Are serum concentrations of vitamin B-12 causally related to cardiometabolic risk factors and disease? A Mendelian randomization study

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ABSTRACT

Background: Several observational studies have shown that low serum vitamin B-12 is associated with increased body mass index (BMI) and adverse cardiometabolic outcomes. However, it is unclear if these associations reflect a causal effect of vitamin B-12 on cardiometabolic risk factors and diseases, latent confounding, or reverse causality.

Objectives: The aims of this study were to investigate *1*) the possible causal relation between vitamin B-12 and indicators of body fat, lipid, and glucose variables; type 2 diabetes (T2D); and cardiovascular disease by using a 2-sample Mendelian randomization (MR) method and *2*) the possible pleiotropic role of fucosyltransferase 2 (*FUT2*).

Design: We selected 11 single nucleotide polymorphisms (SNPs) robustly associated with serum concentrations of vitamin B-12 in a previous genomewide association study (GWAS) in 45,576 individuals. We performed 2-sample MR analyses of the relation between vitamin B-12 and cardiometabolic risk factors and diseases with the use of publicly available GWAS summary statistics for 15 outcomes in $\leq$339,224 individuals. The robustness of results was tested with sensitivity analyses by using MR Egger regression and weighted-median estimation, and by performing additional analyses excluding a variant in the *FUT2* gene, which may be pleiotropic.

Results: We found a suggestive causal relation between vitamin B-12 and fasting glucose and β cell function [homeostatic model assessment (HOMA) of β cell function (HOMA-B)]. However, we found no evidence that serum concentrations of vitamin B-12 were causally related to BMI, waist-to-hip ratio, plasma leptin, body fat, fasting insulin, insulin resistance (from HOMA of insulin resistance), glycated hemoglobin, triglycerides, T2D, coronary artery disease, or HDL, LDL, or total cholesterol.

Conclusions: We found no evidence that serum concentrations of vitamin B-12 are causally related to body weight or the majority of cardiometabolic outcomes investigated. However, vitamin B-12 may have a causal effect on fasting glucose and HOMA-B, although these results will require replication in large independent data sets. This trialwas registered at http://www.isrctn.com/ISRCTN47414943 as ISRCTN47414943. *Am J Clin Nutr* 2018;108:398–404.

Keywords: Mendelian randomization, vitamin B-12, type 2 diabetes, coronary artery disease, glucose, cholesterol, lipids

INTRODUCTION

Vitamin B-12, or cobalamin, is a water-soluble vitamin essential for several cellular functions, such as energy metabolism, DNA synthesis and methylation, and protein, lipid, and carbohydrate metabolism (1). Although dietary composition is a major determinant of serum vitamin B-12 concentrations, several genetic polymorphisms have been associated with concentrations of vitamin B-12 (2–7).

Observational studies have shown a link between low serum vitamin B-12 concentrations and high BMI (8), insulin resistance (9), future type 2 diabetes (T2D) (10), adverse lipid profile (11), and cardiovascular disease (12–15). However, these studies are subject to confounding, reverse causation, and various biases and may therefore not reflect a causal relation (16). Randomized

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Supplemental Figure 1 and Supplemental Tables 1–8 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: *ABCD4*, ATP-binding cassette subfamily D, member 4; CAD, coronary artery disease; *FUT2*, fucosyltransferase 2; GWAS, genomewide association study; HOMA-B, homeostatic model assessment of β cell function; IVW, inverse variance weighted; MR, Mendelian randomization; *MUT*, methylmalonyl-CoA mutase; SNP, single nucleotide polymorphism; T2D, type 2 diabetes;.

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controlled trials circumvent these problems and are considered the gold standard in assessing causality in epidemiology.

Mendelian randomization (MR) is a statistical method that uses genetics to provide information on causality in observational studies. Genetic variants are used as instrumental variable proxies of environmental exposures of interest (17). Mendel's laws of inheritance ensure that genetic variants segregate independently of environmental confounders and assort independently of other traits (18). Therefore, MR studies share some similarities with randomized controlled trials, although in the former case it is genetic variants that are used to stratify individuals into high- or low-risk groups, rather than the physical randomization of participants. Two-sample MR utilizes single nucleotide polymorphisms (SNPs) that have been associated with the exposure in one study and the same SNPs that have been associated with the outcome in another sample of individuals to estimate the causal effect of the exposure on the outcome (19). The 2-sample MR approach allows for the use of summary statistics from large, publicly available genomewide association studies (GWASs) to study the causal relation between an exposure and the outcome, resulting in very large sample sizes with appreciable statistical power (20, 21).

Previous MR studies reported on the relation between vitamin B-12 and ischemic heart disease (22) and BMI (2), but these used either fewer vitamin B-12–associated SNPs or had smaller sample sizes. The study of BMI used variants in the fucosyltransferase 2 (*FUT2*) gene to derive causal estimates of the effect of serum vitamin B-12. The FUT2 protein is involved in the absorption of several nutrients (23–25) and may therefore reflect nutritional status, which, in turn, will affect a multitude of lifestyle-associated traits and diseases. The relation between low vitamin B-12 status and higher BMI disappeared when the variant in *FUT2* was left out of the analysis (2). The aim of this study was to investigate a possible causal relation between vitamin B-12 and indicators of body fat, lipid, and glucose variables; T2D; and cardiovascular disease with the use of 2-sample MR in a very large set of publicly available GWAS results data.

METHODS

Study design and rationale

We extracted summary results data on 11 SNPs (**Table 1**) that were robustly associated with serum concentrations of vitamin B-12 with genomewide significance ($P < 5 \times 10^{-8}$) from the largest GWAS of this phenotype to date (26) ($n = 45{,}576$ individuals of Danish and Icelandic ancestry). The 11 genomewide significant variants explained a total of 5.34% of the variance in serum vitamin B-12 concentrations (3.92% without the *FUT2* variant) (26). The 8 SNPs available in our GWAS results data explained 3.72% of the variation in serum vitamin B-12 (2.33% without the *FUT2* variant). The effect of the individual SNPs on a quantile normalized scale is shown in Table 1. A flowchart of the SNP selection process is shown in **Supplemental Figure 1**.

The use of multiple independent SNPs as our genetic instrument has the potential to increase power, because the instrumental variables will collectively explain more of the observed variance in vitamin B-12 concentrations and provide the opportunity to conduct a range of sensitivity analyses to test the robustness of our results. We performed analyses with and without the known pleiotropic variant rs602662 (*FUT2*) due to concerns that its inclusion could potentially violate one of the core assumptions of MR. When SNPs were not available in outcome data sets, attempts to replace with other SNPs in linkage disequilibrium ($r^2 > 0.8$) were made. The SNP rs1131603 only had 1 proxy rs57017208, $r^2 = 0.92$ CEU (Utah Residents with Northern and Western European Ancestry) population https://analysistools.nci.nih.gov/LDlink, whereas rs34324219, rs7788053, and rs41281112 had none. All SNPs were coded so that the effect allele was associated with increased vitamin B-12.

To assess the strength of our instruments, we approximated first stage F statistics for the individual SNPs reported by Grarup et al. (26) with the use of the estimated βs and SEs from their meta-analysis [i.e., $F \approx (\beta/SE)^2$]. To ensure adequate instrument strength in the MR Egger regression analyses we calculated I^2_{GX} for each of our instrument sets, as previously described by Bowden et al. (27).

We also obtained summary results statistics on the same SNPs from GWASs of 15 different cardiometabolic traits from 9 published studies (**Table 2**). The cardiometabolic outcomes were selected on the basis of the following inclusion criteria: *1*) the outcome having been associated with vitamin B-12 concentration in observational epidemiologic studies and *2*) the availability of large meta-GWAS analyses with publicly available summary statistics on the outcome.

Statistical analysis

We performed inverse variance–weighted (IVW) 2-sample MR analyses of vitamin B-12 with BMI and a variety of other cardiometabolic risk factors and diseases. Analysis was performed with the use of the 2-sample MR package (21) (https://github.com/MRCIEU/TwoSampleMR) in R version 3.3.3 (https://cran.r-project.org/). In order to investigate the robustness of our results, we first investigated the effect that excluding the (pleiotropic) variant at *FUT2* had on our 2-sample MR analyses. We then performed a series of sensitivity analyses with the use of MR Egger (37) regression and weighted-median estimation, which both allow relaxation of the core assumptions on which the MR methodology is based—in particular, the strict requirement for none of the SNPs to exhibit horizontal pleiotropy (38). The weighted-median approach provides an asymptotically consistent estimate of the causal effect when ≤50% of the information contributing to the analysis comes from SNPs that are invalid or exhibit horizontal pleiotropy (38). MR Egger involves regressing estimates of the SNP-outcome association on estimates of the SNP-exposure association. The slope of the weighted regression line provides an estimate of the causal effect of the exposure on the outcome that is robust to the effects from horizontal pleiotropy (37). The intercept estimated in MR regression is a function of the degree of directional pleiotropy present in the data set and statistical tests of the degree to which the intercept differs from zero are equivalent to testing for the presence of directional pleiotropy in the data. Although MR Egger regression allows for any number of SNPs to be invalid (i.e., to display horizontal pleiotropy), it requires the "INstrument Strength Independent of Direct Effect" (INSIDE) assumption to hold—that is, across SNPs, there can be no relation between the strength of association between SNPs and exposure and the strength of association between SNPs and outcome via pathways other than through the

TABLE 1
Effect of SNPs on serum vitamin B-12 concentrations along with putative genes and their potential function[1]

SNP	Gene	Function	Effect	SE	Effect allele	Other allele	EAF	*P*	F-statistic
rs2336573	*CD320*	Transcobalamin receptor; vitamin B-12 uptake; B cell proliferation	0.313	0.019	T	C	0.031	2.89×10^{-60}	267
rs1131603	*TCN2*	Vitamin B-12 transport into cell	0.222	0.015	C	T	0.055	2.11×10^{-48}	112
rs3742801	*ABCD4*	May be involved in intracellular processing of vitamin B-12 and the lysosomal release of vitamin B-12 into the cytoplasm	0.053	0.007	T	C	0.294	2.28×10^{-13}	52
rs2270655	*MMAA*	Involved in translocation of vitamin B-12 into the mitochondria	0.099	0.015	G	C	0.941	5.68×10^{-12}	46
rs12272669	*MMACHC*	Possible role in binding and intracellular trafficking of vitamin B-12	0.510	0.086	A	G	0.0022	1.5×10^{-9}	35
rs34324219	*TCN1*	Facilitate transport of vitamin B-12 into cell	0.235	0.011	C	A	0.881	2.54×10^{-109}	492
rs7788053	*FUT6*	Golgi stack membrane protein	0.050	0.007	A	G	0.254	1.04×10^{-10}	40
rs602662	*FUT2*	Golgi stack membrane protein	0.171	0.007	A	G	0.596	8.15×10^{-138}	623
rs1801222	*CUBN*	Receptor for intrinsic factor–vitamin B-12 complex	0.119	0.007	G	A	0.593	7.24×10^{-74}	329
rs41281112	*CLYBL*	May be involved in vitamin B-12 metabolism	0.181	0.015	C	T	0.948	4.60×10^{-34}	147
rs1141321	*MUT*	Vitamin B-12 dependent enzyme	0.070	0.007	C	T	0.627	5.11×10^{-25}	105

[1]The statistics are derived from linear regression of $n = 45{,}576$ individuals. The effect allele is the allele associated with increased serum vitamin B-12 concentrations. The effect is the mean effect of the increaser allele estimated on a quantile normalized scale. *ABCD4*, ATP-binding cassette subfamily D, member 4; *CD320*, CD320 molecule; *CLYBL*, citrate lyase β-like; *CUBN*, cubilin; EAF, effect allele frequency; *FUT*, fucosyltransferase; *MMAA*, methylmalonic aciduria (cobalamin deficiency) CblA type; *MMACHC*, methylmalonic aciduria (cobalamin deficiency) CblC type, with homocystinuria; *MUT*, methylmalonyl-CoA mutase; SNP, single nucleotide polymorphism; *TCN*, transcobalamin.

exposure. MR Egger also lacks statistical power relative to the IVW MR approach.

We performed tests of heterogeneity in causal effect estimates across SNPs (i.e., in the absence of horizontal pleiotropy all SNPs should produce identical estimates of the causal effect subject to sampling variation). If we can show consistency in causal effect estimates across a wide range of approaches that depend on different sets of assumptions, then we can be increasingly confident in the validity of our causal interpretations.

This trial was registered at http://www.isrctn.com/ISRCTN47414943 as ISRCTN47414943.

RESULTS

Table 3 shows the results of our IVW MR analysis of vitamin B-12 against all of our outcome variables both with and without inclusion of the *FUT2* variant. We found no causal relation between vitamin B-12 and most cardiometabolic outcomes. We found some evidence of a positive causal relation between vitamin B-12 and fasting glucose ($P = 0.05$) and a negative causal relation between vitamin B-12 and homeostatic model assessment of β cell function (HOMA-B) ($P = 0.01$) without *FUT2* in the analysis. The evidence for a causal relation between fasting glucose and vitamin B-12 became weaker with *FUT2* in the analysis. All of the instruments were strong, with F statistics ranging from 35 to 623.

Sensitivity analysis was performed with the use of MR Egger regression (**Supplemental Table 1**) and the weighted-median estimator (**Supplemental Table 2**). These analyses were consistent with the results from the IVW analysis. For fasting glucose and HOMA-B, the effect sizes (i.e., the β coefficients) were similar across the different methods and did not depend on inclusion or exclusion of the SNP rs602662 (*FUT2*) (Table 3, Supplemental Tables 1 and 2), although they were only nominally significant ($P < 0.05$) in the weighted-median MR analysis. No strong evidence of heterogeneity (**Supplemental Table 3**) or directional pleiotropy (**Supplemental Table 4**) was found for these outcomes, although it must be acknowledged that both of

TABLE 2
Overview of outcomes with study details[1]

Outcome	Consortium	Study	*n*	Population
Glucose measures				
Fasting glucose	MAGIC	Dupuis et al. (28)	46,186	European ancestry
Fasting blood insulin	MAGIC	Dupuis et al. (28)	38,238	European ancestry
HOMA-IR	MAGIC	Dupuis et al. (28)	37,037	European ancestry
HOMA-B	MAGIC	Dupuis et al. (28)	36,466	European ancestry
HbA1c	MAGIC	Soranzo et al. (29)	35,920	European ancestry
Lipid measures				
Total cholesterol	GLGC	Global Lipids Genetics Consortium (30)	188,577	Primarily European ancestry
LDL cholesterol	GLGC	Global Lipids Genetics Consortium (30)	188,577	Primarily European ancestry
HDL cholesterol	GLGC	Global Lipids Genetics Consortium (30)	188,577	Primarily European ancestry
Triglycerides	GLGC	Global Lipids Genetics Consortium (30)	188,577	Primarily European ancestry
Anthropometric measures				
BMI	GIANT	Locke et al. (31)	339,224	Primarily European ancestry
Waist-to-hip ratio	GIANT	Shungin et al. (32)	224,459	Primarily European ancestry
Leptin		Kilpelainen et al. (33)	32,161	European ancestry
Body fat		Lu et al. (34)	100,716	Primarily European ancestry
Disease				
Coronary artery disease	CARDIOGRAM	Schunkert et al. (35)	86,995	European ancestry
Type 2 diabetes	DIAGRAM	Morris et al. (36)	149,821	Primarily European ancestry

[1]CARDIOGRAM, Coronary Artery Disease Genome wide Replication and Meta-analysis; DIAGRAM, Diabetes Genetics Replication And Meta-analysis; GIANT, Genetic Investigation of ANthropometric Traits Consortium; GLGC, Global Lipids Genetics Consortium; HbA1c, glycated hemoglobin; HOMA-B, homeostatic model assessment of β cell function; MAGIC, Meta-Analyses of Glucose and Insulin-related traits Consortium.

these tests lack power in our study. Bowden's I^2_{GX} was >0.97 for all SNP combinations in the MR Egger analyses suggesting little impact from measurement error or weak instrument bias (Supplemental Table 1).

Results for the individual SNPs (**Supplemental Table 5–8**) showed some evidence that the results for fasting glucose were driven primarily by variants in the methylmalonyl-CoA mutase (*MUT*) and ATP-binding cassette subfamily D, member 4 (*ABCD4*), genes, whereas the HOMA-B results seemed more consistent across SNPs. Results for the other variables were either not significant or highly inconsistent across the different analyses. For example, weighted-median analyses provided some evidence for a positive causal effect of vitamin B-12 on LDL and total cholesterol (Supplemental Table 2). However, this result depended totally on the inclusion of the variant at *FUT2* (rs602662). Heterogeneity (Supplemental Table 3) and

TABLE 3
Results of IVW analysis[1]

	Without *FUT2*				With *FUT2*				
Outcome	IVs, *n*	β[2]	SE	*P*	IVs, *n*	β[2]	SE	*P*	Individuals, *n*
Glucose measures									
Fasting glucose	6	0.039*	0.020*	0.05*	7	0.032*	0.017*	0.06*	46,186
Fasting blood insulin	6	−0.015	0.014	0.28	7	−0.017	0.012	0.18	38,238
HOMA-IR	6	−0.010	0.016	0.53	7	−0.014	0.013	0.29	37,037
HOMA-B	6	−0.034*	0.012*	0.01*	7	−0.033*	0.011*	1.81×10^{-3}*	36,466
HbA1c	6	0.018	0.012	0.13	7	0.009	0.010	0.41	35,920
Lipid measures									
Total cholesterol	5	0.006	0.030	0.85	6	0.051	0.041	0.21	188,577
LDL cholesterol	5	0.009	0.027	0.73	6	0.048	0.036	0.18	188,577
HDL cholesterol	5	0.024	0.018	0.18	6	0.010	0.017	0.58	188,577
Triglycerides	5	−0.008	0.028	0.77	6	0.026	0.034	0.44	188,577
Anthropometric measures									
BMI	6	0.014	0.017	0.40	7	−0.003	0.019	0.86	339,224
Waist-to-hip ratio	4	0.055	0.036	0.13	5	0.042	0.025	0.10	224,459
Leptin	6	0.034	0.023	0.14	7	0.029	0.020	0.14	32,161
Body fat	7	0.013	0.019	0.48	8	0.008	0.016	0.63	100,716
Disease									
Coronary artery disease	6	0.001	0.110	1.00	7	−0.004	0.087	0.96	86,995
Type 2 diabetes	6	−0.043	0.156	0.78	7	−0.047	0.121	0.70	149,821

[1]*$P < 0.05$ (IVW analysis). *FUT2*, fucosyltransferase 2; HbA1c, glycated hemoglobin; HOMA-B, homeostatic model assessment of β cell function; IV, instrumental variable; IVW, inverse variance weighted.
[2]Estimated causal effect of 1-SD rank-transformed increase in vitamin B-12.

single SNP analyses (Supplemental Table 6) strongly suggested that this variant might exert pleiotropic effects on LDL and total cholesterol. Consistent with this interpretation, MR Egger regression and analyses not including this variant provided no evidence of a positive causal relation between vitamin B-12 and these variables.

DISCUSSION

This 2-sample MR study found no evidence of a causal relation between vitamin B-12 and BMI, waist-to-hip ratio, serum leptin concentrations, body fat, fasting insulin, HOMA-IR, glycated hemoglobin, coronary artery disease (CAD), T2D, or HDL, LDL, or total cholesterol. However, we found a suggestive causal relation between vitamin B-12 and fasting glucose and HOMA-B in a normoglycemic population, although the evidence for fasting glucose was weak and primarily driven by variants in the *MUT* and *ABCD4* genes. Causal effect size estimates were consistent across the different statistical methods and did not depend on whether or not the pleiotropic variant at *FUT2* was included in the analysis.

In observational studies, vitamin B-12 has been associated with cardiovascular disease (12–14), lipid profiles (11), obesity (8, 39), and diabetes (10). However, our findings suggest that these observational associations may be due to confounding or bias. Our finding of no causal relation between vitamin B-12 and BMI is supported by a Danish MR study, which showed that the relation between vitamin B-12 and BMI disappeared when the pleiotropic variant in the *FUT2* gene was left out of the analysis (2). Another MR study found an effect of vitamin B-12 on LDL cholesterol and triglycerides, but not on HDL cholesterol or CAD (22). Although our study supports previous findings that vitamin B-12 has no causal effect on CAD or HDL cholesterol, our results suggest that previous findings that reported a positive causal effect of vitamin B-12 on LDL and total cholesterol may have been driven by pleiotropy at the *FUT2* variant. Removal of this variant from analyses obviated any evidence of a causal association between vitamin B-12 and LDL and total cholesterol.

Vitamin B-12 is involved in many crucial processes in the body, such as regulating the availability of methyl groups for methylation reactions, including DNA methylation, methylation of histones (40), and DNA synthesis (1). It has been shown that vitamin B-12 deficiency can alter DNA methylation (41, 42) and histone modifications (43, 44), and it has been suggested that these epigenetic modifications can account for some of the epidemiologic associations that are observed in relation to vitamin B-12 deficiency (45). One potential mechanism could be an effect of vitamin B-12 on cell cycle and proliferation of pancreatic β cells, resulting in improved insulin secretion among individuals with higher vitamin B-12 concentrations. Although speculative at this point, this result is consistent with our finding that vitamin B-12 is causally related to HOMA-B [a measure of β cell function estimated from fasting glucose and insulin levels (46)].

Methodologic advantages and concerns

Our study has several strengths. First, the MR approach is not subject to confounding and reverse causation, enabling us to test potential causal relations previously found in conventional observational studies.

Second, our instrumental variables explain a moderate proportion of the variation in vitamin B-12 concentrations and our instruments are strong. Weak instrument bias is an important consideration in all MR analyses, including the 2-sample MR that we performed. Each of the vitamin B-12 SNPs used explains a relatively small proportion of the variance in vitamin B-12, but they are all robustly associated with the trait and their effect estimates have been calculated in a large study sample. The F statistics of each of the SNPs from our 2-sample MR study range from 35 to 623, well above $F > 10$ that is widely used in the field to denote a strong instrument (47). In the case of the standard IVW MR analysis we can therefore conclude that weak instrument bias is unlikely to be an issue. In the case of MR Egger regression analysis, the F statistics from the individual markers are not a sufficient indicator of instrument strength. Rather, Bowden et al. (27) showed that an I^2_{GX} statistic can be used to quantify weak instrument bias in MR Egger studies. The authors showed that a high value of I^2_{GX} (i.e., close to 1) suggests that the instrument effect sizes are estimated well and that measurement error or weak instrument bias is unlikely to materially affect the results of standard MR Egger analyses. Our results show high I^2_{GX} (>0.97) and we are therefore confident that our instruments are not weak and that our results do not suffer from weak instrument bias.

The 2-sample MR approach further allows for large sample sizes because it makes it possible to use summary statistics from published GWASs. Our sample sizes are 3- to 10-fold higher than many previous studies, resulting in better statistical power to address these questions. Furthermore, the increase we obtain in statistical power by using the 2-sample MR approach should not adversely affect the accuracy of our analyses, because previous studies have shown high concordance in results from 2-sample MR analyses using GWAS summary data compared with individual-level data (48, 49). In addition, by performing analyses with and without the *FUT2* variant (rs602662), we controlled for potential pleiotropic effects due to this SNP. We also performed a range of sensitivity analyses with the use of MR Egger and weighted-median approaches to gauge the consistency of our results, although we acknowledge that these methods work best with more SNPs than we had available in our analyses.

Our study also has some limitations. First, the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) included only healthy individuals without diabetes and with a fasting glucose value <7 mmol/L. As such, the effect of vitamin B-12 on the full range of fasting glucose and HOMA-B could not be analyzed. Likewise, the Global Lipids Genetics Consortium (GLGC) excluded individuals known to be taking lipid-lowering medications. Hence, we were not able to test the effect of vitamin B-12 on the full range of lipid values, which could lead to potential bias.

Second, the positive results for fasting glucose may be driven by 2 SNPs (i.e., in the genes *ABCD4* and *MUT*). It would be useful to repeat these analyses when the number of known variants associated with vitamin B-12 increases to be sure that they represent a general effect of genetically increasing vitamin B-12 concentrations as opposed to a pleiotropic effect specific to these 2 variants.

We also note that we performed a considerable number of statistical tests in this study and although, to our knowledge,

this is currently the largest MR study on the association between vitamin B-12 and cardiometabolic outcomes, the relation between vitamin B-12 and fasting glucose and HOMA-B would not be significant if we had corrected for multiple testing. It will therefore be important to replicate our results in large independent data sets when they are available in the future.

Conclusions

We found no evidence for a strong causal relation between vitamin B-12 and BMI, waist-to-hip ratio, serum leptin concentrations, body fat, fasting insulin, HOMA-IR, glycated hemoglobin, CAD, T2D, or HDL, LDL, or total cholesterol. This implies that increasing serum concentrations of vitamin B-12 through dietary changes in vitamin supplementation is unlikely to have major effects on these traits. Our results suggest that previously reported associations between vitamin B-12 and cardiometabolic traits from observational studies may be a product of bias, confounding, or chance. However, we did find some evidence for a causal effect of vitamin B-12 on fasting glucose and HOMA-B in normoglycemic individuals. The causal relation between vitamin B-12 and fasting glucose and HOMA-B should be replicated and further tested in a population that includes hyperglycemic individuals before final conclusions are drawn.

The authors' responsibilities were as follows—G-HM: conducted the research and analyzed the data; G-HM, DME, and CS: wrote the manuscript; and all authors: designed the research, and read and approved the final manuscript. None of the authors had a conflict of interest related to this study.

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