Original Article

Genetic predicted causal inferences between antioxidants and birth weight

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Observational studies have suggested a relationship between antioxidants and birth weight. However, the causal association remains unclear. The aim of this study was to assess the causal relationship between antioxidants and birth weight. Genome wide association study (GWAS) summary statistics for 4 endogenous and 7 exogenous antioxidants, as well as birth weight were obtained from GWAS studies and UK biobank. A two-sample Mendelian randomization (MR) analysis was conducted with fixed-effects model inverse variance weighted (IVW) as the primary analytical method, while MR Egger and weighted median used as auxiliary. A series of sensitivity analyses were conducted to verify the robustness of the results. The MR results revealed that genetically predicted higher superoxide dismutase (SOD) (β = 0.025; 95% CI: 0.008, 0.043; *p* = 0.005) and zinc (β = 0.030; 95% CI: 0.013, 0.047; *p* = 0.001) levels were associated with higher birth weight. Sensitivity analysis verified the robustness of the MR results. Our study reinforced the existing evidence supporting a significant positive association between SOD and zinc with birth weight, providing new genetic evidence for antioxidant supplementation during pregnancy to prevent low birth weight infants. Further deeper comprehension studies are warranted to confirm these findings.

***Key Words*: antioxidants, zinc, superoxide dismutase, Mendelian randomization, birth weight**

The effects of a range of intrauterine exposures have a significant impact not only on the development of the fetus during infancy and early childhood, but also have a profound effect on the physical condition of the child and the adult. Studied have confirmed low birth weight (LBW) increases neonatal mortality, as well as a range of infant diseases, including lung and neurological disorders.[1] In addition, the fetal origins of adult diseases have long held that BW has a profound long-term effect on an individual's susceptibility to various diseases in adulthood, as confirmed by numerous observational studies.[2] Adverse effects early in LBW development may lead to permanent physiological and metabolic changes, such as significantly increasing the risk of type 2 diabetes, coronary heart disease, hypertension and tumors in adulthood.[3] Therefore, it is important to explore the risk factors associated with LBW with a view to effective intervention during pregnancy.

The prenatal environment is a sensitive developmental period and oxidative stress (OS) plays an important role in this process.[4] The placenta is in a low-oxygen environment in early pregnancy, and when blood circulation between the embryo and the maternal placenta is fully established, the concentration of oxygen in the placenta increases rapidly, with a concomitant increase in reactive oxygen radicals produced by metabolism. Reactive oxygen species (ROS) are highly reactive molecules produced by the reduction of molecular oxygen and are formed mainly by mitochondrial oxidative phosphorylation, common ROS species are superoxide ($O_2^{\bullet-}$), hydroxide ($^{\bullet}OH-$) and hydrogen peroxide (H_2O_2).[5,6]

ROS is a double-edged sword, at the physiological level, it is involved in cellular signaling pathways that are important for normal development and cellular function, it also involved in the pathology of a variety of female reproductive-related diseases,[5] especially when there is an imbalance between pro-oxidants and antioxidants.[7] Mammals have both enzymatic and non-enzymatic antioxidant system defense mechanisms to neutralize excess free radicals, endogenous enzymatic antioxidants enzymes include glutathione (GSH), glutathione peroxidase (GPX), and superoxide dismutase (SOD), etc.; non-enzymatic antioxidants include vitamins A, C, E, etc.[8] Maintaining a high redox potential is essential for reproductive health, the antioxidant system is activated when minor OS are present.[9] Excessive OS in the placenta and/or the mother may negatively affect all the different stages of pregnancy, leading to the development of several pregnancy-related diseases.[10,11] Several observational studies[12–14] have indicated that the level of OS is significantly and negatively correlated with BW. Significantly higher oxidative damage, mitochondrial dysfunction and impaired angiogenesis were present in the placentas of the LBW group.[15] Some maternal cord blood markers of OS, such as retinol, are inversely correlated with BW and head circumference.[16]

However, due to ethical and other reasons, it is not possible to conduct adequate studies in human pregnancies, the relationship between OS and BW is not clear and the conclusions of related studies are inconsistent.[7] In particular, the causal association of antioxidants with BW has not yet been elucidated. Mendelian randomization (MR) is a novel epidemiological approach based on genome wide association studies (GWAS) using single nucleotide polymorphisms (SNPs) as instrumental variables (IVs) for revealing causal relationships.[17] Based on the principle that alleles follow random assignment during gamete formation, their effect estimates are not affected by confounding factors and reverse causal associations. Currently, no MR studies of antioxidants on BW have been reported. In this study, we explored the causal associations of 11 common antioxidants with BW by a two-sample MR based on publicly available GWAS datasets, providing a new genetic rationale for preventive and therapeutic interventions in LBW infants.

Materials and Methods

Study design and data sources. This study followed the guidelines of Study to Enhance the Use of Mendelian Random-

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ization for Observational Epidemiology (STROBE-MR).[18] We obtained summary statistics of exposure and outcome data from publicly available GWAS datasets. The causal associations of endogenous and exogenous antioxidants with BW were explored by a two-sample MR. Subsequently, the effect of BW on endogenous antioxidant enzymes was explored by reverse MR. The data included in this study were derived from publicly available GWAS datasets, therefore no additional ethical approval was required.

Four endogenous antioxidants, including glutathione *S*-transferase (GST), SOD, GPX and catalase (CAT), all derived from the human plasma proteome of healthy blood donors in the INTERVAL study;[19] tocopherol (α- and γ-, vitamin E) and ascorbate (vitamin C) were obtained from the KORA study;[20] retinol and carotene (vitamin A) were obtained from CLSA cohort study;[21] zinc and selenium were obtained from a study used two adult cohorts from Australia and UK.[22] The GWAS dataset for BW was obtained from UK biobank (https://gwas.mrcieu.ac.uk/). Details of these GWAS datasets were shown in Supplemental Table 1*.

Selection of IVs. Considering that including at least 10 independent SNPs as IVs can maintain sufficient statistical efficiency in MR analysis,[23] when antioxidants were set as the exposure, too few IVs would be included according to the genome wide significant standard ($p<5E^{-8}$), which may affect statistical efficacy. Therefore, referring to previously published literature,[24,25] the selection criteria for IVs was: $p<1E^{-5}$, linkage disequilibrium (LD) $r^2<0.001$ with clump distance = 10,000 kb. In the reverse MR, while BW was used as the exposure, we used the standard $p<5E^{-8}$ and the same LD standard. To fulfill the second hypothesis of MR, the IVs must not associate with any risk factor for the outcome, we retrieved and removed SNPs associated with potential confounders such as body mass index (BMI), obesity, smoking, alcohol consumption, diabetes, hypertension etc. through the Phenoscanner website (http://www.phenoscanner.medschl.cam.ac.uk/). When harmonizing the statistics of the exposure and outcome data, assume all alleles were coded on the forward strand and removed SNPs that were not present in the outcome data. To avoid weak IV bias, the F-statistic was calculated for the selected SNPs with the formula = ($beta^2/se^2$).[26]

Statistical analysis. The fixed-effects inverse variance weighted (IVW) model was used as the main analytical method. If there was heterogeneity in the IVs, the IVW method with a random-effects model was substituted. The IVW works by weighting the causal effects of different genetic variants on traits by inverse variance and then combining the weighted effect estimates assuming that individual genetic variants are independent and valid, which may be biased by horizontal pleiotropy.[27] Therefore, MR Egger and weighted median were used as sensitivity analyses. MR Egger can be used to test for horizontal pleiotropy, the slope coefficients of Egger regressions provide estimates of causal effects, even if all genetic variants are null IVs.[28] Weighted median estimates the causal effects of different genetic variants on a trait are weighted, and the weighted median is then taken as the final causal effect estimate, which yields robust estimates when at least 50% of the information comes from valid IVs, and complements the MR Egger regression approach.[29] The Egger regression intercept was applied as an estimate of the horizontal pleiotropy, with $p<0.05$ indicating that there is no horizontal pleiotropy in the IVs. Cochran's Q in the IVW models was applied to assess the heterogeneity among the included SNPs, with $p>0.05$ indicating there is no heterogeneity. Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) test was used for horizontal pleiotropy test and removed outliers to get a more accuracy estimate.[30] Finally, IVs were removed one by one by leave-one-out analysis, while MR analysis was performed using the remaining SNPs to check whether the results were strongly perturbed by a single SNP.

All GWAS data analyses were performed using R (ver. 4.2.3) statistical software. The "TwoSampleMR" (ver. 0.5.7) and "MRPRESSO" (ver. 1.0) packages were used for MR analysis. $P<0.0045$ (0.05/11) was considered as evidence of statistical significance, while a p value of less than 0.05 was considered as suggestive evidence for a potential causal association.

Results

Effect of antioxidants on the birth weight. The IVs associated with antioxidants were shown in Supplemental Table 2* and the F-statistics were all greater than 10, suggesting that there is no weak IVs bias. The results of MR analysis were shown in Supplemental Table 3* and Fig. 1. The IVW method suggested that among endogenous antioxidants, a higher SOD level was associated with a higher BW (β = 0.025; 95% CI: 0.008, 0.043; $p = 0.005$); Bedsides, although statistically insignificant, the MR Egger (β = 0.009; 95% CI: −0.033, 0.050; $p = 0.683$) and weighted median (β = 0.015; 95% CI: −0.008, 0.037; $p = 0.193$) provided consistent trends with IVW result. Among exogenous antioxidants, the IVW method suggested that high levels of zinc were significantly associated with a higher BW (β = 0.030; 95% CI: 0.013, 0.047; $p = 0.001$), while MR Egger (β = 0.003; 95% CI: −0.048, 0.054; $p = 0.908$) and weighted median (β = 0.028; 95% CI: 0.003, 0.053; $p = 0.031$) provided a consistent trend. Other than that, MR results did not provide evidence of association of other antioxidants with BW.

The sensitivity analysis results (Supplemental Table 4*) suggested that there were no significantly heterogeneity or pleiotropy in IVs of all the antioxidants. The leave-one-out results also suggested that these MR results were not strongly perturbed by a single SNP (Supplemental Table 5*).

We searched through Phenoscanner online for antioxidant-related IVs associated with BW-related confounders such as obesity, BMI, diabetes mellitus, metabolic disorders, hypertension, lipid-lowering medications and alcohol consumption etc. (Supplemental Table 6*). These SNPs were deleted and the MR analysis was repeated. We found all MR results were consistent with the results of the primary analysis (Supplemental Table 7*). Sensitivity analyses indicated no evidence of heterogeneity or pleiotropy (Supplemental Table 8*).

Reverse MR analysis. To exclude confounding by potential reverse causal associations, BW was set as an exposure for reverse MR. The information of BW-associated IVs was shown in Supplemental Table 9*. The IVW method did not find evidence of significant associations between BW with the four endogenous antioxidants (Supplemental Table 10*). Besides, sensitivity analyses (Supplemental Table 11*) suggested the robustness of the MR results.

Discussion

In the present study, based on a two-sample MR study, we explored the causal association of endogenous and exogenous antioxidants with BW. The MR results suggested that higher levels of SOD and zinc showed a causal association with increased BW. No significant associations were observed between other antioxidants with BW. The reverse MR results suggested that BW was not significantly associated with the endogenous antioxidants. To the best of our knowledge, this is the first MR study to explore the causal association of antioxidants with BW using large-scale GWAS data.

The balance between oxidative stress and antioxidants *in vivo* plays an important role in placental homeostasis. Embryonic and placental development begins in a hypoxic environment, and oxygen input is increased for rapid growth beginning in mid-gestation; ROS favor trophoblast proliferation, invasion and angiogenesis on the one hand, yet frequent exposure to oxidative

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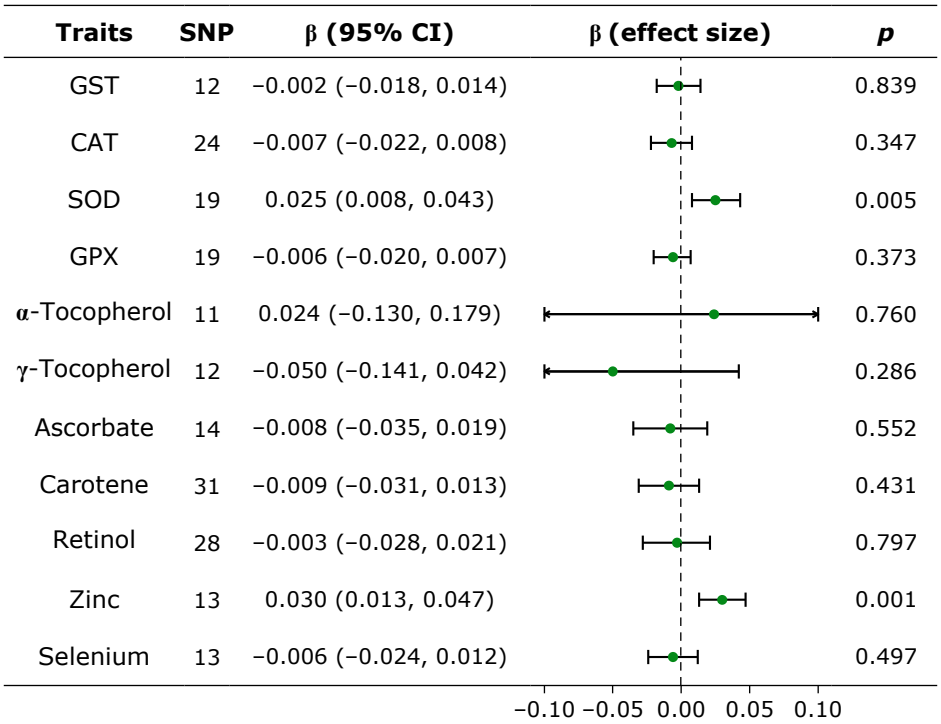

Fig. 1. Mendelian randomization (MR) estimates for the associations of antioxidants on birth weight. SNP, single nucleotide polymorphism. CI, confidence interval; GST, glutathione *S*-transferase; CAT, catalase; SOD, superoxide dismutase; GPX, glutathione peroxidase.

stress impairs placental development.[31] The placenta is the main source of ROS during pregnancy.[32] In late pregnancy, insulin resistance and lipolysis further increase the production of hydrogen peroxide.[33] Large amounts of ROS inactivate biomolecules and disrupt cellular metabolism, leading to endothelial dysfunction and apoptosis of hyper-trophoblastic cells,[34] which causes pregnancy complications including LBW.[35]

The activity of some antioxidants, such as SOD, CAT and GSH, as well as some vitamins and metallic elements (selenium and zinc), are involved in the maintenance of maternal OS homeostasis.[36] In a study conducted in Brazil, it was found that the placentas of the PE group had high levels of hydrogen peroxide and SOD; while GSH levels were positively correlated with BW.[37] An Algerian study found higher plasma hydrogen peroxide levels in LBW compared to normal weight groups, along with significant down-regulation of SOD and CAT, but no significant changes in GPX.[38] Another study conducted in China revealed that in pregnant women with GDM, the level of SOD, but not GSH, was positively associated with BW.[39] A study conducted in Africa to detect plasmodium falciparum-infected placental supernatants found that BW increased significantly with SOD and CAT levels, but decreased with GSH levels.[40] A recently published prospective birth cohort study in southern Spain, which included a normal population free of pregnancy complications, suggested a significant negative correlation between antioxidant enzymes in the prenatal blood and neonatal weight, particularly SOD.[36] In another case-control study, both GSH and SOD levels were found to be decreased in intrauterine growth restriction placentas compared to normal full-term placentas.[32] Among endogenous antioxidant enzymes, our study provides evidence that SOD level was significantly and positively correlated with BW. This is consistent with the findings of two other controlled studies that found that erythrocytes from very LBW infants exhibited lower SOD activity as well as higher oxidative damage results.[41,42] Although there is inconsistency regarding the association of SOD levels with BW, most studies support a positive correlation between SOD levels and BW. Differences in results may be related to different gestational time points, tissue specificity and factors such as race and region. Furthermore, in LBW, increased SOD can be explained as a compensatory mechanism for increased oxidative stress.[37]

Since intestinal absorption does not increase during pregnancy, the additional zinc requirements of fetal and placental tissues must be compensated for by increasing the intake of maternal tissues, and zinc deficiencies caused by inadequate dietary intake are a common phenomenon affecting up to 2 billion people worldwide.[43] Zinc is involved in embryogenesis and formation of the fetus, its low levels may lead to impaired development.[44] Studies have demonstrated that poor zinc status during pregnancy causes intrauterine growth retardation, while placental and serum zinc concentrations are significantly positively correlated with BW.[45] Several studies have revealed a positive correlation between zinc concentration and BW during pregnancy.[32,46,47] However, there is controversy about the effect of zinc on fetal development; the conclusions on the correlation between zinc and BW are inconsistent.[48] In a large birth cohort study based on a Chinese population, it was found that maternal zinc deficiency during pregnancy, especially in the second trimester, increased the risk of LBW infants.[49] Whereas another prospective cohort study in a southern Ethiopian community suggested the occurrence of zinc defects was not associated with LBW, either in mid or late pregnancy.[50] This is consistent with findings from another prospective observational study based on the diets of adolescents of different races that there were no significant differences in plasma zinc concentrations among adolescent mothers who delivered LBW infants.[51] Possible reasons for this are incorrect assessment of maternal zinc status and confounding factors such as dietary or lifestyle habits and intake of other antioxidants that interfere with the determination of an independent effect of zinc on the fetus.[45] Our MR study supports the positive association of zinc with BW. The mechanisms may involve anti-oxidants as well as anti-inflammation. Zinc, as a

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component of SOD, is closely associated with antioxidant function; down-regulated zinc levels may impair its free radical scavenging function.[32] Zinc is also responsible for the DNA-binding capacity of transcription factors through the formation of zinc finger protein molecules; on the other hand, it is also a cofactor of more than 300 enzymes and is widely involved in the regulation of various cellular processes and signal transduction.[48] Zinc is also an important component of many proteins involved in defense against oxidative stress, its deficiency impairs DNA repair mechanisms, thereby enhancing DNA damage and causing growth retardation, reproductive and immune disorders, etc.[52] In terms of anti-inflammatory, a randomized controlled trial found that zinc supplementation reduced the incidence of infection and the production of TNF-α and plasma markers of oxidative stress compared to a placebo group.[53] *In vitro* cellular studies confirm that zinc inhibits NF-κB activation by inducing A20, leading to reduced production of inflammatory cytokines.[54] Maternal serum C-reactive protein, TNF-α and IL-8 levels were significantly higher in LBW patients than in controls, while nuclear NF-κB p65 was significantly upregulated in the placenta.[49]

Lower serum selenium levels in early pregnancy was found to be associated with higher BW risk;[55] but another study found a 28% increase in selenium in the intrauterine growth restriction placenta, with a significant negative correlation with BW and placental weight.[32] It has been suggested that the increase in selenium may be a reactive increase in zinc-deficient conditions.[32] However, this study failed to provide evidence of the relationship between selenium levels with BW, which needs to be confirmed by subsequent studies.

No evidence of causal association of other exogenous antioxidants with BW was found in this study. A case-control study found increased lipid and protein peroxidation, as well as decreased vitamins A, E, C and total antioxidant capacity in cord blood of infants with LBW.[56] Two studies confirm that vitamin A, but not vitamins E and C, in umbilical cord blood has a significant effect on BW and birth height of newborns.[57,58] In mid- and late-gestation, a prospective cohort study evaluated the association of vitamin A levels with BW and found that neither retinol nor β-carotene was significantly associated with BW after adjusting for confounders.[59] Furthermore, previous meta-analysis studies have confirmed that vitamin C and E interventions alone or in combination do not significantly improve pregnancy outcomes as well as BW, which even increase the risk of pregnancy-associated hypertension.[60] This is consistent with other studies that supplementation with antioxidants (vitamins C and E) has no effect on overcoming OS or reversing the disease process.[33] Although generally considered "healthy", vitamin E use may disrupt the physiologic oxidative pregnancy state, which can be detrimental to pregnancy outcomes.[61] Therefore, relevant conclusions still need to be treated with caution, as factors such as different ethnic and dietary habits and pregnancy may interfere with observational findings, and the IVs of MR used in this study may also have insufficient statistical efficacy to bias the conclusions.

This study inevitably has a number of limitations. Firstly, only GWAS data from European populations were included and validation from other populations is needed. Secondly, in order to include more IVs, we used a relaxed *p* value screening criterion, which may reduce the power for the IVs and caused bias. Thirdly, although we used a variety of sensitivity analyses to verify the robustness of the MR results, however, potential horizontal pleiotropy could not be completely ruled out. Fourthly, because of the limitations of the GWAS source, the antioxidants included in the analysis of this study were limited, and follow-up studies are needed to explore the causal associations of other types of antioxidants with BW. At last, the associations explored based on MR were based on these antioxidants as a long-term exposure, which could not simulate exposure during different windows of pregnancy, as factors such as physiological status during different trimesters may bias the results. Therefore, high-quality studies are needed to demonstrate the association and the underlying mechanisms to determine the optimal window and dose of antioxidant supplementation.

Conclusions

Our research findings support the beneficial effects of SOD and zinc on BW. Appropriate SOD and zinc supplementation during pregnancy may prevent LBW infants. However, further studies are needed to confirm our findings and elucidate the underlying mechanisms.

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Abbreviations

BMI	body mass index
BW	birth weight
CAT	catalase
GPX	glutathione peroxidase
GSH	glutathione
GST	glutathione *S*-transferase
GWAS	genome wide association study
IV	instrumental variables
IVW	inverse variance weighted
LBW	low birth weight
LD	linkage disequilibrium
MR	Mendelian randomization
MR-PRESSO	Mendelian randomization pleiotropy residual sum and outlier
OS	oxidative stress
ROS	reactive oxygen species
SNP	single nucleotide polymorphism
SOD	superoxide dismutase
STROBE-MR	Study to Enhance the Use of Mendelian Randomization for Observational Epidemiology

Conflict of Interest

No potential conflicts of interest were disclosed.

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