesity on MS.

## Introduction

An increasing body of evidence supports a role for obesity in the development of multiple sclerosis (MS).[1, 2] However, the mechanisms underlying this association remain unclear. A commonly hypothesized pathway is through vitamin D deficiency, another established MS risk factor,[2] as obesity decreases the bioavailability of 25-hydroxyvitamin D (25OHD).[3] While appealing, these proposed mediating effects of 25OHD have yet to be confirmed or quantified. Obesity is also characterized by a chronic low-grade inflammatory state, driven in part by hormones and cytokines secreted by adipose tissue, such as leptin and adiponectin. These have been implicated in MS pathogenesis and shown to modulate experimental autoimmune encephalomyelitis disease course (reviewed in [4]). However, it remains unclear whether they have a causal role in the disease process or contribute to mediating the association with obesity.

Establishing the relative contributions of obesity and vitamin D deficiency in particular to MS risk has major public health implications, as their prevalence in the US is respectively 41.6% and 39.6%.[5, 6] Furthermore, if a large proportion of the association between obesity and MS is explained by lowered 25OHD levels, then vitamin D supplementation at a population level or in high-risk individuals could become a viable strategy to mitigate the effects of obesity on MS.

To address these questions, we undertook a Mendelian randomization (MR) approach, which uses natural genetic variation as a proxy for an exposure to estimate its effect on an outcome. MR greatly reduces confounding since allelic variants influencing different exposures are randomly allocated at conception.[7] The fact that genotypes are not modifiable by disease onset also limits reverse causation.[8] This makes MR well suited for mediation analysis.[9] In this study, we first

estimated ~~the effect of each of~~whether genetic predisposition towards higher 25OHD, leptin and adiponectin levels ~~on~~ influence the risk of MS, then took forward significantly associated traits into a two-step MR mediation analysis to determine their contribution to the association between obesity and MS.

## Methods

*Data sources*

We identified single-nucleotide polymorphisms (SNPs) for BMI and each potential mediator using large-scale genome-wide association studies (GWAS) as shown in the **Table**. [10-13] As genetic estimates for 25OHD were derived from the UK Biobank cohort,[11] we used a BMI GWAS that did not include this cohort[10] to avoid participant overlap in the mediation analysis, which can lead to inflated type 1 error rates.~~to avoid bias from sample overlap.~~[8, 14] For MS susceptibility, summary genetic estimates were obtained from the discovery cohort of the latest International MS Genetics Consortium meta-analysis, which included 14,802 MS cases and 26,703 controls as previously described.[15] To prevent confounding through population stratification, all genetic estimates were from individuals of European ancestry (white British group for the UK Biobank[11]) and subsequently adjusted for genetic principal components.

We ensured that genetic variants were independent ($r^2 < 0.01$) by using the 1000 Genomes European reference panel[16] and PLINK v1.9[17] (clump command within 10,000 kb distance), ~~[16]~~ except for the 25OHD levels variants for which we used conditionally independent estimates from a COJO analysis.[11] When genetic ~~instruments~~ variants were missing from one of the datasets, we identified proxy SNPs

in linkage disequilibrium (LD; $r^2 > 0.6$) using the same reference panel.[16] We excluded variants within the major histocompatibility complex (MHC) region, as it is strongly associated with MS risk and exhibits complex ~~linkage disequilibrium~~LD which renders it susceptible to bias from pleiotropy. Genetic variants were aligned to the forward strand. For GWAS not originally reported on the Genome Reference Consortium Human Build 37 (BMI, leptin and adiponectin), forward strand alleles for palindromic SNPs were inferred using minor allele frequencies up to 0.42.

The inclusion of genetic variants with smaller effects on the exposure can lead to weak instrument bias, which attenuates MR estimates towards the null. For each phenotype, we evaluated instrument strength using the F-statistic (two-sample conditional F-statistic $F_{TS}$ for multivariable MR), with values greater than 10 indicating adequately strong instruments. As a sensitivity analysis, we also measured the effect of BMI on the risk of MS using the latest GWAS meta-analysis by Yengo and colleagues[18] (n=681,275) which included UK Biobank participants and thus was not used for the mediation analysis, as discussed above. This study identified 941 near-independent SNPs, of which 548 were included after filtering out MHC variants, marginal effects below genome-wide significance and correlated variants.

*Statistical analysis*

We first carried out univariable inverse-variance weighted (IVW) MR to examine the effect on MS of genetically determined BMI, 25OHD, leptin and adiponectin levels individually. For each genetic variant, the effect on MS was estimated using the SNP effect coefficients via the ratio method, with standard errors derived using the delta method.[19] These individual MR estimates were combined into a summary measure using random-effect inverse-variance weighted meta-analysis. In addition, we

applied the MR-Egger and weighted median MR methods to assess for potential bias from pleiotropic effects, whereby genetic ~~instruments~~ variants affect the outcome independent of the risk factor.[8] We also performed the MR-Pleiotropy RESidual Sum and Outlier (MR-PRESSO) outlier test to identify and remove potentially pleiotropic variants.[20]

As leptin and adiponectin displayed no measurable effect on MS risk, only 25OHD level~~s were~~ was taken forward into the mediation analysis. We measured the indirect effect of BMI on MS mediated by 25OHD levels using the product of coefficients method via two-step MR (**Figure 1**).[9] This involved first estimating the effect of BMI on 25OHD levels, then multiplying this by the effect of 25OHD levels on MS risk adjusting for BMI using regression-based multivariable MR.[21] The multivariable MR included genetic effects of both 25OHD levels and BMI for variants associated with either phenotype. The proportion mediated was estimated by dividing ~~this~~ the indirect effect by the total effect of BMI on MS. Outlier-corrected estimates were used for univariable analyses.

To further ensure that our estimates were not biased by pleiotropy, we repeated the mediation analysis using ~~only~~ 6 genetic variants for 25OHD levels identified in a previous GWAS,[22] most of which have a well-defined role in vitamin D synthesis (*DHCR7/NADSYN1* [rs12785878]; *CYP2R1* [rs10741657]), transportation (*GC* [rs3755967]), or degradation (*CYP24A1* [rs17216707]). Lastly, we calculated the effect of BMI on MS risk adjusting for 25OHD levels using multivariable MR.

All statistical analyses were performed in R (version 3.6.0). We used the MendelianRandomization[23] (version 0.4.2), TwoSampleMR[24] (version 0.5.4) and MVMR (version 0.2)[25] R packages. The alpha-level for statistical significance was set to 0.05. The data sources used in this study obtained

informed consent from all participants. Separate institutional review board approval was not required for this study.

## Results

*Effects of vitamin D, leptin and adiponectin levels on MS*

**Figure 2** shows the total effect on odds of MS for BMI, 25OHD levels, leptin and adiponectin levels as estimated by univariable MR. A significant effect was ~~only~~ observed for BMI (odds ratio [OR]=1.33~~40~~ per standard deviation [SD] increase in BMI, 95% CI 1.09 to 1.63, $P$=4.8x10$^{-3}$ ~~1.16 to 1.67, $P$=3.12x10$^{-4}$~~) and 25OHD levels (OR=0.72 per SD increase in log-transformed 25OHD, 95% CI 0.60 to 0.87, $P$=6.2~~1~~x10$^{-4}$). To guide clinical interpretation, the SD for BMI was 4.7 kg/m$^2$,[10] while for 25OHD it corresponded to a 29.2 nmol/L increase for individuals with a baseline level of 50 nmol/L. Additional equivalences for clinically relevant vitamin D thresholds are presented in the **Supplementary Table S1**. In sensitivity analyses, the MR-Egger intercept was centered around zero for both phenotypes (BMI: intercept=-0.00~~64~~, 95% CI -0.020 to 0.008~~0.007 to -0.17~~, $P$=0.43~~50~~; 25OHD levels: intercept=0.006, 95% CI -0.003 to 0.005 ~~to -0.003~~, $P$=0.22). **Figure 2** shows that the MR-Egger regression coefficient, ~~and~~ weighted median and MR-PRESSO outlier-corrected estimates were consistent with the main IVW analysis. F-statistics were greater than 10 for all phenotypes (range 22.2-167.9) and are reported in the ~~F-statistics for each phenotype are reported in the~~ S**upplementary Table S2**. The effect of BMI on MS risk was replicated using the larger set of 548 variants from the latest BMI GWAS meta-analysis (OR=1.29, 95% CI 1.15 to 1.46, $P$=2.6x10$^{-5}$).

*Mediation of the effect of BMI on MS by vitamin D*

Higher BMI was associated with lower 25OHD levels (IVW: β=-0.082 per SD unit increase in BMI, 95% CI -0.118 to -0.046, $P$=7.6~~8.71~~x10$^{-6}$; outlier-corrected: β=-0.081, 95% CI -0.111 to -0.051,

*P*=2.6x10$^{-7}$). In contrast, genetically increased 25OHD levels did not influence BMI (β=0.027, 95% CI -0.018 to 0.073, *P*=0.24). In line with this, the effect of 25OHD levels on MS remained robust after adjusting for BMI in multivariable MR (OR=0.81, 95% CI 0.68 to 0.97, *P*=0.02~~OR=0.80, 95% CI 0.67-0.96, *P*=0.01~~). The slightly weaker adjusted compared to unadjusted estimate is due to the reduced number of variants ($N_{SNPs}$=78) also present in the BMI dataset (**Supplementary Table S3**). The proportion of the effect of BMI on MS mediated by 25OHD levels was 5.2% (95% CI 0.3% to 31.0%) ~~5.4% (95% CI 0.4% to 30.5%)~~. Using only the 6 previously identified SNPs for 25OHD,[22] we obtained similar estimates of the mediated proportion (5.9%, 95% CI 0.3% to 36.3%) ~~(6.4%, 95% CI 0.7% to 33.7%)~~.

In multivariable MR, an effect of higher BMI on MS persisted after adjusting for 25OHD levels (OR=1.28 per SD unit increase in BMI, 95% CI 1.05-1.55, *P*=0.01), consistent with incomplete mediation (**Supplementary Table S3**). The two-sample conditional F-statistic $F_{TS}$ was 77.5 and 33.0 for 25OHD levels and BMI respectively.

None of the individual variants associated with BMI, 25OHD, leptin or adiponectin levels were genome-wide significant in the discovery cohort of the MS susceptibility GWAS (**Supplementary Tables S4-7**). Of the genes reported to be associated with MS (based on distance from their corresponding variants), 2 overlap with 25OHD levels (*CYP24A1* and *TNFAIP8*) and 1 with BMI (*ADCY3*). While the lead variants for MS and those phenotypes were not in LD (**Supplementary Table S8**), a previous study reported a coding variant in *CYP24A1* which strongly reduces 25OHD levels and also increases risk of MS.[26] *CYP24A1* encodes an enzyme that catalyzes the inactivation of calcidiol and calcitriol.

## Discussion

This study provides genetic support for a ~~causal~~ role of increased BMI and lowered 25OHD levels in the development of MS, using updated genetic estimates from large-scale cohorts. When considering both phenotypes in a mediation framework, we estimated that ~~only~~ 5.~~4~~2% (and up to a third) of the association between obesity and MS susceptibility can be explained by lowered vitamin D levels. In contrast, the results show no effect on the risk of the disease for lifelong genetically related increases in leptin or adiponectin levels. Therefore, the majority of the effect of obesity remains unexplained.

Previous MR studies on the effects of 25OHD levels and BMI on MS risk have found directionally consistent results with overlapping estimate confidence intervals.[27-31] Similarly, our previous MR study of adiponectin levels using an independent MS genetic cohort found no effect on MS risk.[32] While some observational studies have described increased leptin and lowered adiponectin levels in MS compared to controls, these have generally been small and included prevalent MS cases, making them susceptible to reverse causality.[4] That said, small effects by those cytokines on MS risk may still exist given their wider estimate confidence intervals.

~~Despite the large body of observational studies on vitamin D deficiency and obesity in MS, to our knowledge none has addressed the question of mediation between these risk factors.~~ A previous study in pediatric-onset MS and another in adult-onset MS reported independent effects of genetically determined BMI and 25OHD levels using multivariable regression.[28, 31] However, they did

not quantify the mediated effects and employed an approach which may be more susceptible to measurement error in the intermediate phenotype. Moreover, both studies used genetic estimates for 25OHD previously adjusted for BMI, making them ~~ill~~ less suited to assess mediation of obesity.

A major strength of this work is the MR approach which helps overcome many of the challenges facing traditional mediation analysis by reducing bias from unmeasured confounding, reverse causality and measurement error. It also enabled us to estimate the effects of each phenotype using large-scale genetic studies totaling more than 800,000 participants, while alleviating the need for ~~all~~ direct measurements of those phenotypes ~~to be performed~~ in a single cohort. We also acknowledge a number of limitations. First, potential bias from pleiotropy cannot be entirely excluded. While the genetic variants included are reliably associated and predictive of their respective phenotypes, their functional consequences (or those of strongly correlated variants in the same region) are not known in most cases and may include pleiotropic effects. These cannot be directly tested; h~~H~~owever, our sensitivity analyses and the consistent estimates obtained using the reduced set of SNPs mapped to genes with well-characterized roles in vitamin D metabolism decrease the likelihood of pleiotropic bias. Second, a binary outcome can lead to biased mediation estimates due to the non-collapsibility of odds ratio, although this is lessened by the use of product of the coefficients method and the rare prevalence of MS.[9] Third, MR estimates the risk associated with lifelong differences in the exposure, and as such may not fully capture effects that are time-specific. Fourth, although the risk of weak instrument bias is low given the F-statistics, the lower variance explained for leptin, as well as the smaller GWAS sample size for leptin and adiponectin, have contributed to reduced statistical power compared to other phenotypes, as indicated by the wider confidence intervals. As such, small effects by those cytokines in MS risk may still exist. That said, the genetic variants used in this study

adequately capture differences in BMI in early adulthood,[33] the time period where obesity is most strongly associated with MS risk, while for vitamin D deficiency associations have been reported from in utero through early adulthood.[2] Lastly, we assume that the associations between MS, obesity and 25OHD levels are linear (assumptions supported by previous studies[34-36]) and without interaction. Confirmation of these findings in observational studies with direct phenotype measurement, studies investigating potential interactions between BMI and 25OHD levels, and exploration of the mechanistic pathways underlying the association between obesity and MS are needed.

## Conclusion

This MR study found that a small proportion of the effect of obesity on the risk of MS is mediated by decreasing levels of vitamin D, while leptin and adiponectin had no measurable effect on MS susceptibility. This suggests that widespread vitamin D supplementation would only lead to modest reduction in the association between obesity and MS, most of which remains unexplained.

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## Figure Legends

**Figure 1. Directed acyclic graph of the MR mediation analysis.**

Increased BMI may affect the risk of MS through lowering 25OHD levels (indirect pathways in red), or independently from 25OHD (pathway in blue). The indirect effect can be calculated by multiplying $\alpha$ times $\beta$, where $\alpha$ is the effect of BMI on 25OHD levels, and $\beta$ the effect of 25OHD levels on MS adjusted for BMI using multivariable MR. The proportion mediated can be estimated by dividing the indirect effect by the total effect of BMI on MS. 25OHD=25-hydroxyvitamin D; MS = multiple sclerosis; SNP = single nucleotide polymorphism

**Figure 2. Forest plot showing the MR estimates investigating the effect of BMI and its potential mediators on the risk of MS.**

There were too few variants to apply the MR-Egger method for leptin levels, and no outliers were identified by MR-PRESSO except for BMI. The unit change for each phenotype associated with reported odds ratio can be found in **Table 1**. 25OHD=25-hydroxyvitamin D; CI=confidence intervals; IVW=inverse-variance weighted; MR=Mendelian randomization; MS=multiple sclerosis; $N_{SNPs}$=number of singe nucleotide polymorphisms included in each analysis; OR=Odds ratio.

## Tables

**Table 1. Summary of the phenotypes and summary genetic data used as exposures in the MR analyses**

| Phenotype | Sample size | $N_{SNPs}$ [a] | Units | Data source |
|---|---|---|---|---|
| BMI | 322,154 | 74 | SD increase in BMI, adjusted for age, age$^2$ and sex | GIANT consortium [10] |
| 25OHD | 401,460 | 138 | SD increase in standardized log-transformed levels, adjusted for vitamin D supplementation, age, sex, season of measurement and assessment center (as proxy for latitude) | UK Biobank [11] |
| Adiponectin | 38,276 | 11 | Unit increase in log-transformed levels, adjusted for age, sex and BMI | ADIPOGen consortium [12] |
| Leptin | 52,126 | 4 | Unit increase in log-transformed levels, adjusted for age, age$^2$ and sex | Kilpeläinen et al., 2016 [13] |

[a] Genome-wide significant SNPs, after exclusions due to linkage disequilibrium, MHC region or palindromic SNPs. For 25OHD levels, this represents the number of conditionally independent variants from COJO analysis. BMI=body mass index; MHC=major histocompatibility complex; MR=Mendelian randomization; SD=standard deviation; SNPs=single nucleotide polymorphisms