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Clinical Research Article

Physical Activity, Sedentary Behavior, and Type 2 Diabetes: Mendelian Randomization Analysis

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Abstract

Context: The causality and pathways of the associations between physical activity and inactivity and the risk of type 2 diabetes remain inconclusive.

Objective: We conducted an updated mendelian randomization (MR) study to explore the associations of moderate-to-vigorous physical activity (MVPA) and leisure screen time (LST) with type 2 diabetes mellitus (T2DM).

Methods: Genetic variants strongly associated with MVPA or LST with low linkage disequilibrium were selected as instrumental variables from a genome-wide meta-analysis including more than 600 000 individuals. Summary-level data on T2DM were obtained from the DIAbetes Genetics Replication And Meta-analysis consortium including 898 130 individuals. Data on possible intermediates (adiposity indicators, lean mass, glycemic traits, and inflammatory biomarkers) were extracted from large-scale genome-wide association studies (n = 21 758-681 275). Univariable and multivariable MR analyses were performed to estimate the total and direct effects of MVPA and LST on T2DM. Methylation MR analysis was performed for MVPA in relation to diabetes.

Results: The odds ratio of T2DM was 0.70 (95% CI, 0.55-0.88; $P = .002$) per unit increase in the log-odds ratio of having MVPA and 1.45 (95% CI, 1.30-1.62; $P = 7.62 \times 10^{-11}$) per SD increase in genetically predicted LST. These associations attenuated in multivariable MR analyses adjusted for genetically predicted waist-to-hip ratio, body mass index, lean mass, and circulating C-reactive protein. The association between genetically predicted MVPA and T2DM attenuated after adjusting for genetically predicted fasting insulin levels. Two physical activity-related methylation biomarkers (cg17332422 in *ADAMTS2* and cg09531019) were associated with the risk of T2DM ($P < .05$).

Conclusion: The study suggests causal associations of MVPA and LST with T2DM that appear to be mediated by obesity, lean mass, and chronic low-grade inflammation.

Key Words: inflammation, mendelian randomization, obesity, physical activity, sedentary behavior, type 2 diabetes

Abbreviations: BMI, body mass index; CRP, C-reactive protein; ELPD, expected log pointwise posterior density; FG, fasting glucose; FI, fasting insulin; GWAS, genome-wide association study; HbA_{1c}, glycated hemoglobin A_{1c}; IL-1RA, interleukin-1 receptor antagonist; IL-6, interleukin-6; LD, linkage disequilibrium; LST, leisure screen time; mQTL, methylation quantitative trait loci; MR, mendelian randomization; MVPA, moderate-to-vigorous physical activity; OR, odds ratio; RCT, randomized controlled trial; SNV, single-nucleotide variation; T2DM, type 2 diabetes mellitus; WHR, waist-to-hip ratio.

Type 2 diabetes mellitus (T2DM) is a global pandemic that causes a heavy burden on the health system as well as the whole of society [1]. Population-based epidemiological studies have revealed several modifiable lifestyle factors, such as cigarette smoking, unhealthy diets, and physical inactivity, for T2DM [2], which provide evidence bases for disease prevention [3]. Regarding physical activity and sedentary behaviors, meta-analyses of observational studies found that higher levels of physical activity and lower levels of sedentary behaviors were associated with a reduced risk of T2DM [4-6]. However, whether these associations are causal remains undetermined because of potential biases, such as reverse causality and confounding, in observational studies. For the association between physical activity and diabetes risk, randomized controlled trials (RCTs) have generated conflicting evidence. A systematic review of RCTs found no firm evidence that physical activity alone compared to standard treatment influenced the risk of T2DM [7] even though some trials revealed significant associations of higher levels of physical activity with a lower risk incidence of diabetes

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and insulin resistance [8, 9]. Other trials found a consistent protective effect of lifestyle intervention on diabetic incidence; however, the intervention usually simultaneously acted on multiple lifestyle factors, like diet, physical activity, and treatments [7, 10, 11], which hinders the assessment of the independent role of physical activity in diabetes. In addition, despite RCT being regarded as the gold-standard design for causal inference, it may be confined by a short intervention period and low adherence.

Physical activity and sedentary lifestyle have been associated with obesity [12], lean mass [13], glycemic status [14], and inflammation [15], which are vital factors for the development of T2DM. Nevertheless, whether and how these factors mediate the associations of physical activity and sedentary behaviors with diabetes risk are unestablished. A clear appraisal of these pathways may benefit the development of more precise preventive and therapeutic strategies.

Mendelian randomization (MR) analysis is an epidemiological approach that can reinforce causal inference using observational genetic data [16]. Using genetic variants as an instrumental variable for an exposure (eg, moderate-to-vigorous physical activity [MVPA]), the MR approach can diminish confounding and reverse causality since germline genotypes are usually not associated with environmental or self-adopted factors and cannot be modified by the onset of disease [16]. A previous MR study examined the associations of accelerometer-based physical activity average accelerations, vigorous physical activity, and sedentary behavior with T2DM and 4 glycemic traits and found none of the associations [17]. However, findings were conflicting in another MR study, in which the associations of moderate and vigorous physical activity and television watching with T2DM were identified [18]. Epigenetics, including DNA methylation, plays an important role in human health and has been revealed to mediate the association between exercise and diabetes [19]. Therefore, we conducted this MR study based on larger data sets to 1) determine the associations of MVPA and leisure screen time (a long time presenting sedentary behavior) with the risk of T2DM; 2) explore potential pathways of these associations; and 3) explore how physical activity affects diabetes from the epigenetics perspective.

Materials and Methods

Study Design

The present study was based on publicly available summary-level genome-wide association study (GWAS) data and thus required no ethical permit. Individual studies included in the GWASs have been approved by an ethical review board. We first estimated the genetic correlations between MVPA, LST, and T2DM. We then used MR-CAUSE analysis to compare the fit of genetic sharing and causal pathways [20]. To strengthen causal inference, we conducted MR analyses to explore the associations of MVPA and LST with T2DM. To reveal potential pathways, we examined the MR associations of MVPA and LST with possible intermediates (ie, obesity, lean mass, glycemic traits, and chronic inflammation biomarkers) and further performed multivariable MR analyses adjusting for associated traits to estimate the mediation effects. To reveal possible methylation mechanisms, we also performed a DNA methylation MR analysis. T2DM was the primary outcome, and all intermediates were secondary outcomes. The MR design and its assumptions are displayed in Fig. 1.

Genetic Instrumental Variable Selection

Summary-level data for MVPA and LST were obtained from a genome-wide meta-analysis of up to 606 820 and 526 725 individuals of European ancestry, respectively [21]. MVPA and LST were measured by domain- and intensity-specific questions. MVPA was defined as a binary variable with being active (~48% of studied participants) and inactive status after harmonizing the phenotype across more than 40 included studies that used different questionnaires. Detailed definitions of MVPA in each study can be found in the original GWAS [21]. LST included time spent watching television, playing videogames, sitting at the computers, etc and was treated as a continuous variable with a mean (SD) of 3.53 (1.87) hours/day. GWAS analysis was performed in each study by sex and with adjustment for age, age [2], genetic principal components, and study-specific covariates [21].

Single-nucleotide variations (SNVs; formerly single-nucleotide polymorphisms [SNPs]) associated with MVPA or LST at the genome-wide significance threshold ($P < 5 \times 10^{-8}$) were extracted from the corresponding GWAS data set. Linkage disequilibrium (LD) of selected SNVs for each trait was calculated based on the 1000 Genomes European reference panel [22]. To increase the power of the analysis by including more genetic variants, SNVs without or in low LD (defined as $R^2 < 0.1$) were used as instrumental variables, leading to 22 SNVs for MVPA and 152 SNVs for LST. We also selected SNVs in lower LD ($R^2 < 0.01$) as supplementary instruments. SNVs for MVPA and LST were combined and pruned using the same selection criteria to select instruments for multivariable MR analysis with mutual adjustment. Selected SNVs are shown in Supplementary Table S1 [23].

We also used rs1625595 as an instrumental variable for MVPA in the supplementary analysis to minimize pleiotropy. Rs1625595 is strongly associated with MVPA ($P = 1.9 \times 10^{-11}$) and located approximately 300 kb upstream of the *ACTN3* gene that is highly expressed in skeletal muscle (GTEx; $P = 6.6 \times 10^{-5}$) [21]. A missense variant of this gene makes the α-actinin-3 filaments more flexible, resulting in low susceptibility to exercise-induced muscle damage and thereby facilitating a more active lifestyle [21].

Data Sources for Type 2 Diabetes

Summary-level data on the DNA-diabetes associations were obtained from the DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) consortium including 898 130 individuals (74 124 patient cases and 824 006 controls) of European descent with a mean age of 55 years, 51.8% of whom are men [24]. The genetic associations were adjusted for genetic principal components, relatedness, and study-specific covariates [24]. Patient cases with T2DM were defined by previous clinical diagnosis (self-reported and medical records), antidiabetic medication, and cutoff of certain glycemic biomarkers in specific studies [24].

Data Sources for Adiposity, Lean Mass, Glycemic Traits, and Inflammatory Biomarkers

Two adiposity indicators (waist-to-hip ratio [WHR] and body mass index [BMI]), lean mass, 3 glycemic traits (fasting insulin [FI], fasting glucose [FG], and glycated hemoglobin A_{1c} [HbA_{1c}]), and 3 inflammatory biomarkers (C-reactive protein [CRP], interleukin-1 receptor antagonist [IL-1RA], and interleukin-6 [IL-6]) were treated as possible intermediates

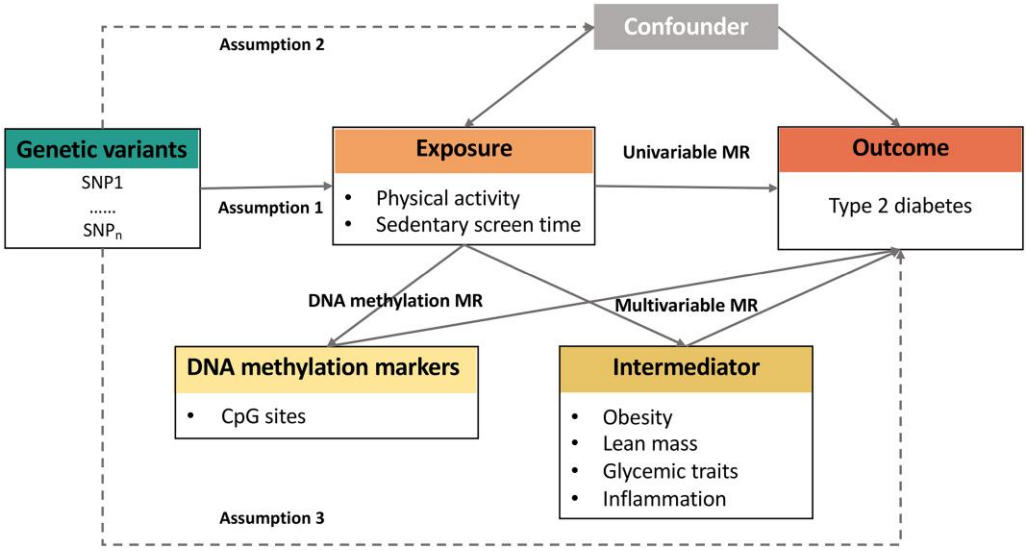

Figure 1. Multivariable mendelian randomization (MR) design overview. SNP, single-nucleotide polymorphism (now known as single-nucleotide variation [SNV]). Assumption 1 indicates that the used genetic variants as instrumental variables should be strongly associated with the exposure; assumption 2 indicates that the used genetic variants should not associated with confounders; and assumption 3 indicates the used genetic variants should affect the outcome merely via the exposure, not partly or entirely via other alternative pathways.

[25]. Summary-level data on these phenotypes were obtained from the Genetic Investigation of ANthropometric Traits (GIANT) Consortium for WHR (n = 224 459) [26] and BMI (n = 681 275) [27], a genome-wide association analysis in the UK Biobank study (n = 450 243) for lean mass measured by the bioelectrical impedance analysis approach [28], the Meta-Analysis of Glucose and Insulin-related Traits Consortium (MAGIC, n = up to 46 613) for glycemic traits [29], the Neale Lab GWAS in UK Biobank for CRP (n = 343 524), and the Systematic and Combined AnaLysis of Olink Proteins (SCALLOP) consortium (n = up to 21 758 participants) for IL-1RA and IL-6 [30].

Blood DNA Methylation Marker Identification

Blood DNA methylation markers (CpG sites) of MVPA were obtained from an epigenome-wide association study with 5513 individuals [31]. DNA methylation levels were measured by Illumina Infinium HumanMethylation450 (HM450) BeadChip array, and the MVPA-methylation associations were estimated after adjustment for age, sex, birthplace, socioeconomic status, smoking status, alcohol consumption, sample type, and estimated white blood cell composition [31]. Seven CpG sites were found to be associated with MVPA at $P < 1 \times 10^{-5}$ [31]. We further identified methylation quantitative trait loci (mQTL) that regulate the methylation levels of identified MVPA-related CpG sites based on the Accessible Resource for Integrative Epigenomic Studies Genetics of DNA Methylation Consortium (GoDMC) [32]. The Matrix eQTL software was used to test the preliminary association of SNVs with CpG sites [32]. SNVs identified at the epigenome-wide association significance level were further analyzed using exact linear regression adjusted for covariates in PLINK 1.07, and genome-wide complex trait analysis was performed to obtain the most representative independent locus in relation to each CpG site [33]. Methylation levels at MVPA-associated CpG sites were treated as the exposure, which was instrumented by their mQTLs. Given no epigenome-wide association study on LST, corresponding methylation MR analysis could not be performed.

Data Analysis

Genetic correlations were calculated using LDSC software [34]. MR-CAUSE [20] based on summary-level data was used to compare the fit of genetic sharing and causal pathways of the association between MVPA, LST, and T2DM. This method holds the assumption that the genetic relationship between 2 phenotypes is a mixture both of causal and shared correlated pleiotropy. The analysis estimates posterior distributions of a null effect, a shared effect, and a causal effect, and compares the model fit by estimating Δ expected log pointwise posterior density (ELPD) [20].

SNVs were harmonized by effect alleles and corresponding allele frequency of the exposure genetic associations. SNVs with ambiguous matches (ie, allele mismatch or palindromic pair with minor allele frequency of 0.45-0.55) between the exposure and outcome data were removed from the analysis. We used the inverse variance weighted method with multiplicative random effects as the primary method. To detect the minimize horizontal pleiotropy, 3 sensitivity analyses, including the weighted median [35], MR-Egger [35], and MR-PRESSO [36], were used. The weighted median can provide accurate estimates when more than half of the SNVs are valid [35]. MR-Egger can generate estimates after correction for possible horizontal pleiotropy detected by its embedded intercept test even though the estimates are usually underpowered [35]. MR-PRESSO can detect SNV outliers that introduce pleiotropy and generate estimates after the removal of these outlying SNVs [36]. Cochran's Q test was used to assess the heterogeneity of SNV estimates. Multivariable MR analysis with mutual adjustment was performed to minimize the mutual influence of MVPA and LST on the outcomes. Further, we performed multivariable MR adjusting for related intermediates to estimate the direct effect of MVPA and LST on T2DM, where the same sets of SNVs as those used in

univariable MR were employed due to no interest in the effects of intermediates on T2DM [16]. Multivariable MR was performed for each intermediator in turn. Mediation was calculated as the direct effect divided by the total effect and subtracted from 1. The Wald ratio method was used to estimate the methylation MR association where the odds ratio (OR) of T2DM was scaled per SD increase in MVPA-reduced methylation levels. Associations with P less than .05 were considered statistically significant. All statistical tests were 2-sided, and analyses were performed using CAUSE, TwoSampleMR, MendelianRandomization, and MR-PRESSO packages in R software (4.4.1).

Results

MVPA was inversely ($rg = -0.24$; $P = 5.54 \times 10^{-20}$) and LST positively ($rg = 0.34$; $P = 4.41 \times 10^{-55}$) correlated with T2DM. MR-CAUSE analysis revealed both genetic sharing and causal pathways of the associations of MVPA and LST with T2DM ($P < .05$) and supported that the causal pathways had a better fit ($P = 1.70 \times 10^{-6}$ for MVPA and 8.70×10^{-13} for LST; Table 1).

Associations of Moderate-to-Vigorous Physical Activity and Leisure Screen Time With Diabetes

Genetically predicted MVPA and LST were associated with the risk of T2DM (Fig. 2). The OR of T2DM was 0.70 (95% CI, 0.55-0.88; $P = .002$) per unit increase in the log-OR of having MVPA and 1.45 (95% CI, 1.30-1.62; $P = 7.62 \times 10^{-11}$) per SD increase in genetically predicted LST. The associations were stable in sensitivity analyses (see Fig. 2). High heterogeneity (Cochran's $Q = 83$ for MVPA and 409 for LST) was observed but there was no indication of horizontal pleiotropy detected by MR-Egger intercept tests ($P > .201$). Five and 6 SNV outliers were identified by MR-PRESSO analyses; however, the association persisted after removal of these outliers. The association for MVPA became stronger in the supplementary analysis using rs1625595 with few pleotropic effects as the instrumental variable (OR 0.27; 95% CI, 0.15-0.48; $P = 1.21 \times 10^{-5}$). The associations were stable in the analysis based on SNVs in LD less than 0.01 (Supplementary Table S2) [23]. The associations attenuated slight but remained statistically significant in multivariable MR analysis with mutual adjustment (see Fig. 2).

Table 1. MR-CAUSE analysis on the associations of moderate-to-vigorous physical activity and leisure screen time with type 2 diabetes

Model 1[a]	Model 2[a]	Δ ELPD[b]	SE (Δ ELPD)	Z score	P
MVPA (2349 SNVs)					
Null	Sharing	−27.69	5.27	−5.26	7.30E-08
Null	Causal	−34.81	6.73	−5.17	1.10E-07
Sharing	Causal	−7.11	1.53	−4.65	1.70E-06
P value testing indicates that causal model is a better fit: 1.7E-06					
LST (3297 SNVs)					
Null	Sharing	−73.49	9.45	−7.77	3.80E-15
Null	Causal	−81.44	10.51	−7.75	4.50E-15
Sharing	Causal	−7.95	1.13	−7.05	8.70E-13
P value testing indicates that causal model is a better fit: 8.7E-13					

Abbreviations: ELPD, expected log pointwise posterior density; LST, leisure screen time; MVPA, moderate-to-vigorous physical activity; SNV, single-nucleotide variation.
[a]Model 1 and model 2 refer to the models being compared (null, sharing, or causal).
[b]Model fit is measured by Δ ELPD and negative values indicate that model 2 is a better fit.

Associations of Moderate-to-Vigorous Physical Activity and Leisure Screen Time With Intermediators

Genetically predicted MVPA and LST were associated with some of the possible intermediators (Fig. 3). Genetically predicted MVPA was associated with lower levels of WHR (β = −0.14; 95% CI, −0.23 to −0.05; $P = .004$), FI (β = −0.12 pmol/L; 95% CI, −0.17 to −0.07; $P = 7.21 \times 10^{-6}$), and CRP (β = −0.52 mg/L; 95% CI, −0.76 to −0.28; $P = 2.54 \times 10^{-5}$), and higher levels of lean mass (β = 0.15 kg; 95% CI, 0.05-0.25; $P = .004$). One SD increase in genetically predicted LST was associated with higher levels of WHR (β = 0.21; 95% CI, 0.17-0.26; $P = 1.78 \times 10^{-18}$), BMI (β = 0.14; 95% CI, 0.11-0.18; $P = 4.19 \times 10^{-5}$), CRP (β = 0.75 mg/L; 95% CI, 0.42-1.07; $P = 7.29 \times 10^{-6}$), IL-1RA (β = 0.17 SD; 95% CI, 0.07-0.25; $P = .001$), and IL-6 (β = 0.13 SD; 95% CI, 0.03-0.23; $P = .008$), and less lean mass (β = −0.09 kg; 95% CI, −0.14–−0.05; $P = 1.48 \times 10^{-4}$). Moderate-to-high heterogeneity was observed in the associations of genetically predicted MVPA and LST with BMI, lean mass, glycemic traits, and CRP; however, there was no indication of horizontal pleiotropy (MR-Egger intercept $P > .05$) (Supplementary Table S3) [23]. These associations remained stable in sensitivity analyses (Supplementary Table S3) [23] and attenuated but were overall consistent when MVPA and LST were mutually adjusted (Fig. 3).

Direct Effects of Moderate-to-Vigorous Physical Activity and Leisure Screen Time on Diabetes and Mediation Effects

The associations of genetically predicted MVPA and LST with T2DM attenuated and became nonsignificant in multivariable MR analysis adjusted for related intermediates (Table 2). Most of the association between MVPA and T2DM was mediated by WHR (66%), followed by CRP (63%), FI (43%), BMI (22%), and lean mass (9%) (see Table 2). BMI (53%) mediated the most effect of LST on T2DM, followed by WHR (43%), CRP (27%), IL-1RA (12%), and lean mass (10%) (see Table 2). Of note, because some included mediators are genetically correlated, the total mediation percentage exceeded 100%.

Associations of Moderate-to-Vigorous Physical Activity–related DNA Methylation With Diabetes

Seven mQTLs were identified to be associated with MVPA-associated CpG sites and included in the methylation MR analysis (Table 3). Two CpG sites (cg17332422 in *ADAMTS2* and cg09531019 in an unknown gene) were associated with the risk of T2DM ($P < .05$). Per SD increase in methylation levels associated with having no MVPA, the OR of diabetes was 2.78 (95% CI, 1.02-7.69) for cg17332422 and 2.86 (95% CI, 1.05-7.69) for cg09531019.

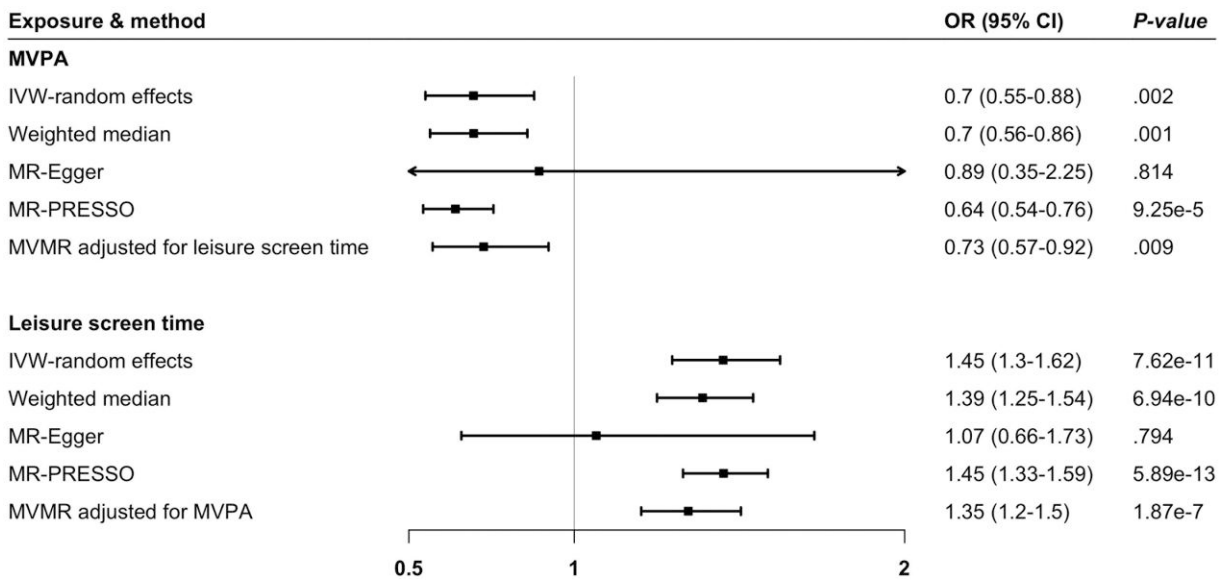

Figure 2. Associations of moderate-to-vigorous physical activity (MVPA) and leisure screen time (LST) with type 2 diabetes. OR, odds ratio.

Discussion

Using data from large-scale genetic studies, this updated MR study identified potential causal associations of MVPA and LST with T2DM. Our multivariable MR analysis revealed that adiposity, lean mass, and chronic inflammation mediated the associations. Insulin levels also appeared to partly mediate the association between MVPA and T2DM. Increased levels of DNA methylation at cg17332422 in the *ADAMTS2* gene and cg09531019 in relation to low physical activity levels were associated with a higher risk of T2DM.

Our findings are consistent with many observational associations of high levels of physical activity and low levels of sedentary behavior with reduced risk of T2DM [4-6, 37] and evidence from some RCTs [8, 9] even though a systematic review of RCTs summarized that physical activity plus diet instead of physical activity alone reduced or delayed the incidence of T2DM [7]. Our MR results strengthened the potential causality of the associations between physical activity and the risk of diabetes. Given that diabetes may be more effectively modified by multiple factors, a comprehensive recommendation including physical activity, diet, and treatments should be made in the clinical setting to the population with a high risk of diabetes [38]. Additionally, our multivariable MR analysis identified obesity as an important mediator in these associations even though some clinical trials failed to observe significant body mass change after exercise training or a combined lifestyle modification including promoting physical activity in patients with T2DM [39, 40]. The possible explanation for this discrepancy may be the different obesity measurements used between studies. Body weight employed as an indicator of obesity in the aforementioned trials might not capture the change in body fat disposition caused by increased levels of physical activity [41]. Other factors, such as insufficient levels of physical activity as well as low adherence to the intervention, may also contribute to the observed disagreement. Except for adiposity, the present MR study also identified a small mediation by lean mass. Exercise and moderate-to-high levels of training maintained and improved fat-free body mass [42], and high levels of lean mass were found to be associated with a low risk of T2DM [43].

Chronic low-grade inflammation, featured by high CRP levels, is another mediator of the associations of MVPA and LST with T2DM in this study. This is in line with previous data [44]. For example, a meta-analysis of 83 controlled trials found that exercise training is associated with a decrease in CRP levels [45]. Likewise, sedentary behavior, like long-time television viewing, was associated with an increase in CRP levels [46]. Except for CRP, this MR analysis additionally found associations of genetically proxied longer LST with increased levels of 2 other inflammatory biomarkers, IL-1RA and IL-6, that have previously been shown to be associated with the risk of T2DM [25]. However, our mediation analysis indicated that only IL-1RA appeared to partly mediate the association between LST and T2DM. All these findings support the high potential of promoting physical activity and reducing sedentary behavior for diabetes prevention and on the other side imply that dampening systemic inflammation in immobile individuals with low physical activity levels may be a strategy for lowering the risk of T2DM.

Different types of physical activity, such as free-living walking and high-intensity interval training, were associated with an improved glycemic profile in patients with T2DM [14, 47, 48]. However, the present MR study did not observe clear associations of genetically predicted MVPA and LST with any of the studied glycemic traits, except for a positive association between genetically predicted MVPA and FI levels. The reason for the discrepancy may be that GWASs on glycemic traits were conducted in individuals without T2DM.

Our methylation analysis revealed 2 methylation signals (cg17332422 and cg09531019) in support of low levels of physical activity and high risk of diabetes. The cg17332422 site is in the *ADAMTS2* gene. In line with our findings, an epigenome-wide association study found that another methylation site in *ADAMTS2* was associated with T2DM in HIV-infected individuals [49]. CpG cg09531019 was not

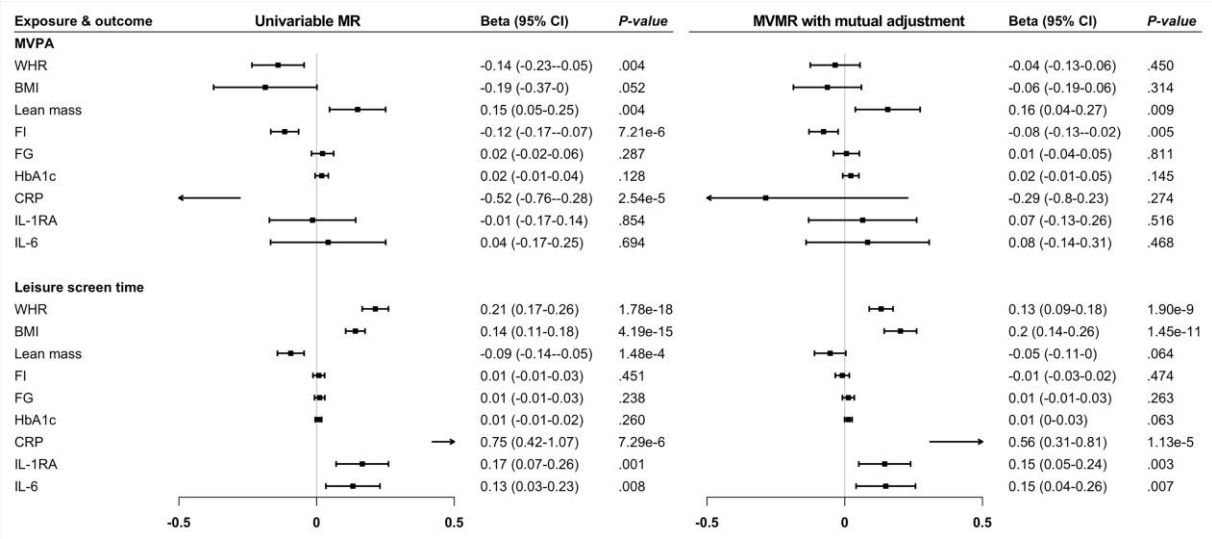

Figure 3. Associations of moderate-to-vigorous physical activity (MVPA) and leisure screen time (LST) with obesity, glycemic traits, and inflammatory biomarkers. BMI, body mass index; CRP, C-reactive protein; FG, fasting glucose; FI, fasting insulin; HbA_{1c}, glycated hemoglobin A_{1c}; IL-1RA, interleukin-1 receptor antagonist; IL-6, interleukin-6; OR, odds ratio; WHR, waist-to-hip ratio.

Table 2. Associations of moderate-to-vigorous physical activity and leisure screen time with type 2 diabetes after adjusting for intermediates and mediation effect

Adjustment	Association after adjusting for intermediate			Mediation
	OR	95% CI	*P*	
MVPA				
None	0.70	0.55-0.88	.002	-
WHR	0.89	0.60-1.31	.540	66
BMI	0.75	0.54-1.05	.098	22
Lean mass	0.72	0.50-1.03	.071	9
FI	0.81	0.61-1.09	.166	43
CRP	0.87	0.64-1.19	.396	63
LST				
None	1.45	1.30-1.62	7.62E-11	-
WHR	1.23	1.06-1.43	.006	43
BMI	1.19	1.01-1.41	.037	53
Lean mass	1.40	1.25-1.56	2.45E-09	10
CRP	1.31	1.17-1.47	3.55E-06	27
IL-1RA	1.39	1.24-1.55	1.11E-08	12
IL-6	1.45	1.29-1.63	2.59E-10	0

Abbreviations: BMI, body mass index; CRP, C-reactive protein; FI, fasting insulin; IL-1RA, interleukin-1 receptor antagonist; IL-6, interleukin-6; LST, leisure screen time; MVPA, moderate-to-vigorous physical activity; OR, odds ratio; SNV, single-nucleotide variation; WHR, waist-to-hip ratio.

Table 3. Associations of moderate-to-vigorous physical activity–related CpG sites and risk of type 2 diabetes

CpG site	SNV	β	SE	OR	95% CI	*P*
cg17332422	1	1.03	0.52	2.78	1.02-7.69	.046
cg18954163	1	−0.04	0.26	0.96	0.58-1.59	.866
cg00174845	1	0.32	0.55	1.37	0.47-4.00	.559
cg09531019	1	1.06	0.51	2.86	1.05-7.69	.039
cg10868567	1	−0.50	0.77	0.61	0.13-2.78	.516
cg17757602	1	−0.57	0.41	0.56	0.25-1.25	.160

Abbreviations: OR, odds ratio; SNV, single-nucleotide variation.

annotated to a gene. Thus, more study is needed to verify this association.

The merits of this study include data sources from larger-scale studies, the combination of traditional and methylation MR analyses, as well as the utilization of multivariable MR analysis to explore the mediation pathways. Limitations need to be considered when interpreting our findings. First, the present analysis was based on European populations, which limits the generalizability of our results to other populations. Second, glycemic trait data were obtained from individuals without T2DM, which confines the generalizability of these findings to diabetic populations. Third, we used a comparative relaxed LD threshold to select genetic instruments to increase the power, which might inflate the rate of type 1 error. However, the associations were consistent in the sensitivity analysis using stringent instrument selection criteria. Fourth, we might have overlooked other important methylation sites linking physical activity and T2DM given the small sample size of the epigenome-wide association study on physical activity [31]. Fifth, the study could not differentiate the comparative effects of different types of physical activity on the risk of T2DM based on the summary-level data. Sixth, we could not examine the sex- or ethnicity-specific associations of physical activity or sedentary behaviors with the risk of T2DM because of data unavailability. Seventh, different genotyping arrays/methods were used in the included GWASs, which might influence our MR results, even though this concern is limited since different genotyping arrays have been found not to be related to the usability of GWAS [50].

In summary, this updated MR study found associations of genetically predicted MVPA and LST with the risk of

T2DM, and these associations were mediated by adiposity, chronic inflammation, and lean mass. Our findings suggest promoting physical activity and lowering sedentary behavior may be a strategy for diabetes prevention.

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Author Contributions

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Disclosures

The authors have nothing to disclose.

Data Availability

All data analyzed in this study can be obtained by reasonable request to corresponding authors.

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