

RESEARCH ARTICLE

Effect of immunophenotype-mediated physical activity on depression: A two-step Mendelian randomization study

Min Liu^{1,†}, Yijun Dong^{1,†}, Yi Yin¹, Yixiang Shao¹, Jianfeng Xia², Shenbin Dai³, Rong Wang^{1,*}

¹School of Health, Nantong Institute of Technology, Nantong, Jiangsu, China. ²Huichun Tang Pharmacy, Rugao, Nantong, Jiangsu, China. ³Hai'an Zhangduo Hospital, Nantong, Jiangsu, China.

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Depression is a major psychiatric disorder with complex interactions among behavioral, neurological, and immune-related factors. Although physical activity has been associated with a lower risk of depression, the biological mechanisms underlying this association, particularly the potential mediating role of immune-cell phenotypes, remain unclear. This study aimed to determine whether physical activity causally affected the risk of depression and evaluate whether specific immunophenotypes mediated this association. A two-sample, two-step Mendelian randomization (MR) analysis was conducted using genetic instruments for physical activity, immunophenotypes, and depression. Causal effects were primarily estimated using the inverse-variance weighted method. MR-Egger regression, weighted-median, weighted-mode, and cML_MA_BIC methods were also applied to assess the consistency of the findings. MR-PRESSO and Cochran's Q test were used to evaluate potential horizontal pleiotropy, outliers, and heterogeneity. The results showed that genetically predicted physical activity was causally associated with the risk of depression. Three immunophenotypes were identified as potential mediators of this association including terminally differentiated CD4⁺ CD8⁺ T cells, the absolute count of CD28⁺ CD4⁺ CD8⁺ T cells, and CD20 expression on IgD⁺ CD24⁺ B cells. These immunophenotypes mediated 5.18%, 4.35%, and 4.79% of the total effect, respectively. The results obtained using complementary MR methods, and sensitivity analyses supported the robustness of the principal findings. These findings provided genetic evidence that specific T cell and B cell immunophenotypes might partly mediate the causal relationship between physical activity and depression. The study advanced the understanding of the interplay among physical activity, immune regulation, and depression and identified immune-cell phenotypes that warranted further mechanistic and clinical investigation.

Keywords: causal effects, depression, physical activity, Mendelian randomization.

*Corresponding author: Rong Wang, School of Health, Nantong Institute of Technology, Nantong, Jiangsu 226001, China. Email: 20210149@ntit.edu.cn.

[†]These authors contributed equally to this work.

Introduction

Depression is a common and disabling psychiatric disorder that affects more than 341.1 million people worldwide and imposes a substantial burden on individuals, healthcare systems, and

society [1]. Despite advances in pharmacological and psychological treatments, the prevention of depression remains challenging because few well-established and modifiable protective factors have been identified. Physical activity has emerged as a potentially important preventive

target because it is accessible, modifiable, and associated with broad physical and mental health benefits. Accumulating epidemiological evidence supports an inverse association between physical activity and depression. Meta-analytic and prospective cohort studies have consistently suggested that regular physical activity is associated with a lower risk of incident depression and fewer depressive symptoms [2, 3]. A meta-analysis of 111 prospective cohort studies involving more than three million adults reported that physically active individuals had an approximately 21% lower risk of developing depression or experiencing increased subclinical depressive symptoms after adjustment for relevant confounders [2]. Nevertheless, observational associations may be influenced by residual confounding, measurement error, and reverse causation. In addition, the biological mechanisms through which physical activity may reduce depression risk remain insufficiently understood.

Immune dysregulation has increasingly been implicated in the pathophysiology of depression. Peripheral inflammatory activation, elevated concentrations of proinflammatory mediators, altered immune cell counts, and abnormal antibody responses have been observed in individuals with depressive disorders [4, 5]. Persistent activation of the peripheral immune system including activation associated with malignancies, systemic infections, or autoimmune diseases may also increase the risk of major depressive disorder in susceptible individuals [6]. Alterations in leukocyte number and function together with changes in cytokine expression, have therefore been proposed as potential biomarkers of depression and other stress-related psychiatric disorders [7]. The immune system is highly responsive to environmental and behavioral exposures including physical activity [8]. Regular exercise may improve immune regulation by reducing chronic low-grade inflammation, attenuating obesity-related immune dysfunction, and counteracting age-related immunosenescence and inflammaging [9]. Population-based studies

have further suggested that regular moderate-intensity physical activity is associated with a reduced risk of immune-mediated and inflammatory disorders, multimorbidity, and premature mortality [10]. These findings indicate that immune-cell characteristics may represent an important biological link between physical activity and depression. However, the causal relationships among physical activity, immunophenotypes, and depression have not been clearly established. Most available evidence is derived from observational studies, which cannot fully exclude confounding or reverse causation. Furthermore, although both physical activity and depression have been associated with immune system alterations, it remains unclear whether specific immunophenotypes mediate the potential effect of physical activity on depression risk. Identification of such mediating pathways may improve understanding of the biological mechanisms underlying this association and help identify potential targets for prevention or intervention.

Mendelian randomization (MR) uses genetic variants as instrumental variables to estimate the causal effect of an exposure on an outcome and is less susceptible to confounding and reverse causation than conventional observational analyses [11]. A two-sample, two-step MR framework can additionally evaluate whether an intermediate phenotype mediates the causal relationship between an exposure and an outcome. This study applied a two-sample, two-step MR design to investigate the causal relationships among physical activity, immune cell phenotypes, and depression to determine whether genetically predicted physical activity causally influenced the risk of depression and identify immunophenotypes that might mediate this association. Multiple MR methods and sensitivity analyses were applied to assess the consistency and robustness of the causal estimates. By clarifying the potential mediating role of immune cell phenotypes, this study provided new insights into the biological pathways linking physical activity with depression and supported the development of

mechanism-based preventive strategies.

Materials and methods

Physical activity data

Genome-wide association study (GWAS) summary statistics for objectively measured physical activity were obtained from the UK Biobank cohort (<https://www.ukbiobank.ac.uk/>). Participants were originally recruited through 22 assessment centers across England, Scotland, and Wales with the age from 40 to 69 years old between 2006 and 2010. The accelerometer assessment was conducted between 2013 and 2015, when participants were approximately 45 – 79 years old. The physical activity GWAS included 91,084 participants of European ancestry with 54.5% of females. Physical activity was objectively assessed using an Axivity AX3 wrist-worn triaxial accelerometer (<https://axivity.com/product/ax3>). Participants received written instructions and were asked to wear the device continuously on the dominant wrist for seven consecutive days while maintaining their usual daily activities. The device recorded raw triaxial acceleration at a sampling frequency of 100 Hz. Average acceleration was derived after calibration, removal of gravitational acceleration and sensor noise, and identification of wear and non-wear periods. Participants were excluded if they had fewer than 72 hours of valid recordings, lacked data for any one-hour period of the 24-hour cycle, had accelerometer data that could not be adequately calibrated, or had average acceleration values more than four standard deviations from the sample mean. A minimum of 72 hours of wear time was required because simulation analyses indicated that this duration provided an estimate within 10% of the complete seven-day measurement [12]. In the final GWAS sample, the mean average acceleration was 27.98 milli-gravities with a standard deviation of 8.14 milli-gravities [13].

Immunophenotype data

GWAS summary statistics for immune cell traits were obtained from IEU OpenGWAS database

(<https://opengwas.io/>) and NHGRI-EBI GWAS Catalog (<https://www.ebi.ac.uk/gwas/>). The corresponding GWAS Catalog accession numbers ranged from GCST90001391 to GCST90002121. The original GWAS was based on 3,757 participants from the SardiNIA longitudinal cohort, a general population study comprising 6,602 individuals (57% females, 43% males) ranging from 18 to 102 years, all native to the central east coast of Sardinia, Italy. Participants were recruited from four towns in the Lanusei Valley in the Ogliastra region. Participants were genotyped using high-density arrays, and genotype imputation was performed using a Sardinian sequence-based reference panel, resulting in approximately 22 million genetic variants [14]. The association analyses were adjusted for age, age squared, and sex. A total of 731 immunophenotypes were examined and classified into four groups including 118 absolute cell counts, 192 relative cell counts, 32 morphological parameters, and 389 median fluorescence intensity measurements reflecting cell surface antigen expression. These phenotypes covered major immune cell populations including B cell and T cell maturation subsets, monocytes, myeloid cells, dendritic cells, and TBNK populations comprising T cells, B cells, and natural killer cells.

Depression data

GWAS summary statistics for depression were obtained from the Psychiatric Genomics Consortium meta-analysis [15]. Major depression was defined on the basis of lifetime diagnoses established through structured diagnostic interviews, clinician-administered assessments, or medical-record review. To minimize sample overlap with the physical activity GWAS, the UK Biobank samples were excluded from the depression dataset. The 23andMe (<https://www.23andme.com/>) samples were also excluded because the corresponding summary statistics were subject to access restrictions. The final depression dataset included 143,265 participants of predominantly European ancestry, comprising 45,591 depression cases and 97,674 controls. No

participants from UK Biobank or 23andMe were included in this analytical dataset.

Instrumental variable selection

This study employed a three-variable analytical framework with depression as the primary outcome, objectively measured physical activity as the exposure, and comprehensive immunophenotypic profiles as potential mediating factors. Genetic variants were especially well-suited to serve as instrumental variables (IVs) in MR analysis because of their distinctive properties with these instruments typically consisting of one-genetic variants, collections of multiple variable samples. For an IV to be considered valid, three main assumptions should be met, which included that it was associated with the exposure, unassociated with confounders, and affected the outcome only through the exposure [16]. For the selection of suitable genetic variants in MR analysis, several standards were applied including setting a genetic significance threshold of 5.0×10^{-8} for extracting IVs. However, due to the limited number of Single-nucleotide polymorphisms (SNPs) related to immunophenotypes meeting with this threshold, a more lenient significance level of 1.0×10^{-5} was adopted to more thoroughly explore the potential mediating effects of immunophenotypes in the link between physical activity and depression [17]. For physical activity and depression, the stringent genome-wide significance threshold of 5.0×10^{-8} was applied. SNPs were clumped ($r^2 < 0.001$, window = 10,000 kb) using 1,000 Genomes European reference data to ensure independence. To enhance robustness, SNPs with a minor allele frequency (MAF) of 0.01 or lower were excluded [17]. Those with (A/T) or (G/C) polymorphisms, ambiguous allele frequencies were omitted in the harmonization process for the exposure and outcome data on non-palindromic (SNPs). Additionally, for results reaching significance, the constrained maximum likelihood and model averaging based on the Bayesian information criterion (cML_MA_BIC) (<https://rdrr.io/github/xue-hr/MRcML/>) method was incorporated to further investigate and

enhance detection.

TSMR and 2-step MR-analyses

This study employed several methods to evaluate causal on a risk of relationship between physical activity and depression, while investigating role of potential mediators. The analytical methods included inverse-variance weighted (IVW), MR-Egger, weighted-median, simple or weighted model. IVW method is commonly employed in MR studies due to its ability to provide reliable causal estimates and its robustness against pleiotropy [18], which estimated causal effects using the Wald ratio based on individual genetic IVs, and these estimates were then combined using a fixed-effects model to produce an overall causal effect estimate [19]. Additionally, MR-Egger, weighted median, simple mode, and weighted mode were implemented as complementary methods to further explore the causal association between the exposure and outcome [20]. For results that reached significance, the cML_MA_BIC method was also included to enhance the detection process [21].

Sensitivity analyses

In this MR study, the pleiotropy test was crucial for ensuring that IVs did not influence a risk on depression through individual biological approach and other confounding factors unrelated to physical activity mediators. To detect horizontal pleiotropy in the IVs, whether the intercept differed significantly from zero was assessed with a significant deviation from 0 indicating the existence of horizontal pleiotropy. MR pleiotropy residual sum and outlier (MR-PRESSO), an overall tool designed to systematically assess pleiotropy in MR studies, was employed. MR-PRESSO consisted of three key components of the global test, outlier, and distortion assessment. *P* value less than 0.05 was used to identify IVs and might show pleiotropism at different levels. Additionally, MR-Egger regression, using the intercept term, was conducted to determine whether directional pleiotropy was present in the MR results [22]. To evaluate the differences between the IVW method and the results obtained by MR-Egger,

the Cochrane Q test was applied, which assessed the degree of heterogeneity in the estimates from these two approaches [23]. *P* value less than 0.05 indicated a lack of statistically significant heterogeneity. To ensure robustness of causal inferences, reverse MR was used to test whether depression risk could drive changes in physical activity, associations between physical activity and immunophenotypes, and between immunophenotypes and depression. This multi-step approach provided stronger evidence for the directionality of these associations.

Counting the mediation proportion

In the two-step-MR-method, first-stage involved estimating the β_1 -value, which represented the relationship between physical activity and immunophenotype. In second stage, the β_2 -value was calculated to signify the association between immunophenotypes and depression. In addition, the β_3 -value was determined to capture the direct association between physical activity and depression, accounting for the potential mediating effect. The mediating ratio of immune cells was quantified below to evaluate its mediating effect.

$$\text{Mediation proportion} = \frac{\beta_1 \times \beta_2}{\beta_3} \quad (1)$$

When the direction of the mediation effect was opposite to the total effect, a masking effect was considered with the calculation as follows.

$$\text{Masking proportion} = |\beta_1 \times \beta_2 / (\beta_3 - \beta_1 \times \beta_2)| \quad (2)$$

Evaluation of genetic-instrumental-variables

The variance R^2 for each genetic instrument was calculated as follows.

$$R^2 = 2 \times EAF \times (1 - EAF) \times \beta^2 \quad (3)$$

where *EAF* was the effect allele frequency. β was the effect estimate of the genetic variant in the exposure GWAS. Additionally, the *F*-statistics for each variant were calculated below.

$$F = R^2(N - 2)/(1 - R^2) \quad (4)$$

where *N* was the number of individuals in the exposure GWAS. These calculations were carried out to evaluate the efficacy of the selected tools.

Results

Causal effect of physical activity on depression

Seven SNPs associated with physical activity at the genome-wide significance threshold were initially identified. During data harmonization, two SNPs were excluded because their alleles could not be aligned unambiguously. Therefore, five SNPs were retained as instrumental variables for the two-sample Mendelian randomization analysis, which were rs11012732, rs12522261, rs148193266, rs34517439, and rs9293503. The IVW analysis showed that genetically predicted physical activity was associated with a reduced risk of depression ($\beta = -0.047$, OR = 0.954, 95% CI: 0.918–0.991; *P* = 0.015). The weighted-median method yielded a similar result ($\beta = -0.054$, OR = 0.947, 95% CI: 0.904–0.992; *P* = 0.022). The cML-MA-BIC analysis also supported an inverse association between physical activity and depression ($\beta = -0.045$, OR = 0.956, 95% CI: 0.928–0.984; *P* = 0.002) (Table 1). The causal estimates obtained using all five conventional MR methods were negative, indicating a consistent direction of association between higher genetically predicted physical activity and lower depression risk. Although the MR-Egger estimate did not reach statistical significance, its effect direction was consistent with the IVW result. Similarly, the simple-mode analysis produced an inverse but nonsignificant estimate. The weighted-mode estimate was also inverse but did not reach statistical significance after accounting for the small number of genetic instruments. Overall, the consistency in effect direction across the different MR methods supported a potential protective causal effect of physical activity on depression (Figure 1A). The SNP-specific causal estimates varied in precision with wider confidence intervals than the pooled estimates. Nevertheless, the individual estimates were generally consistent with an inverse association between physical activity and

Table 1. MR estimates of the causal effect of physical activity on depression.

Method	nSNP	Beta	OR (95% CI)	P value	Cochran's Q statistic (P value)	Egger intercept (P value)	Global test P value
Inverse variance weighted	5	-0.047	0.954 (0.918, 0.991)	0.015	0.884	0.393	0.794
MR Egger		-0.112	0.894 (0.782, 1.022)	0.198	0.982		
Weighted median		-0.054	0.947 (0.904, 0.992)	0.022			
Simple mode		-0.064	0.938 (0.878, 1.002)	0.130			
Weighted mode		-0.064	0.938 (0.860, 1.024)	0.113			
cML_MA_BIC		-0.045	0.956 (0.928, 0.984)	0.002			

Note: MR: Mendelian randomization. SNP: single nucleotide polymorphism. OR: odds ratio.

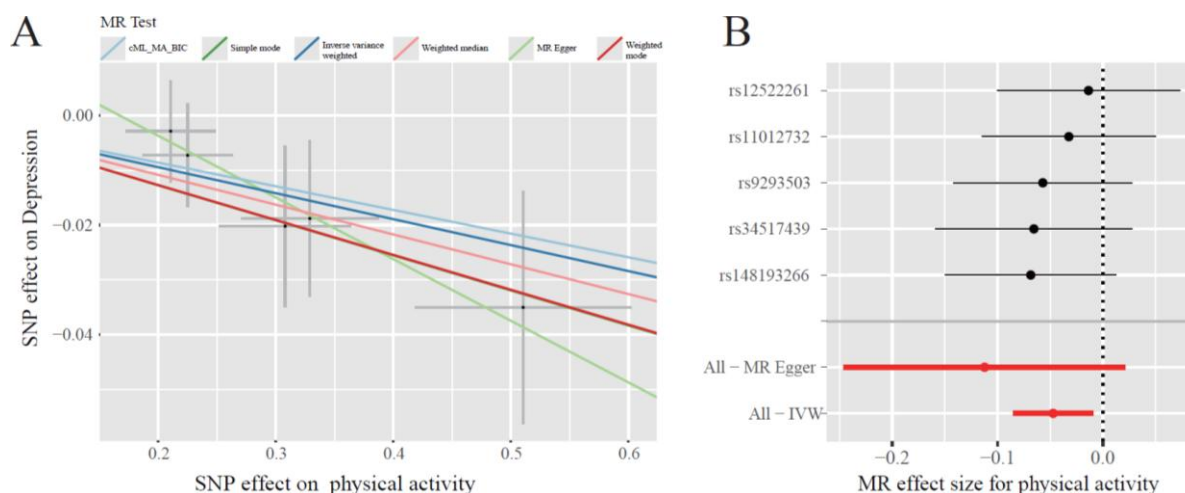


Figure 1. Scatter and forest plot for causal relationship between physical activity and depression. **A.** Scatter-plot showed the influences of SNPs on physical activity and their relationship to depression with each line's slope representing the MR effect estimated by each method. Data were presented as raw β -values along with 95% CIs. **B.** Forest-plot illustrated MR-estimated effect sizes for individual and combined SNPs. The effect sizes reflected the log odds of depression for every one standard deviation increase in average physical activity with 95% CIs indicated by the error bars.

depression. The combined IVW estimate was located below the null value and had a relatively narrow confidence interval, indicating greater precision than most individual SNP estimates (Figure 1B). The pooled results therefore suggested that the observed association was not attributable solely to a single genetic instrument but reflected the combined contribution of the five included SNPs. Sensitivity analyses showed no evidence of substantial heterogeneity or horizontal pleiotropy. Cochran's Q tests did not identify significant heterogeneity among the SNP-specific estimates under either the IVW model or the MR-Egger model. The MR-PRESSO global test detected no evidence of horizontal

pleiotropy, and no influential outliers were identified. In addition, the MR-Egger intercept test provided no evidence of directional horizontal pleiotropy. Reverse-direction MR analysis did not support a causal effect of genetic liability to depression on physical activity.

Causal effects of physical activity on immunophenotypes

During instrumental-variable selection, 110 of the 731 immunophenotypes were excluded because no eligible genetic instruments were available, leaving 621 immunophenotypes for the MR analyses. The results obtained using six MR methods of IVW, MR-Egger, simple mode,

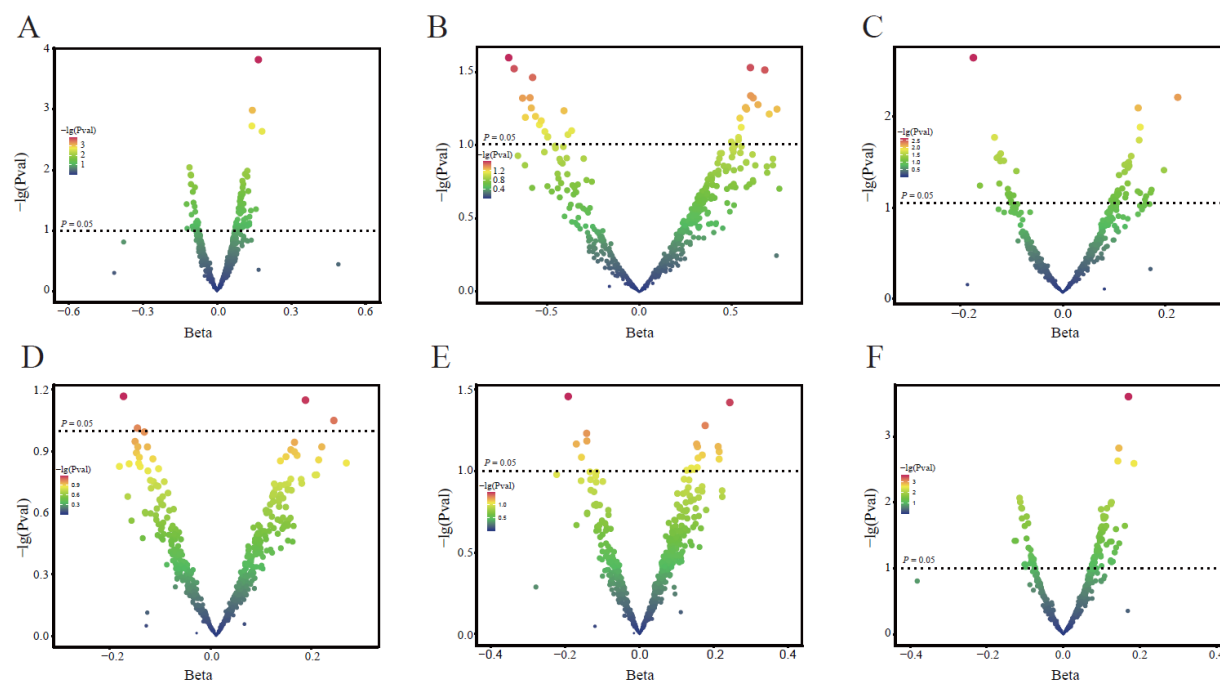


Figure 2. Volcano plot of the MR results for physical activity on the immunophenotypes. **A to F** represented MR analysis with IVW, MR-Egger, Simple-mode, Weighted median and mode, and cML-MA-BIC.

weighted median, weighted mode, and cML-MA-BIC demonstrated that, overall, the effect estimates for most immunophenotypes were close to the null, whereas a subset showed nominal associations with genetically predicted physical activity at $P < 0.05$ with both positive and inverse associations observed (Figure 2). In accordance with the predefined analytical framework, IVW was used as the primary method, while MR-Egger, simple mode, weighted median, and weighted mode were applied as complementary analyses. Associations reaching nominal significance in IVW analysis were further evaluated using cML-MA-BIC to assess their robustness.

(1) Inverse-variance weighted analysis

IVW analysis identified nominal associations between genetically predicted physical activity and 43 immunophenotypes ($P < 0.05$), of which 32 showed positive associations and 11 showed inverse associations. The strongest statistical evidence was observed for CD25 expression on IgD⁺ CD24⁻ B cells ($\beta = 0.167$, 95% CI: 0.081–0.253; $P < 0.001$), CD25 expression on naive-

mature B cells ($\beta = 0.143$, 95% CI: 0.057–0.228; $P = 0.001$), CD25 expression on IgD⁺ CD38dim B cells ($\beta = 0.141$, 95% CI: 0.052–0.229; $P = 0.002$), CD25 expression on IgD⁺ CD38⁻ naive B cells ($\beta = 0.182$, 95% CI: 0.065–0.299; $P = 0.002$), and the absolute count of CD62L⁻ plasmacytoid dendritic cells ($\beta = -0.112$, 95% CI: -0.195 to -0.028; $P = 0.009$).

(2) cML-MA-BIC analysis

The cML-MA-BIC analysis supported 42 of the 43 associations identified by the IVW method. The effect directions and magnitudes were generally consistent between the two methods. cML-MA-BIC confirmed the associations with CD25 expression on IgD⁺ CD24⁻ B cells ($\beta = 0.170$, 95% CI: 0.079–0.261; $P < 0.001$), CD25 expression on naive-mature B cells ($\beta = 0.145$, 95% CI: 0.056–0.235; $P = 0.001$), CD25 expression on IgD⁺ CD38dim B cells ($\beta = 0.143$, 95% CI: 0.051–0.235; $P = 0.002$), CD25 expression on IgD⁺ CD38⁻ naive B cells ($\beta = 0.185$, 95% CI: 0.065–0.305; $P = 0.003$), and the absolute count of CD62L⁻ plasmacytoid dendritic cells ($\beta = -0.112$, 95% CI: -0.197 to -0.027; $P = 0.010$). The association with the

absolute count of CD8dim natural killer T cells did not reach statistical significance in the cML-MA-BIC analysis, although its effect direction was consistent with the IVW estimate.

(3) Sensitivity and reverse-direction analyses

The MR-PRESSO global test for all 43 immunophenotypes did not detect significant horizontal pleiotropy. Additionally, Cochran's Q test and MR-Egger intercept did not reveal significant heterogeneity in the associations. Reverse MR identified six immunophenotypes with a reverse causal association with physical activity among the 43 tested, which included CD25 on IgD⁺ CD38dim B cell, CD25 on IgD⁺ B cell, CD25 on IgD⁺ CD24⁺ B cell, CD8dim Natural Killer T Absolute Count, CD3 on Effector Memory CD4⁺ T cell, and CD3 on Central Memory CD4⁺ T cell. Although these phenotypes were included in the next stage of analysis, no evidence was found that they acted as mediators on the correlation between physical activity and depression.

Causal effects of immunophenotypes on depression

Among the 43 immunophenotypes associated with physical activity in the primary analysis, three showed nominal evidence of a causal association with depression in the IVW analysis. Terminally differentiated CD4⁺ CD8⁺ T cells as a percentage of CD4⁺ CD8⁺ T cells were inversely associated with depression risk ($\beta = -0.027$, OR = 0.973, 95% CI: 0.950–0.997; $P = 0.028$). This association was also supported by the weighted-median method (OR = 0.964, 95% CI: 0.931–0.999; $P = 0.042$) and cML-MA-BIC (OR = 0.973, 95% CI: 0.950–0.996; $P = 0.021$), whereas the MR-Egger, simple-mode, and weighted-mode estimates were not statistically significant. The absolute count of CD28⁺ CD4⁺ CD8⁺ T cells was positively associated with depression risk in the IVW analysis ($\beta = 0.024$, OR = 1.024, 95% CI: 1.001–1.048; $P = 0.037$). However, this association was not supported by MR-Egger, weighted median, simple mode, weighted mode, or cML-MA-BIC, all of which yielded P values greater than 0.05. CD20 expression on IgD⁺ CD24⁺ B cell was also positively associated with

depression risk in IVW analysis ($\beta = 0.026$, OR = 1.027, 95% CI: 0.999–1.054; $P = 0.049$). Consistent associations were observed using the weighted-median method (OR = 1.035, 95% CI: 1.006–1.065; $P = 0.019$), weighted-mode method (OR = 1.035, 95% CI: 1.006–1.064; $P = 0.027$), and cML-MA-BIC (OR = 1.034, 95% CI: 1.012–1.055; $P = 0.002$), whereas the MR-Egger and simple-mode estimates were not statistically significant. None of the remaining 40 immunophenotypes showed nominal evidence of association with depression in the primary IVW analysis. Sensitivity analyses indicated no significant heterogeneity for terminally differentiated CD4⁺ CD8⁺ T cells or the absolute count of CD28⁺ CD4⁺ CD8⁺ T cells based on Cochran's Q tests under both IVW and MR-Egger models. The MR-Egger intercept and MR-PRESSO global tests also provided no evidence of horizontal pleiotropy for these two immunophenotypes. For CD20 expression on IgD⁺ CD24⁺ B cells, Cochran's Q tests indicated significant heterogeneity under both IVW ($P = 0.013$) and MR-Egger ($P = 0.009$) models. However, the MR-Egger intercept test showed no evidence of directional horizontal pleiotropy, and the MR-PRESSO global test did not reach statistical significance. Reverse-direction MR analyses did not identify significant causal effects of depression on any of the three immunophenotypes.

The masking effect of mediating factors connection on physical activity-depression

A two-step MR study was conducted to explore the mediating role of immunophenotypes connection on physical activity-depression. IVs for physical activity were used to estimate the causal connection between exposure and various immunophenotypes. Further, IVs for potential immunophenotypes were used to assess the causal connection between these mediators and the risk of depression. Among the potential mediators, only three immunophenotypes of terminally differentiated CD4⁺ CD8⁺ T-cell % T-cell (CD4⁺ CD8⁺), T-cell-Absolute-Count (CD28⁺ CD4⁺ CD8⁺), and B-cell (CD20 on IgD⁺ CD24⁺) reached significance. Since the mediation effects of these three immunophenotypes were in the opposite

Table 2. Evaluation of the mediating effect of immunophenotype on the impact of physical activity on depression.

Mediation (M)	Direct effect E-M	Direct effect M-O	Mediation effect	Direct effect E-O	Total effect E-O	Mediation effect/Direct effect E-O ×100%
Terminally differentiated CD4 ⁺ CD8 ⁺ T cell % CD4 ⁺ CD8 ⁺ T cell	-0.095	-0.027	0.003	-0.050	-0.047	5.18%
CD28 ⁺ CD4 ⁺ CD8 ⁺ T cell Absolute Count	0.089	0.024	0.002	-0.049	-0.047	4.35%
CD20 on IgD ⁺ CD24 ⁺ B cell	0.091	0.026	0.002	-0.049	-0.047	4.79%

direction to the total effect, they were considered to exert a suppression effect on the physical activity - depression connection. The results found that masking effect for terminally differentiated T-cell (CD4⁺ CD8⁺) % T-cell (CD4⁺ CD8⁺) was 0.003 with a suppression ratio of 5.18%. For T-cell-Absolute-Count (CD28⁺ CD4⁺ CD8⁺), the suppression effect was 0.002 with a suppression ratio of 4.35%. For B-cell (CD20 on IgD⁺ CD24⁺), the suppression effect was 0.002 with a suppression ratio of 4.79% (Table 2).

Discussion

Depression is a prevalent psychiatric disorder that adversely affects daily functioning, occupational performance, and interpersonal relationships. In recent years, the prevalence of depression has been rising, so from a public health and social perspective, prevention and treatment of depression are both very important. Research suggests that physical activity plays a significant role in reducing the onset and progression of depression [24]. Physical activity is believed to counteract the effects of depression by stimulating various neuroplasticity mechanisms or increasing cerebral blood flow. Additionally, the anti-inflammatory effects of exercise may play a crucial role in the pathophysiology of depression [25]. Sufficient levels of sustained physical activity exert positive immunomodulatory effects and have an antidepressant effect. In contrast, a lack of physical activity promotes the accumulation of visceral fat, infiltration of adipose tissue by pro-inflammatory immune cells, and persistent low-

grade inflammation [26]. While there is evidence that the immune system may play a potential mediating role between physical activity and depression, the exact causal relationship between physical activity, immune phenotypes, and depression remains unresolved. This study used a two-step Mendelian randomization framework to assess whether immune phenotype mediated the association between physical activity and depression. The results indicated that physical exercise could reduce the risk of depression, which was consistent with the findings of most existing observational studies. Studies have shown that exercise is associated with a reduced risk of depression. Compared to people who do not exercise, those who exercise can reduce their risk of depression by 30% [27]. A recent study followed a large population of 33,908 adults over 11 years with none of them having symptoms of common mental disorders or significant physical health issues. The results revealed that regular physical activity was connected to a decreased likelihood of experiencing depression later, though it did not affect anxiety. This protective effect was consistent across different exercise intensities, as well as among participants of varying sex and age. Assuming causality, the data suggested that 12% of the depression cases seen during the follow-up might have been averted if every participant had exercised for at least an hour weekly [28]. Regular exercise is now regarded as a key lifestyle activity that can mitigate the negative effects of chronic illnesses on physical well-being and lessen the adverse impacts of depression [29].

CD4⁻ CD8⁻ T cells, also known as double-negative (DN) T cells, like all T lymphocytes, express either the $\alpha\beta$ or $\gamma\delta$ chain of the T cell receptor (TCR). This ability helps them recognize pathogens and respond to them, thereby generating a functional adaptive immune response. Terminally differentiated DN T cells are closely associated with disease progression and aging [30]. The worsening of a disease or the aging of the body deterioration fosters the undifferentiated DN T cells. These differentiated DN T cells play a crucial role in the immune surveillance and elimination of viral infections and tumor cells, resulting in a markedly higher proportion of terminally differentiated DN T cells relative to their undifferentiated counterparts. Children have a notably higher percentage of DN T cells compared to adults. A higher BMI is associated with a lower percentage of DN T cells, but engaging in physical activity can raise their percentage [31]. The findings of this study aligned with these previous reports, showing that physical activity increased percentage of DN T cells, which in turn decreased the proportion of terminally differentiated DN T cells. In addition, this study also found a protective causal effect of the percentage of terminally differentiated DN T cells against depression. Currently, studies on the influence of DN T cells in neurological diseases remain limited. Zhang *et al.* identified and characterized antigen-specific DN T cells, demonstrating their ability to regulate immune responses to alloantigen both *in vitro* and *in vivo* and selectively eliminated activated syngeneic CD4 and CD8 T cells [32]. Lu *et al.* demonstrated that DN T cells might play a key mediating role in the onset and progression of depression by regulating immune responses, thereby promoting systemic inflammation and tissue injury, which held significant implications for autoimmune and inflammatory diseases [33]. CD28, a well-researched co-stimulatory receptor, plays a significant role in T cell activation, proliferation, and survival. CD28 deficient T cells display unique features including diminished TCR diversity and compromised proliferation in response to antigen stimulation. Despite these deficits, CD28⁻ T cells show increased cytotoxicity

and possess regulatory properties. Additionally, CD28 plays a critical role in modulating immune responses by regulating T cell activity [34]. This study found that increased physical activity raised the DN T cells' absolute count of CD28⁻, and this cellular expansion showed a significant association with increased depression risk. Prolonged and intensive training can cause a reduction in secretory immunoglobulin A (IgA), thereby increasing the body's susceptibility to diseases. CD28⁻ DN T cells possess enhanced cytotoxic capabilities and immunoregulatory functions, and they have been shown to play a role in various human diseases [35]. CD20, a member of the MS4A protein family, is a 33-37 kDa non-glycosylated-surface-protein expressed on both healthy and neoplastic B lymphocytes. Following mitogen stimulation, this molecule becomes heavily phosphorylated, potentially facilitating its role as a calcium channel during B cell activation [36]. Emerging evidence suggests CD20 serves as a critical mediator of B cell receptor (BCR) signaling, enabling efficient signal transduction in B lymphocytes [37]. Balogh *et al.* observed an elevated naive B cell frequency among healthy university students after a 14-week endurance training program, potentially attributable to either augmented hematopoietic activity or bone marrow-derived naive B cell mobilization [38]. Studies have found an increased density of brain-infiltrating B lymphocytes in patients with depression [39]. Additionally, extensive research indicates that the significant infiltration of B cells in the central nervous system contributes to the pathological mechanisms of various neurological conditions, such as stroke, multiple sclerosis, Parkinson's disease, and Alzheimer's disease, ultimately resulting in cognitive decline [40].

This study underscored the pivotal involvement of immune cells in mediating the association between physical activity and depression. The results not only validated involvement of immunophenotypes in mediating this connection but also underscored the complexity and heterogeneity of the relationship. Amid the uncertainties surrounding the mechanisms of

depression, the findings of this research offered new insights into the intricate interactions between physical activity, immunophenotypes, and depression. The observed association between immune phenotype regulation and physical activity and depression highlighted the regulatory role of the immune system in the pathophysiology of depression. The findings indicated that immunomodulatory interventions could potentiate the antidepressant effects of physical activity by enhancing protective immune mechanisms. However, certain inherent limitations of this study must be recognized as warranting consideration. Due to the relatively small sample size of the immunophenotype GWAS, individual genetic variants could potentially introduce weak instrument bias although the robust F-statistics confirmed the instruments' sufficient predictive strength. Lowering the *P* value threshold might enhance the statistical power of MR analyses. Future studies utilizing expanded immunophenotype GWAS datasets could implement stricter variant selection criteria. Additionally, the exclusive use of European ancestry cohorts, while reducing population stratification bias, might constrain the generalizability of results across diverse ethnic populations. This study did not perform multiple validation correction because only three immunophenotypes were included after the two-step MR screening. Given the small number of hypothesis tests, applying multiple corrections could set overly stringent significance thresholds, potentially excluding all biologically relevant associations. However, this also implied that the results might carry some risk of false positives, a limitation that should be considered in the interpretation of these findings.

Conclusion

In this research, the causal association between physical activity and depression risk mediated through immunophenotypes was confirmed, which added valuable insights to sports medicine and mental health research. While previous studies have explored these connections, the

present findings highlighted the need in future research to focus on detailed interactions between physical activity, immune function, and mental health. Addressing the underlying molecular mechanisms will be crucial for developing more targeted interventions in depression prevention and treatment. Despite challenges such as sample heterogeneity and the complexity of immunophenotypes, this study strengthened the body of evidence and offered a new perspective on how physical activity might influence mental health through immune pathways.

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