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Genetically proxied vitamin B12 and homocysteine in relation to life course adiposity and body composition

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ABSTRACT

Objective: Observational studies explore the association between vitamin B12 and obesity. However, causality is not reflected by such observations. We performed a bi-directional Mendelian randomization (MR) study to elucidate the causal relationship of vitamin B12 and homocysteine (Hcy) with life course adiposity and body composition.

Methods: Two-sample MR analysis was conducted. Independent genetic variants associated with vitamin B12 and Hcy from large-scale genome-wide association studies (GWASs) were utilized as genetic instruments, and their causal effects on five life course adiposity phenotypes (birth weight, body mass index (BMI), childhood BMI, waist circumference, waist-to-hip ratio) and three body compositions (body fat mass, body fat-free mass, body fat percentage) were estimated from UK Biobank, other consortia, and large-scale GWASs. The inverse variance weighting (IVW, main analysis), bi-directional MR, and other six sensitivity MR analyses were performed.

Results: Genetically proxied higher vitamin B12 concentrations were robustly associated with reduced BMI (Beta = −0.01, 95% confidence interval (CI) −0.016 to −0.004, $P = 7.60E-04$), body fat mass (Beta = −0.012, 95%CI -0.018 to −0.007, $P = 1.69E-05$), and body fat percentage (Beta = −0.005, 95%CI -0.009 to −0.002, $P = 4.12E-03$) per SD unit by IVW and other sensitivity analyses. Stratification analysis showed that these results remained significant in females and at different body sites (all $P < 0.05$ after Bonferroni correction). Bi-directional analyses showed no reverse causation.

Conclusions: This study provides strong evidence for the causal effect of vitamin B12 on adiposity. This gives novel clues for intervening obesity in public health and nutrition.

1. Introduction

Vitamin B12 has been explored as having influences on many health and disease outcomes, including obesity, cognitive impairment, and metabolic-related outcomes [1–3]. Vitamin B12, mainly in two forms in humans: 5′-deoxyadenosylcobalamine and methyl cobalamin, is reported to be involved in one-carbon metabolism, which presents a process where folate transfers 1-carbon units to participate in a wide range of biological processes encompassing DNA synthesis and methylation [4]. Vitamin B12 interacts with folate as methyl donors in this network, and a lack of either folate or vitamin B12 can contribute to an elevated circulating level of the relevant metabolite, homocysteine (Hcy) [4], which has been indicated as an independent risk factor for obesity [5–7]. Given that obesity has emerged as a very severe global health problem [8], a better understanding of its causes and consequences seems worthwhile. Partially motivated by the joint associations between vitamin B12 deficiency, increased Hcy levels, and obesity, numerous studies have assessed the relationship between vitamin B12 status and obesity, whereas there appears to be inconsistent on this issue in the literature [2,9–11]. For inconsistency in different studies, a variety of limitations, including selection bias, confounding factors, potential reverse causality, and residual confounding, were unable to be fully explained by observational studies, so controversial results deriving from observational studies could not supply the causal inference in the association between vitamin B12 concentrations and obesity.

Because of the shortcomings of the observational studies, it is tough to estimate whether associations are causal or explained by residual confounding. Understanding whether associations of vitamin B12

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concentrations with obesity are biased by reverse causation, as well as comprehending whether there exists a bidirectional effect, is crucial in itself. Notably, the Mendelian randomization (MR) approach used in this study should be able to circumvent these issues. MR is a causal inference method that utilizes germline genetic variants correlated with potentially exposures as instruments to evaluate causal effects on outcomes. MR is less vulnerable to biases induced by conventional observational analyses, such as confounding and reverse causation, in spite of there are some assumptions that are able to produce biased estimates when violated [12,13].

On the basis of a study of elderly twins, genetic variants account for an extensive component of the heritability of B vitamin levels, with evaluations of 59% and 66% for vitamin B12 and Hcy, respectively [14]. Hence, identification of genetic variants that influence circulating levels of B vitamins could give more perspectives on the interplay of genetics and human health. Genome-wide association studies (GWASs) of serum vitamin B12 and blood Hcy have detected and replicated several single nucleotide polymorphisms (SNPs) associated with these biomarkers [15,16]. The related genetic variants are able to be utilized as instruments to infer biomarker concentrations, as well as examination of their associations with obesity can help strengthen the causal inference [2,17].

Obesity, usually defined by the body mass index (BMI), has been widely applied in clinical practice and epidemiological research [18]. However, BMI has been argued for its limited ability to distinguish between fat mass and fat-free mass [18]. Individuals with the same BMI perhaps have crucial differences in body components as well as in regional fat distribution, which results in various health risks [19]. Thus, a series of measurements of obesity, including waist circumference (WC), waist-to-hip ratio (WHR), body fat mass, and body fat-free mass, may comprehensively reflect different morphological characteristics and fat distributions of the body.

In the present study, we hypothesize that vitamin B12 and Hcy concentrations have distinct causal associations with life course adiposity and body composition. We conducted bi-directional MR analyses by use of powerful genetic instruments from multiple consortiums and summary statistics from large-scale GWASs in European populations to explore the causal effects of vitamin B12 and Hcy on 5 life course adiposity phenotypes (Birth weight, childhood BMI, WC, WHR, adult BMI) and 3 body compositions (body fat mass, body fat-free mass, body fat percentage).

2. Methods

2.1. Study design

We conducted a two-sample MR study based on summary statistics of genome-wide association analyses on vitamin B12 levels [15], Hcy concentrations [16], life course adiposity (birth weight, childhood BMI, WHR (waist-to-hip ratio) adjusted for BMI, waist circumference adjusted for BMI, and BMI), and body composition (body fat percentage, body fat mass, and body fat-free mass) from published large-scale GWASs [20–22]. Data sources are from diverse studies and consortia encompassing the UK Biobank study, the GIANT consortium, the InCHIANTI study, and so on (Supplementary Table 1). We first computed genetic correlations of vitamin B12 and Hcy concentrations with life course adiposity and body composition. Then, a forward MR analysis was performed to estimate the causal relationships of genetically proxied higher vitamin B12 and Hcy levels with life course adiposity and body composition. Then, in case that adiposity might have an impact on vitamin B12 and Hcy levels through the inflammatory reaction and related pathways [23], a reverse MR analysis was conducted to evaluate the associations of genetic liability to life course adiposity and body composition with vitamin B12 and Hcy concentrations. Furthermore, as gender and body sites are vital considerations for obesity, we further carried out hierarchical analysis stratified by gender and body sites, together with the bioinformatics analysis, to give plausible explanations for the significant results. Fig. 1 shows the specific flow chart for the study design. The cited GWASs comprising related review boards, approved all the studies. No necessity for ethnic approval because of the summary-level data in this MR analysis.

2.2. Data sources of vitamin B12 and Hcy

According to the literature on exposures to serum vitamin B12 (n = 45,576) [15], and blood Hcy (n = 44,147) [16], SNPs that are correlated with the corresponding exposures at the genome-wide significant level (p value < 5E-08) were adopted as the candidate genetic instruments, respectively. Then, we evaluated the linkage disequilibrium (LD) for these SNPs for each exposure with the PLINK clumping approach based on the 1000 Genome European reference panel as the subjects in these GWASs are mainly from European origin. Meanwhile, we also eliminated palindromic variants with equivocal strands. Consequently, 14 independent SNPs without LD ($r^2 < 0.01$) were found and performed as alternative instruments for vitamin B12 and Hcy, respectively. The instruments that we conducted account for approximately 6.0% of phenotypic variance for vitamin B12 and Hcy, respectively, and have been used in previous MR studies [24,25]. Given the possibility that horizontal pleiotropic effects consisted in these instruments, namely, the chosen SNPs of vitamin B12 and Hcy are associated with the outcome across other paths besides vitamin B12 and Hcy, we tested the pleiotropy for these instruments through the internet resource (PhenoScanner V2) [26]. As a result, five SNPs (rs7130284, rs2275565, rs548987, rs1047891, and rs1801222) were found to be associated with other phenotypes and might own pleiotropic effects (Supplementary Table 2), and they were removed, resulting in 13 independent SNPs for vitamin B12 and 9 independent SNPs for Hcy as instruments eventually (Supplementary Table 3). As exposures, vitamin B12 and Hcy were changed to one standard deviation (SD) unit for standardization. We calculated F-statistics for vitamin B12 and Hcy for testing the strength of genetic instruments. F-statistics was much larger than 10 (Supplementary Table 4), suggesting supportive evidence for the strength of genetic instruments. Summary-level data of vitamin B12 and Hcy levels in the reverse MR analysis were acquired from the BioVU and InCHIANTI study [27,28] as insufficient SNPs correlated with adiposity and body composition, which acted as exposures, were selected from these two GWAS meta-analysis [15,16].

2.3. Data sources of life course adiposity and body composition

Five traits of life course adiposity were involved in the subsequent analysis, including birth weight (n = 133,903), childhood BMI (n = 39,620), WHR adjusted for BMI (n = 210,082), WC adjusted for BMI (n = 231,353), BMI (referring to the adult BMI as following) (n = 454,884). Additionally, three body composition traits, including body fat mass (n = 454,137), body fat-free mass (n = 454,850), and body fat percentage were also encompassed (n = 454,633) [20–22,29].

Genetic variants for birth weight were extracted from 30 GWASs and UK Biobank [20]. Birth weight is the weight of a baby at birth, which was obtained from measurements by obstetric records, medical practitioners, interviews with the mother and adult self-report. 50 SNPs without LD ($r^2 < 0.001$) that were significantly associated with birth weight (p value < 5E-08) were identified. Genetic variants for childhood BMI were drawn from a GWAS meta-analysis with 39,620 European children aged between 2 and 10 years [21]. We identified 17 SNPs without LD ($r^2 < 0.001$) that had significant associations with childhood BMI (p value < 5E-08). Genetic instruments for all adult traits (BMI, WHR adjusted for BMI, and WC adjusted for BMI) applied in two-sample MR were extracted from the Genetic Investigation of ANthropometric Traits (GIANT) consortia and UK Biobank, with participants of self-reported European ancestry. BMI was defined as weight (in kilograms) divided by the square of height in meters. A total of 448 independent SNPs for BMI, 38 independent SNPs for WHR adjusted by BMI,

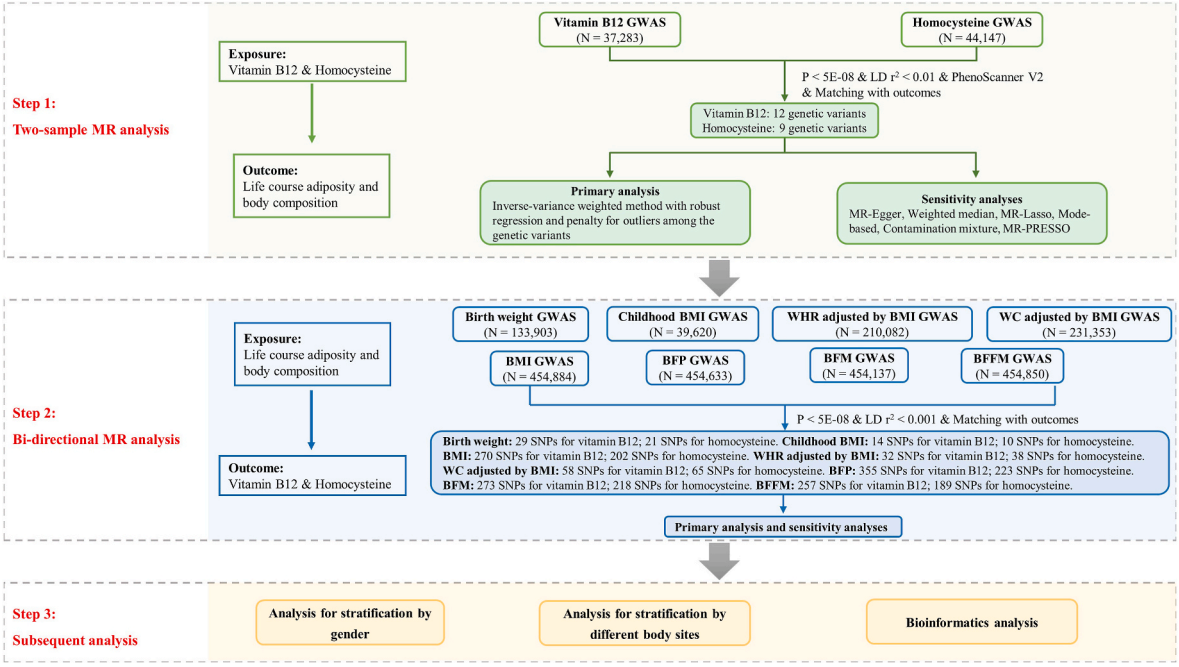

Fig. 1. Flowchart for the study design. Step 1, two-sample MR analysis was performed for vitamin B12 and homocysteine to investigate associations with life course adiposity and body composition; Step 2, bi-directional MR analysis was conducted to test the genetic associations of life course adiposity and body composition with vitamin B12 and homocysteine; Step 3, further analysis for stratification by gender, different body sites, and bioinformatics for the above significant results. GWAS, genome-wide association study; IVW, inverse variance weighted; MR, Mendelian randomization; BMI, body mass index; WHR, waist-to-hip ratio; WC, waist circumference; BFP, body fat percentage; BFM, body fat mass; BFFM, body fat-free mass.

65 independent SNPs for WC adjusted for BMI reached genome-wide significance (p value < 5E-08). Body composition from UK Biobank participants was estimated using the Tanita BC418MA body composition analyzer. The specific information of the GWAS pipeline for the full UK Biobank (version 3, March 2018) genetic statistics can be obtained at https://data.bris.ac.uk/data/dataset/pnoat8cxo0u52p6ynfaekeigi.

GWASs were performed using linear mixed model (LMM) association approach as implemented in BOLT-LMM (v2.3), adjusting for sex and genotyping array. We screened out 435, 556, and 395 instrumental variables for body fat mass, body fat-free mass, and body fat percentage, respectively, using the parameters (p value < 5E-08, LD r^2 < 0.001). Supplementary Table 6 displayed all the acquired genetic instruments of life course adiposity and body composition used to infer causal relationships. We applied MR Steiger directionality test to compute R2 values (variance of exposure explained by the instruments), and F statistics were also adopted to evaluate the strength of relationships between instrumental variables and adiposity and body composition (Supplementary Table 7) [30].

2.4. Mendelian randomization methods

One of the primary assumptions of MR defines that the genetic instruments should only be correlated to the outcome of interest across the instrumented exposure, namely, a lack of horizontal pleiotropy [31]. Horizontal pleiotropy is usual and may exist when a genetic variant influences the exposure and the outcome through independent mechanisms (uncorrelated pleiotropy) or by means of a shared heritable course or pathway (correlated pleiotropy) [32]. The present study adopted the complementary approaches of two-sample MR, which makes diverse assumptions about horizontal pleiotropy.

Generally, inverse variance-weighted (IVW) approach was used as the main analysis, whereby a combined evaluation of the causal effect from every variant is acquired from the slope of a regression via the weighted instrument-mean exposure versus instrument -mean outcome associations, with the regression line restricted to having an intercept of zero [33]. This study employed IVW with robust regression using the function "lmrob" from the R package "robustbase", rather than standard linear regression (lm), in combine with a penalty applied to the weights to down-weight the contribution of genetic variants with outlying ratio estimates to the analysis. Apart from IVW method, weighted median [34] affords some horizontal pleiotropy of any kind; MR-Egger [35] and MR-PRESSO [36] (Mendelian Randomization Pleiotropy RESidual Sum and Outlier) afford for directional uncorrelated pleiotropy; contamination mixture method [37] takes uncorrelated and correlated pleiotropy into consideration simultaneously; the mode-based estimate [38] and MR-Lasso method [39] could provide a consistent result even if the most of the instruments are invalid.

In the present analyses, IVW, weighted median, mode-based estimate, MR-Egger, contamination mixture, and MR-Lasso, as well as estimates for directional horizontal pleiotropy through Egger-intercept were performed using R package "MendelianRandomization" version 0.5.1 [40] with default parameters (LD r^2 > 0.8 for proxy genetic variants if available, and minor allele frequency ≤0.3 for strand aligning for palindromic variants when necessary). MR-PRESSO was carried out using the MR-PRESSO R package version 1.0 (https://github.com/rondolab/MR-PRESSO). We set the number of distributions at 10,000 and the threshold at 0.05. We gave a corrected estimate by excluding the outliers if the p value for the MR-PRESSO global test was lower than 0.05 and also provided a distortion test, which evaluated whether the results with or without outliers were similar.

2.5. Bi-directional Mendelian randomization

We performed bi-directional MR analyses to investigate whether there existed any evidence for causal effects of life course adiposity and body composition on vitamin B12 and Hcy. For every trait, variants with p value < 5E-08, LD r^2 < 0.001 (clumping window 10,000 kb) were drawn (Supplementary Table 6). Similarly, IVW, weighted median, MR-Egger, MR-Lasso, mode-based, contamination mixture, and MR-PRESSO were applied.

Population substructure can bias genetic instrument-outcome associations; hence, it was minimized through constricting analyses to European ancestry subjects and applying GWAS data that had been adjusted for principal components denoting different ancestral subpopulations.

As samples from GWASs of vitamin B12, Hcy, life course adiposity, and body composition were scarcely overlapped, sample overlap between each pair of exposure and outcome was negligible; therefore, analyses between these studies should not violate the independence assumption in two-sample MR.

2.6. Statistical analyses

The MR approaches were used to assess the causal effects of genetically proxied vitamin B12 and Hcy on each outcome of life course adiposity and body composition in turn, and vice versa for bi-directional MR. Each effect denotes the estimated causal beta of birth weight, childhood BMI, WHR adjusted for BMI, waist circumference adjusted for BMI, BMI, body fat percentage, body fat mass, and body fat-free mass in response to per SD increase of vitamin B12, or Hcy, respectively.

A consistent effect evaluation across the MR approaches is not likely to be a false positive. IVW was conducted as the primary analysis, weighted median, mode-based estimate, MR-Egger, contamination mixture, MR-PRESSO and MR-Lasso were performed as sensitivity analyses. We regarded the associations as significant if the directions of the estimates through these 7 methods were consistent, the IVW approach passed the Bonferroni-corrected significance level (0.05/8 = 6.25E-03), no significant pleiotropy was identified by MR-Egger (the p value of Egger-intercept term was $\geq$0.05) or p value of the MR-Egger regression denoted <0.05, and p values of more than half of sensitivity analyses were lower than 0.05.

For the significant results, we further explored the causal effects stratified by gender, or body sites for adiposity, to give more evidence about the significant findings.

All the statistical analyses were performed by R version 4.1.0 utilizing R packages "MendelianRandomization" version 0.5.1 [40] and "MRPRESSO" version 1.0 [36]. This study was performed in accordance with Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [41].

2.7. Bioinformatics analysis

For significant findings detected in the MR analysis, bioinformatics analysis was conducted to investigate the underlying biological mechanisms. First, to detect whether the instruments impose effects across modifying the expression of located genes, we carried out expression quantitative trait loci (eQTL) analysis on the basis of the GTEx v8 release (https://gtexportal.org/home/), which is composed of normalized gene expression data within whole blood tissue. In brief, the *cis*-eQTL mapping window was determined as 1 MB up- and downstream of the transcription start site. The effect sizes were denoted as the slopes of the linear regression calculated as the effect of the alternate allele compared to the reference allele [42]. Subsequently, to investigate the interactions between exposure-associated genes and outcome-associated genes, we applied STRING (https://string-db.org/) to establish a protein–protein interaction (PPI) network via utilizing the instruments located genes and the top 10 associated genes of exposure and outcome [43]. We used the GeneCards website (https://www.genecards.org/) [44] to search the top 10 associated genes of exposure and outcome. As these genes may be involved in certain biological processes, we performed the pathway enrichment analysis, which consisted of the tissue expression enrichment, the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Reactome analysis [45–47]. For multiple testing, FDR correction was implemented in the bioinformatics analysis and an adjusted p value < 0.05 was considered as a cutoff threshold.

3. Results

In MR analyses estimating causal effects of vitamin B12 and Hcy on each outcome of life course adiposity and body composition in turn and vice versa, the numbers of instruments utilized in the final analyses ranged between 7 and 12 for vitamin B12, 9 for Hcy (Supplementary Table 4), between 21 and 29 for birth weight, between 202 and 270 for BMI, between 10 and 14 for childhood BMI, between 32 and 38 for WHR adjusted for BMI, between 58 and 65 for WC adjusted for BMI, between 223 and 355 for body fat percentage, between 218 and 273 for body fat mass, and between 189 and 257 for body fat-free mass (Supplementary Table 7). F statistics ranged between 46 and 1279, which are much bigger than 10 and suggest little possibility of weak instrument bias (Supplementary Tables 4 and 7). Sample overlap between each pair of exposure and outcome was negligible (Supplementary Table 1); hence, no worry about overlapping samples between two datasets would bias the estimated causal effects in this two-sample MR.

3.1. Causal effects of vitamin B12 and Hcy on life course adiposity and body composition

We observed significant causal effects of the genetically proxied increased concentrations of vitamin B12 on decreased BMI, body fat mass, and body fat percentage (Fig. 2 and Supplementary Table 5). For 1-SD increase in the genetically proxied vitamin B12 levels, the evaluated betas were −0.01 (95%CI, −0.016 to −0.004, P = 7.60E-04) for the 1-SD BMI, −0.012 (95%CI, −0.018 to −0.007, P = 1.69E-05) for the 1-SD body fat mass, and −0.005 (95%CI, −0.009 to −0.002, P = 4.12E-03) for the 1-SD body fat percentage. Results remained directionally consistent by all the sensitivity analyses for weighted median, mode-based estimate, MR-Egger, contamination mixture, MR-PRESSO and MR-Lasso (Supplementary Table 5). We noticed that there is no pleiotropy through the MR-Egger and MR-PRESSO methods for causal effects of vitamin B12 on BMI (Egger intercept p = 0.333; MR-PRESSO global p = 0.462), BMI (Egger intercept p = 0.486; MR-PRESSO global p = 0.425), and body fat mass (Egger intercept p = 0.307; MR-PRESSO global p = 0.533) (Supplementary Table 5). Leave-one-out analysis, scatter plot, and funnel plot revealed that the significant causal effects of vitamin B12 levels on BMI, body fat mass, and body fat percentage were not affected by single SNPs associated with vitamin B12 concentrations (Supplementary Figs. 1–9). The genetically proxied vitamin B12 concentrations had no effect on other adiposity and body composition except for BMI, body fat mass, and body fat percentage (Supplementary Table 5). These results suggested that the effects of vitamin B12 on body fat mass might outweigh those of BMI. In addition, we observed no effect of genetically proxied Hcy levels with BMI, birth weight, childhood BMI, WHR adjusted by BMI, WC adjusted by BMI, body fat mass, body fat-free mass, and body fat percentage, separately (Supplementary Table 5). Sensitivity analyses gave consistent results (Supplementary Table 5).

3.2. Causal effects of adiposity and body composition on vitamin B12 and Hcy

In bi-directional MR evaluating causal effects of life course adiposity and body composition on vitamin B12 and Hcy levels, there were possibly causal paths from BMI to Hcy, and WHR to vitamin B12. We observed that the effect size of BMI in per SD change was −0.531 (95% CI, −0.936 to −0.126, P = 0.01) for 1-SD increase of Hcy concentrations, and the effect size of WHR in per SD change was 0.138 (95%CI, 0.008 to 0.269, P = 0.037) for 1-SD increase of vitamin B12 concentrations in the main analysis (Fig. 3 and Supplementary Table 8). However, after Bonferroni-correction, there was little evidence to support adiposity traits causally affecting vitamin B12 or Hcy levels. Other sensitivity MR analyses persistently indicated the lack of significant causal effect (Supplementary Table 8). No detected horizontal pleiotropy was displayed in the MR-Egger analysis (p for intercept >0.1), and no outlier

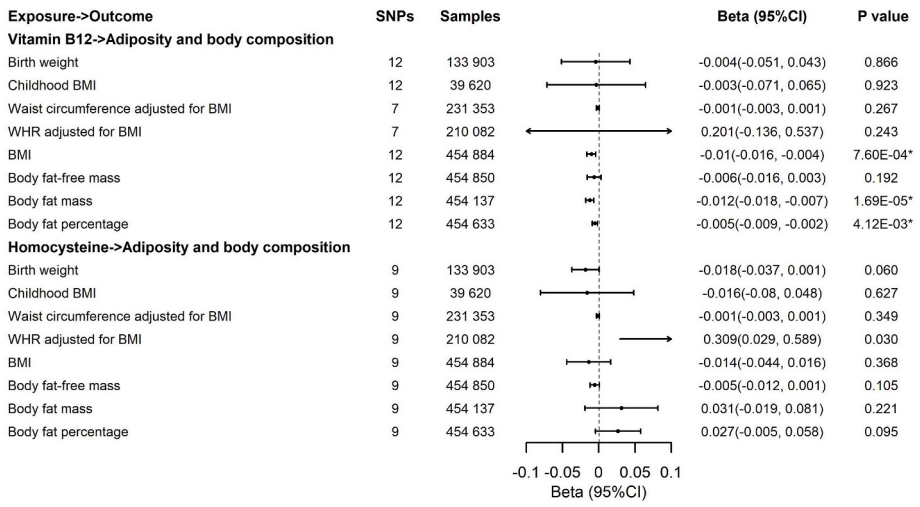

Fig. 2. Forest plots illustrating causal effects of genetically proxied vitamin B12 and homocysteine on life course adiposity and body composition by IVW. BMI, body mass index; CI, confidence interval; IVW, inverse-variance weighted method with robust regression and penalty for outliers among the genetic variants; SNP, single-nucleotide polymorphism; *The P-value reached the significant level after Bonferroni correction.

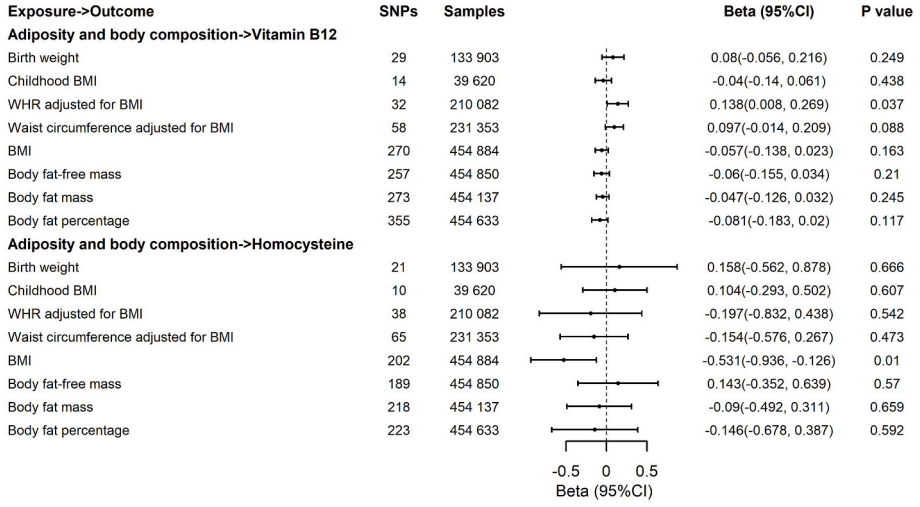

Fig. 3. Forest plots elucidating causal effects of genetically proxied life course adiposity and body composition on vitamin B12 and homocysteine by IVW. BMI, body mass index; CI, confidence interval; IVW, inverse-variance weighted method with robust regression and penalty for outliers among the genetic variants; SNP, single-nucleotide polymorphism.

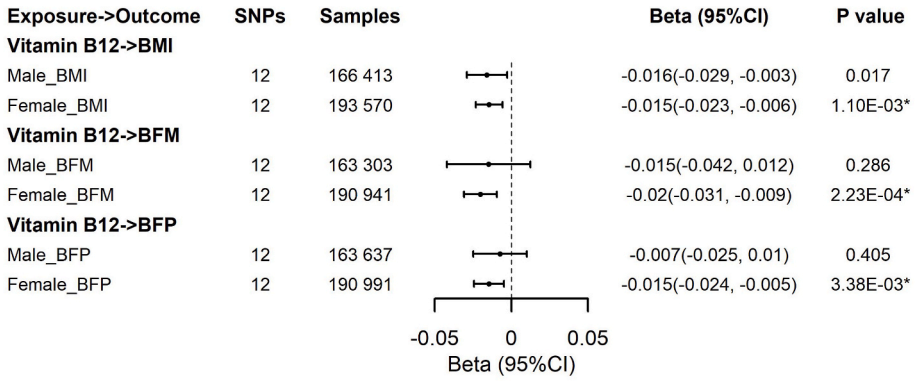

Fig. 4. Forest plots illustrating causal effects of genetically proxied vitamin B12 on BMI, BFM, and BFP stratified by gender by IVW. BMI, body mass index; BFM, body fat mass; BFP, body fat percentage; CI, confidence interval; IVW, inverse-variance weighted method with robust regression and penalty for outliers among the genetic variants; SNP, single-nucleotide polymorphism; *The P-value reached the significant level after Bonferroni correction.

was identified during the MR-PRESSO analysis (Supplementary Table 8).

3.3. Analysis for stratification by gender

For the significant causal effects of vitamin B12 on BMI, body fat mass, and body fat percentage, we further explored the causal effects through population stratification by gender and different body sites for body fat mass. The causal effects of genetically proxied vitamin B12 on BMI, body fat mass, and body fat percentage in different gender are shown in Fig. 4. We observed the significant causal effects of genetically proxied elevated vitamin B12 on lower BMI in female (Beta, −0.015; 95%CI, −0.023 to −0.006; $P = 1.10E-03$), and in male (Beta, −0.016; 95%CI, −0.029 to −0.003; $P = 0.017$), lower body fat mass in female (Beta, −0.02; 95%CI, −0.031 to −0.009; $P = 2.23E-04$), and lower body fat percentage in female (Beta, −0.015; 95%CI, −0.024 to −0.005; $P = 3.38E-03$). No significant effect was found in vitamin B12 with body fat mass and body fat percentage in male (Fig. 4 and Supplementary Table 9). The significant finding of causal effect of vitamin B12 on body fat mass in female kept consistent significantly by majority of sensitivity approaches, including MR-Lasso, weighted median, mode-based, contamination mixture, MR-PRESSO (Supplementary Table 9). Other results remained directionally consistent by all the sensitivity analyses (Supplementary Table 9). No pleiotropy is existed across the MR-Egger and MR-PRESSO approaches for causal effects of vitamin B12 on BMI, body fat mass, body fat percentage stratified by gender (Egger intercept $p > 0.08$; MR-PRESSO global $p > 0.5$) (Supplementary Table 9).

3.4. Analysis for stratification by different body sites

The causal effects of genetically proxied vitamin B12 on body fat mass in different body sites are shown in Fig. 5. There were causal paths from vitamin B12 to body fat mass in different body sites. The effect sizes of genetically proxied vitamin B12 in per SD change were −0.012 (95% CI, −0.016 to −0.008, $P = 5.61E-09$) for per SD decrease of left arm fat mass, −0.009 (95%CI, −0.013 to −0.006, $P = 1.34E-06$) for per SD decrease of right arm fat mass, −0.01 (95%CI, −0.015 to −0.005, $P = 7.10E-05$) for per SD decrease of left leg fat mass, −0.009 (95%CI, −0.013 to −0.004, $P = 4.43E-04$) for per SD decrease of right leg fat mass, and −0.011 (95%CI, −0.018 to −0.005, $P = 6.52E-04$) for per SD decrease of trunk fat mass (Fig. 5 and Supplementary Table 10). The majority of sensitivity approaches supported the significant findings of causal effects of vitamin B12 on body fat mass in different body sites (Supplementary Table 10). There was no pleiotropy through MR-Egger and MR-PRESSO methods during assessing the causal effects (Egger intercept $p > 0.1$; MR-PRESSO global $p > 0.2$) (Supplementary Table 10).

3.5. Bioinformatics analysis

By utilizing gene expression data from GTEx, we identified that 5 vitamin B12-associated genetic instruments encompassing rs1141321, rs56077122, rs34528912, rs41281112, and rs2336573 were associated with the expression of *MUT*, *CUBN*, *TCN1*, *CLYBL*, and *CD320* (Fig. 6A). The protein-protein interaction analysis detected interactions between the vitamin B12-associated genes (*MUT* and *CUBN*) and BMI, body fat mass, or body fat percentage-associated genes (*TP53*, *VDR*, *PPARG*, and *FTO*) (Fig. 6 B, C, D). Pathway analysis indicated that these genes were significantly enriched in biological process relevant to AMPK signaling pathway, propionyl-CoA catabolism, and metabolism of lipids (Supplementary Table 11), which play primary role in energy metabolism of lipids, amino acids and other molecules; therefore, alterations in these pathways may result in the imbalance of energy metabolism, as well as the development of fat accumulation. Bioinformatics analysis also revealed these genes were highly expressed in subcutaneous adipose tissue and abdominal adipose tissue. These data indicate possible mechanism of action that may underlie the inferred associations between vitamin B12 and obesity-related traits.

4. Discussion

In the present study, we have examined the genetic variants of exposures (SNPs-vitamin B12 and SNPs-Hcy) and outcomes (SNPs-birth weight, SNPs-childhood BMI, SNPs-WC, SNPs-WHR, SNPs-BMI, SNPs-body fat mass, SNPs-body fat-free mass, and SNPs-body fat percentage) from different large-scale GWASs data sources and carried out the corresponding MR analysis to explore the causal effects of vitamin B12 and Hcy concentrations on the changes of life course adiposity and body composition in turn and vice versa. The results of our study showed that higher, genetically proxied vitamin B12 concentrations had causal effects on reduced BMI, body fat mass, and body fat percentage. Additionally, through analysis for stratification by gender and distinct body sites, we found genetically proxied vitamin B12 concentrations causally affected body fat mass in different body sites as well as influenced BMI, body fat mass, and body fat percentage in females.

4.1. Public health and clinical implications

Our findings exerted a potentially substantial influence on public health and had clinical implications. Nowadays, many chronic diseases encompassing hypertension, diabetes mellitus, and cardiovascular diseases are closely associated with obesity, and being obese can strongly increase the likelihood of these diseases, which have been a serious public health issue globally [48]. Identifying valuable biomarkers that caused obesity could be greatly significant not only in understanding its developmental pathogenesis but also in affording more effective interventions to decrease the incidence of obesity. Our results indicated that elevated serum vitamin B12 caused reduced BMI, body fat mass and body fat percentage, suggesting vitamin B12 may serve as a potential biomarker for obesity, although the dysregulation of metabolism in relevant pathways may be the driver of higher concentrations of vitamin B12 in the circulation.

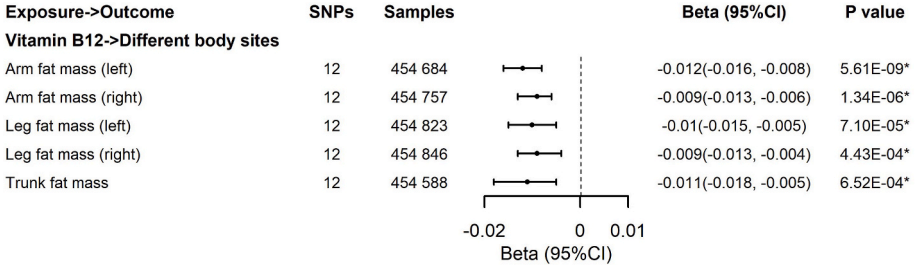

Fig. 5. Forest plots elucidating causal effects of genetically proxied vitamin B12 on body fat mass in different sites by IVW. CI, confidence interval; IVW, inverse-variance weighted method with robust regression and penalty for outliers among the genetic variants; SNP, single-nucleotide polymorphism; *The P-value reached the significant level after Bonferroni correction.

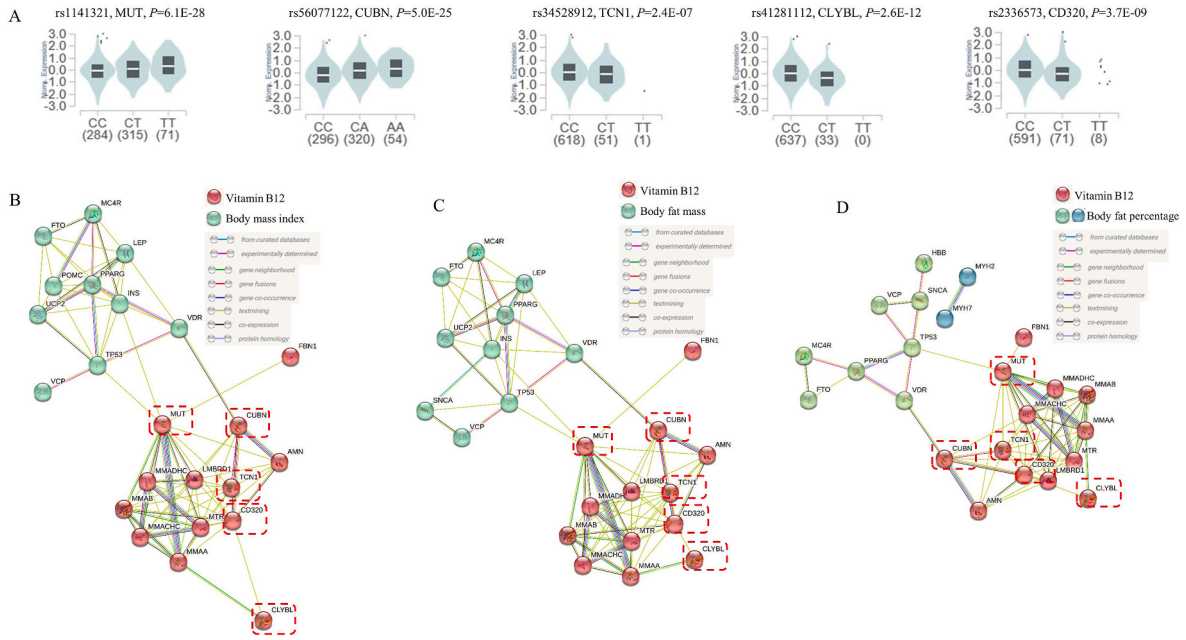

Fig. 6. Bioinformatics analysis for vitamin B12-associated instrumental variants. (A) eQTL violin plots of the associations between vitamin B12-associated SNPs and expression of located genes. (B) Diagram of the interactions between vitamin B12-associated genes and body mass index-associated genes. (C) Diagram of the interactions between vitamin B12-associated genes and body fat mass-associated genes. (D) Diagram of the interactions between vitamin B12-associated genes and body fat percentage-associated genes. CD320, CD320 molecule; CLYBL, citramalyl-CoA lyase; CUBN, cubilin; eQTL, expression quantitative trait loci; MUT, methylmalonyl-CoA mutase; SNP, single-nucleotide polymorphism; TCN1, transcobalamin 1.

In general, a better comprehension of the complex relationship between nutrients and adiposity traits is able to be helpful for individuals who struggle to keep healthy diets or a healthy weight, build up overall health, and accordingly reduce the economic burden on our health care system.

4.2. Comparison with related studies

Previous literatures concerning the association between serum vitamin B12 concentrations and obesity in adults presented controversial findings [9,49–59]. The results in present study were supported by some studies in which serum vitamin B12 concentrations had an inverse association with obesity [9,51–54,56–59]. Nevertheless, a number of the results from those mentioned studies were unable to extend to general populations as they were limited to a specific population, such as pregnant women [53,54,57,58]. To some degree, it is consistent with our significant results for females. Additionally, several studies on children and adolescents were also in support of our results [52,56]. Previously, we have created genetic statistics to identify genetic variation in complex diseases and utilized MR methods to assess the causal effects between complex disease traits, encompassing the casual effects of vitamin B12 and Hcy on obesity and related diseases [2,5,24,60–66]. Notably, the majority of the previous studies utilized BMI to define obesity. Although BMI is widely employed in clinical practice and epidemiology, some studies have manifested the phenomenon of the survival benefit of obesity, which is so-called “obesity paradox” [67]. In other words, BMI is not an ideal measurement for representing anthropometric characteristics, because it can not distinguish between body fat mass and body fat-free mass. Estimating body composition is pivotal, as fat tissues have distinct physiological properties from non-fat tissues, and they could have different effects on health [18]. Body composition examination can better predict chronic cardiovascular diseases than BMI or WC alone [68]. This study not only included BMI, but also body composition and other measurement indicators (i.e. WC and WHR), which can comprehensively and accurately describe the status of obesity. By use of MR analysis, we gave the strong evidence of plausible effects of serum vitamin B12 levels on BMI, body fat mass, and body fat percentage, especially in females.

4.3. Explanations and implications

Numerous studies have demonstrated the importance of vitamin B12 concentrations in regulating one-carbon metabolism [4,69]. In this pathway, low serum vitamin B12 concentrations would induce folate as 5-methyltetrahydrofolate, hinder the generation of methionine from Hcy, and thus decrease protein synthesis and relevant tissue deposition [70]. It is also possible that low vitamin B12 concentrations may contribute to adipocyte dysfunction through cellular inflammation [58,71]. Another possibility is that reducing dietary intake or absorption, increasing catabolism as well as sequestration in adipose tissue are the reasons for lower serum vitamin B12 levels leading to obesity [53]. In addition, alterations in the gut microbiota profiles could influence the metabolism of vitamin B12 [72]. Recently, studies in the Danish population revealed that lower serum vitamin B12 levels had a significant association with higher BMI, but a genetic risk score based on variants correlated with vitamin B12 levels revealed no association with BMI [9]. It indicated that some genotypes might influence the association between vitamin B12 levels and obesity through pleiotropic effects on gut flora [9]. The possibility of different mechanisms of vitamin B12 influence on obesity among different gender populations may be gender differences in vitamin B12 metabolism. In the *trans*-sulfuration pathway, vitamin B12 and vitamin B6 serve as cofactors to help homocysteine convert to cysteine by cystathionine-b-synthase and by cystathionine -γ-lyase. It has been indicated that women have greater flux of homocysteine through the *trans*-sulfuration pathway and thus higher concentrations of vitamin B12 [73]. In comparison, men have a higher demand for creatine synthesis because they have greater muscle mass, contributing to the lower concentrations of vitamin B12. Therefore, gender seems to be an important factor affecting vitamin B12 concentrations to the advantage of women. Higher concentrations of vitamin B12 in women may be another contributing factor to the discrepancy between women and men with respect to the risk of developing obesity.

Furthermore, we performed bioinformatics analysis to find more explanations for our findings (Fig. 6 and Supplementary Table 11). Consequently, many variants related to vitamin B12 and obesity traits were enriched in adipose tissue, and the metabolic pathways of energy, such as propionyl-CoA catabolism and the AMPK signaling pathway, were observed by KEGG and Reactome enrichment analyses. The aforementioned evidence may, to some degree, explain the possible mechanisms by which vitamin B12 causes obesity risk.

4.4. Strengths

The strengths of the present study lie in the use of MR, which could reduce residual confounding and diminish the possibility of false negatives in large-scale GWASs. To the best of our knowledge, this is the first study utilizing the bi-directional MR analysis to explore the causal effects of genetically proxied vitamin B12 and Hcy concentrations on life course adiposity and body composition in turn and vice versa by means of several large independent data sources. Furthermore, MR assumptions were totally explored with the application of additional sensitivity analyses such as MR-Egger and MR-PRESSO, the results of which give evidence for the robustness of our results. The genetic summary statistics used for all traits in the present study were acquired from the largest available GWASs while still keeping zero overlap between exposure and outcome datasets. Combining distinct non-overlapping data sources and applying more SNPs associated with exposures as instruments would guarantee the sample size, interpret the phenotypic variance, respectively, as well as ensure statistical power, even though some weak associations have been overlooked. As horizontal pleiotropy was a non-negligible factor in MR analysis, strict screening criteria were used for selecting SNPs as instruments (Supplementary Table 2) to avoid it with respect to the possible genetic intermediate effect between exposure and outcome.

4.5. Limitations

Limitations should be mentioned. The populations in this study were confined to individuals of European ancestry to decrease population structure bias, which prevented the generalization of our findings to other populations. In addition, although we were thorough in evaluation of MR assumptions employing various sensitivity analyses (weighted median, mode-based estimate, MR-Egger, contamination mixture, MR-PRESSO and MR-Lasso), we were not able to directly estimate the independence of instruments from undetectable confounding factors. Given that MR employs germline instruments, it is highly considered that these will not be impacted by confounders. Minimizing population stratification could contribute to alleviating concerns of independence assumption violation [74], but it is tough to estimate in a two-sample MR framework. Furthermore, the dose-effect relationship between serum vitamin B12 concentrations and adiposity, together with dietary intake or lifestyles, should be further explored for clinical application in the future. As previously mentioned, the causal effects of serum vitamin B12 concentrations on adiposity and body composition may differ in populations of distinct ancestries. More studies focused on subjects of non-European descent are needed. Although we took the effects of gender and body sites on adiposity and body composition into account, further studies need to explore data in separated populations based on gender and menopausal status within the different gender groups (i.e., women >55 years old) because hormonal status may be an important issue to consider.

5. Conclusion

In conclusion, the present study elucidated the causalities of genetically proxied vitamin B12 levels with reduced BMI, body fat mass, and body fat percentage, especially in females, as well as genetically proxied vitamin B12 elevation in relation to decreased body fat mass in different body sites, suggesting that serum vitamin B12 concentrations may play a crucial role in the pathogenesis and process of adiposity in distinct body regions and genders. Further studies are nevertheless needed to explore and figure out the potential mechanisms by which vitamin B12 regulates adiposity. The vitamin B12-associated genetic variants impose their effects on the expression of genes that play a crucial part in the development of the outcomes, which may be potential biological mechanisms underlying the identified causal effects.

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Author contributions

Study concept and design: JM and LF; Acquisition of data: LF, LG, HC, and XZ; Analysis and interpretation of data: LF; Drafting of the manuscript: LF and JM; Critical revision of the manuscript for important intellectual content: LF, LG, HC, XZ, and JM; Funding recipients: LF and JM.

Data availability statement

The authors thank the UK Biobank study, and the summary statistics data of UKB can be download from Neale lab (http://www.nealelab.is/uk-biobank). The authors thank other GWAS summary-level data, which have been downloaded from https://www.ebi.ac.uk/gwas. The authors thank all the reviewers and editors for their useful suggestions for improving this study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2023.102883.

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