Original Article

# Relationship between perinatal antioxidant vitamin and heavy metal levels and the growth and cognitive development of children at 5 years of age

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To evaluate how prenatal exposure to antioxidant vitamins and heavy metals affects subsequent development. Maternal serum and cord serum levels of antioxidant vitamins (A, E, and C) were determined. Full-state, performance, and verbal intelligence quotients (FSIQ, PIQ, and VIQ, respectively) of 97 children were assessed at 5 years of age. The placental transport ratio (PTR: cord level relative to maternal serum level) of vitamin A (VA) was associated positively with FSIQ score ($p$=0.041), vitamin E (VE)-PTR was associated positively with PIQ ($p$=0.002) and FSIQ ($p$=0.025) scores, and cord serum cadmium (Cd) level was correlated negatively with VIQ score ($p$=0.025) at 5 years of age. High VE-PTR protected against low PIQ (OR=0.025; $p$=0.021) and FSIQ (OR<0.001; $p$=0.004). High maternal age was a protective factor against low VIQ (OR=0.661; 95% CI, 0.500-0.875; $p$=0.004) and FSIQ (OR=0.700; 95% CI, 0.512-0.957; $p$=0.025). A higher maternal education (OR=0.038; 95% CI, 0.003-0.458; $p$=0.010) and economic level (OR=0.047; 95% CI, 0.004-0.579; $p$=0.017) were protective against a low FSIQ score. VA-PTR predicted physical growth. VA-PTR and VE-PTR predicted intelligence test performance at 5 years old. High Cd in cord blood may negatively affect subsequent intelligence.

**Key Words: antioxidant vitamins, heavy metals, intelligence quotients, cord blood, children**

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## INTRODUCTION

Abnormalities in the levels of micronutrients, including vitamins and minerals, have been associated with many medical conditions, as well as increased risks of developing influenza, pneumonia, certain cancers, cardiovascular disease, and musculoskeletal pain. Many epidemiological studies in adults have suggested that there are internal relationships between neurodegenerative diseases and the reduced intake and blood levels of dietary nutrients, such as the antioxidant vitamins A, C, and E.[1,2] Nevertheless, supplementation of antioxidant vitamins does not improve cognitive impairment reliably.[3-7]

A possible reason for this phenomenon is that dietary antioxidants influence cognition and behaviour development primarily during a critical period early in life. Reduced exposure to antioxidant vitamins during this early critical period may be associated with an increased incidence of neurodegenerative disease later in life.[8,9] However, such a model does not exclude the possibility of minor micronutrient effects early in life. Moreover, possible roles for antioxidants in disease initiation have not been defined and it is not known whether supplemental vitamins affect cognitive function. There may be conditions under which excess levels of particular antioxidants are harmful to development. Oxygen radicals are needed for many metabolic and physiological processes. Thus, excess antioxidation could disrupt the equilibrium between the production of a biologically important radical and its inactivation. Notably, immune cell functions are highly sensitive to the antioxidant/oxidant balance.[3,4]

Heavy metals, such as lead (Pb), mercury (Hg), and cadmium (Cd), may enter the circulation of an infant or fetus and affect their development.[10,11] Recently, multiple lines of evidence have indicated that the intelligence quotient (IQ) development of children may be influenced by multiple factors during pregnancy.[11] Although the results of these studies are not consistent, most research supports

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the conclusion that early-life lead, mercury, or cadmium exposure is strongly associated with lower child IQ scores.[12-14] Another study concluded that elevated pre- and postnatal serum lead levels were associated with small decrements in the intelligence of young children.[15]

To clarify the conclusions of these previous studies, we conducted a cohort study to determine whether antioxidant vitamin and heavy metal levels in the perinatal period influenced subsequent intelligence performance, as assessed with Wechsler intelligence scales, in children at 5 years of age.

## METHODS

### *Subjects and ethical approval*

The present work represents a 5-year follow-up cohort study in Chongqing from March, 2003 to December, 2008. A total of 150 pairs of healthy and non-smoking Chinese mothers and their children were recruited into this study. All of the mothers gave birth at 4 hospitals in Tongliang County between March 4, 2003 and June 19, 2003. Maternal exclusion criteria included: hypertension, thyroid disease, pregnancy toxaemia, diabetes mellitus, bronchial asthma, active hepatitis, chronic renal failure, and heart failure. All mothers came from the same area (Tongliang County, Chongqing, China), had similar socioeconomic situations (low to middle class), and had similar nutritional habits and statuses.

A total of 149 mothers completed the interview and contributed a cord blood sample at the time of delivery. A total of 97 mother-infant pairs remained in the study for 5 years after the follow-up visits were completed. All mothers in the study provided written informed consent and were allowed to withdraw from the study at any time for any reason. The research protocol was reviewed and approved by the Institutional Ethics Committee of the Children's Hospital of Chongqing Medical University in Chongqing, China (approval number: 002/2004).

### *Personal interview*

A trained interviewer administered a 45-min questionnaire after delivery. The questionnaire included demographic information, lifetime residential history (location of birth and duration of each residency), smoking status (active or passive) during pregnancy and maternal exposure to home and workplace chemicals, medications, and alcohol during each trimester of pregnancy. The study also collected socioeconomic information related to maternal age, educational level, height, weight before pregnancy, income, and educational level. The tester estimated gestational age based on the maternal report of the last menstrual period combined with ultrasound measurement by obstetricians.

### *Anthropometric measurements*

Anthropometric measurements were obtained by trained anthropometrists from the Children's Hospital of Chongqing Medical University. The head circumference, birth weight, and length (prior to and after 3 years of age) or height (after 3 years of age) of children was collected. Weight measurement was obtained by a digital weight scale to the nearest 50 g, with subjects in minimum clothing and bare feet. Length (for neonates) or height (after 3 years of age) was measured with a standing or supine scale to the nearest 0.1 cm in a standard position. Head circumference was measured to the nearest 0.1 cm with a non stretch plastic tape measure. All of the measurement tools were calibrated daily if necessary.

### *Blood sampling and biochemical assessment*

Maternal blood (10 mL) was collected within 24 h after delivery. Umbilical cord blood (40-60 mL) was collected at delivery. All blood samples were immediately separated from the buffy coat. The packed red blood cells and serum were stored in the dark at -70°C. Serum retinol and α-tocopherol concentrations were measured by high-performance liquid chromatography (HPLC), according to the methods of Miller and Yang, with slight modifications.[16] Briefly, after deproteinized with ethanol, retinol was extracted with hexane, and the sample was blown dry with nitrogen gas. The residue was dissolved in 0.1 mL of methanol.

An aliquot of the sample (20 μL) was injected into the column (Symmetry Shield RPl8 3.9 × 150 mm) installed with the HPLC apparatus (Waters 1525 Binary HPLC Pump, Waters Breeze, USA). The mobile phase was a methanol-$dH_2O$ mixture (95:5) (for α-tocopherol: 98:2). The retinol concentration was determined by spectrophotometry (Waters 2487 Dual λ Absorbance Detector, USA) at 315 nm (280 nm for α-tocopherol). The serum ascorbic acid concentration was measured by HPLC, according to the method described by Zhanguo and Esteve,[8,17] in which 100 mmol/L potassium dihydrogen phosphate was used as the mobile phase at pH 3.5 with phosphoric acid. The detected absorbance λ was 254 nm. In all procedures, care was taken to protect the serum from light.

One out of 10 of the samples were measured repeatedly, and the estimated variability was 0.02 μmol/L. Three control serum samples, with low (0.70 μmol/L), medium (1.40 μmol/L), and high (2.79 μmol/L) serum retinol concentrations, were generated by using retinol standard solution (Sigma, USA) with pooled serum. Intraday coefficients of variation (CVs) for the low, medium and high concentrations of serum retinol were 5.68%, 3.16%, and 1.85%, respectively; for 10, 20, and 40 μmol/L serum α-tocopherol the CV's were 4.6%, 2.2%, and 2.0%, respectively; and for 30, 60, and 120 μmol/L serum ascorbic acid the CV's were 3.2%, 2.8%, and 1.1%, respectively.

Blood mercury levels were analyzed with an AMA-254 liquid/solid mercury analyzer. Serum lead and cadmium contents were determined with a PE-800 Zeeman atomic absorption spectrometer. All biochemical factors were measured by professional technicians who worked in the Pediatric Laboratory of Chongqing Medical University, Chongqing, China.

### *Neurodevelopmental measures*

The Wechsler Preschool and Primary Scale of Intelligence is a standardized IQ test designed for children aged 4 to 6 years and 7 months of age. The Shanghai version of the Wechsler Preschool and Primary Scale of Intelligence (1985), which was standardized against the Shanghai population, was administered at age 5 years. The test has three main scales: a verbal intelligence quotient (VIQ) scale, which measures acquired knowledge, verbal reasoning and comprehension, and attention to verbal stimuli; a performance intelligence quotient (PIQ) scale, which measures fluid reasoning, spatial processing, attentiveness to detail, and visual-motor integration; and a Full Scale Intelligence Quotient (FSIQ), which measures general intellectual function. Raw scores were converted to composite scores, and age equivalencies and percentile ranks were estimated by using standardized norms. Each child was assigned an IQ in each of the three scales. The mean of the standardized IQ is 100, with a standard deviation (SD) of 15. Scores of <70 are classified as extremely low, 70-79 as borderline, 80-89 as low average, 90-109 as average, 110-119 as high average, 120-129 as superior, and ≥130 as very superior. The IQ tests were performed by two qualified psychologists who were blinded to the serum retinol concentration of the participants.

### *Statistical analysis*

The Kormogorov-Smirnov goodness-of-fit test was used to test the distribution of each set of data for normality before analysis. Results are presented as the mean±SD for data with a normal distribution and rate (%) for data with a non-normal distribution. Tests of significance were two-tailed, and a *p*-value <0.05 was considered statistically significant. A two-sample *t*-test was used to compare the inclusion and exclusion groups with respect to normal distribution. Wilcoxon nonparameter tests were used for data that were not normally distributed. All categorical variables were analyzed with the chi-squared test.

Multivariable regression models were used to estimate the effects of independent variables on the IQ scores of children. Adjusted odds ratios (ORs) were calculated for the relationship of IQ score with the cord blood concentrations and placental transport rates (PTRs) of antioxidant vitamins and heavy metals. These ORs were determined by multiple logistic regression models, after adjustment was made for the same covariates in the aforementioned final multivariable regression models. The variance inflation factor (VIF) of every independent variable was calculated, and variables with more than 10 VIFs were excluded from the models. VIF quantifies the severity of multicollinearity in an ordinary least squares regression analysis. It provides an index that measures how much the variance (the square of the estimate's standard deviation) of an estimated regression coefficient is increased because of collinearity. All statistical analyses were performed with the SAS 9.0 software package for Windows.

## RESULTS

### *Population characteristics*

Of the 150 mother-child pairs enrolled, complete epidemiological and clinical data were obtained from 97 pairs. All 97 children completed the Wechsler IQ test at their 5-year follow-up. The sociodemographic, clinical, and environmental characteristics of the cohorts are provided and compared in Tables 1 and 2. Briefly, the mothers were generally in their twenties with a healthy body mass index (BMI) before pregnancy. The education and economic levels did not vary greatly among the mothers, with marginally less than half having completed at least high school and a majority having a high economic status. All of the children included in our analysis were born full term, with a near even gender ratio. The FSIQ scores averaged near the population average, with only four children having FSIQ scores below 70. The subjects included in the analysis did not differ (*p*<0.05) from those not included with respect to the examined characteristics, except that children in the inclusion group had a higher vitamin C PTR (Wilcoxon rank sum test, *p*=0.017).

### *Effects of antioxidant vitamins and heavy metals on growth*

The results of multiple regression analysis of the relationship between the growth of children and their earlier exposure to antioxidant vitamins and heavy metals are reported in Tables 3 and 4. After adjusting for confounding factors, the vitamin A-PTR (VA-PTR; i.e. the ratio of vitamin A concentration in cord serum to that in maternal serum) was associated positively with birth weight (β=0.198; *p*=0.046) and the head circumference of children at 5 years of age (β=0.748; *p*=0.025). Baby boys were, on average, longer than baby girls at birth (β=0.758; *p*=0.026). Maternal BMI index before pregnancy was associated with increased neonatal head circumference (β=0.145; *p*=0.004). Maternal age was correlated posi-

**Table 1.** Characteristics of study participants (n=150)

| Characteristic | Mean value (SD) or percentage (ratio)* | |
|---|---|---|
| | Recruited (n=97) | Dropped out (n=53) |
| Maternal age (years)* | 25.4 (3.10) | 25.7 (3.36) |
| Height (cm)* | 157.7 (3.49) | 158.3 (4.35) |
| Weight before pregnancy (kg)* | 49.3 (5.84) | 50.2 (5.72) |
| BMI before pregnancy (kg/m$^2$)* | 19.9 (2.18) | 20.0 (2.17) |
| Head circumference (cm)* | 54.6 (1.36) | 54.5 (1.24) |
| Maternal education level (%) | | |
| <High school | 55.7 (54/97) | 43.4 (23/53) |
| ≥High school | 44.3 (43/97) | 56.6 (30/53) |
| Family economic level (%) | | |
| Low economic level | 42.7 (41/96) | 46.2 (24/52) |
| High economic level | 57.3 (55/96) | 53.8 (28/52) |
| Passive smoking exposure (%) | 57.0 (53/93) | 56.8 (21/37) |

*There was no significant difference between recruited participants and those who dropped out. Wilcoxon test was used for the comparison of continuous variables, and the $\chi^2$ test was used for categorical variables.
BMI: body mass index.

**Table 2.** Characteristics of offspring (n=150)

| Characteristic | Value[†] | | | |
|---|---|---|---|---|
| | Recruited (n=97) | | Dropped out (n=53) | |
| | Mean | SD | Mean | SD |
| Gestational age (days) | 278 | 10.2 | 276 | 13.1 |
| Birth length (cm) | 50.3 | 1.58 | 50.4 | 1.89 |
| Birth weight (kg) | 3.31 | 0.37 | 3.38 | 0.42 |
| Birth head circumference (cm) | 33.8 | 0.96 | 33.7 | 1.27 |
| Height at 5 years old (cm) | 109 | 4.31 | 110 | 4.35 |
| Weight at 5 years old (cm) | 18.0 | 2.12 | 18.1 | 2.16 |
| Head circumference at 5 years old (kg) | 50.0 | 1.32 | 49.7 | 1.38 |
| Cord serum antioxidant vitamin levels (μg/mL) | | | | |
| Vitamin A | 0.73 | 0.18 | 0.68 | 0.12 |
| Vitamin C | 98.4 | 37.4 | 101 | 46.5 |
| Vitamin E | 16.0 | 7.73 | 14.5 | 6.62 |
| Cord serum heavy metal levels (μg/L) | | | | |
| Pb | 34.5 | 11.7 | 45.1 | 30.9 |
| Cd | 0.44 | 0.43 | 0.37 | 0.31 |
| Hg | 6.88 | 3.19 | 7.38 | 3.56 |
| PTR of antioxidant vitamins and heavy metals | | | | |
| Vitamin A | 0.80 | 0.39 | 0.80 | 0.34 |
| Vitamin C[‡] | 1.74 | 1.02 | 1.36 | 0.88 |
| Vitamin E | 0.91 | 0.63 | 0.79 | 0.45 |
| Pb | 0.85 | 0.55 | 0.94 | 0.82 |
| Cd | 1.68 | 3.40 | 2.96 | 12.4 |
| Hg | 1.46 | 0.68 | 1.46 | 0.86 |
| Intelligence test performance | | | | |
| VIQ | 98.3 | 15.0 | 96.2 | 13.5 |
| PIQ | 100 | 15.8 | 98.9 | 18.8 |
| FSIQ | 99.4 | 14.4 | 97.3 | 15.2 |
| Gender of neonate (% males) | 50.5 (49/97) | | 51.2 (22/43) | |

[†] Wilcoxon test was used for the comparison of continuous variables, and the $\chi^2$ test was used for categorical variables. There was no significant difference between recruited participants and those that dropped out, except for PTR of vitamin C.

[‡] $p$=0.017.

PTR refers to the ratio of concentration between maternal and cord serum.

VIQ, PIQ, and FSIQ: verbal, performance, and full-scale intelligence quotient, respectively.

**Table 3.** Multiple regression analysis of anthropometric measurements at birth (n=97)

| Dependent variable | Independent variable | Regression coefficient (β) | | *p*-value | $R^2/R^2_{adj}$ |
|---|---|---|---|---|---|
| | | Parameter estimate | Standardized estimate | | |
| Birth length (cm) | Neonate gender | 0.758 | 0.237 | 0.026 | 0.003/0.002 |
| Birth head circumference (cm) | Maternal BMI pre-pregnancy (kg/m$^2$) | 0.145 | 0.301 | 0.004 | 0.015/0.011 |
| | Maternal ETS exposure | 0.298 | 0.151 | 0.144 | |
| Birth weight (g) | Cord serum vitamin E level (μmol/L) | -0.009 | -0.190 | 0.073 | 0.006/0.003 |
| | Placental transport ratio of vitamin A | 0.198 | 0.212 | 0.046 | |

PTR refers to the ratio of vitamin or metal concentrations between maternal and cord serum.

BMI: body mass index; ETS: environmental tobacco smoke.

tively with the height (β=0.361; *p*=0.012) and weight (β=0.180; *p*=0.021) of children at 5 years of age. The heights and head circumferences of children at 5 years of age were associated with the height and head circumference of the mothers, respectively.

### *Multiple regression analysis for relationship of IQ with perinatal exposure to antioxidant vitamins and heavy metals*

The results of multiple regression analysis after adjusting for potential confounders are shown in Table 5. The variance inflation factor (VIF) of every independent variable was calculated, and variables with more than 10 VIFs were excluded from the models. All independent variables together ($R^2$) explained 14.2%, 13.0%, and 16.8% of the variability in VIQ, PIQ, and FSIQ in children at 5 years of age, respectively. A 1% increase in the vitamin E-PTR (VE-PTR) was associated with a 23.8-point increase in PIQ (*p*=0.002) and a 15.0-point increase in FSIQ (*p*=0.025), respectively. Similarly, a 1% increase in the VA-PTR was associated with a 9.8-point increase in FSIQ (*p*=0.041). High maternal age was associated with increased VIQ (β=1.87; *p*=0.004), whereas maternal education level was correlated positively with VIQ (β=7.93; *p*=0.031), with PIQ (β=13.4; *p*=0.000), and with FSIQ (β=9.96; *p*=0.004). There was a negative correlation between cord serum Cd level and VIQ (β=-10.5; *p*=0.025). In the same model, high Pb-PTR was associated with low

**Table 4.** Multiple regression analysis of the anthropometric measurements of children at 5 years old (n=97)

| Dependent variable | Independent variable | Regression coefficient (β) |  | $p$-value | $R^2/R^2_{adj}$ |
|---|---|---|---|---|---|
|  |  | Parameter estimate | Standardized estimate |  |  |
| Height at 5 years old (cm) | Maternal height (cm) | 0.245 | 0.212 | 0.041 | 0.021/0.013 |
|  | Maternal age (years) | 0.361 | 0.258 | 0.012 |  |
|  | VA-PTR | 1.75 | 0.166 | 0.107 |  |
| Head circumference at 5 years old (cm) | Maternal age (years) | 0.067 | 0.154 | 0.137 | 0.035/0.022 |
|  | Maternal head circumference (cm) | 0.240 | 0.262 | 0.011 |  |
|  | VA-PTR | 0.748 | 0.230 | 0.025 |  |
|  | Maternal exposure to ETS | 0.503 | 0.196 | 0.061 |  |
| Weight at 5 years old (kg) | Weight before pregnancy (kg) | 0.078 | 0.207 | 0.054 | 0.021/0.011 |
|  | Maternal age (years) | 0.180 | 0.250 | 0.021 |  |
|  | Maternal education level | 0.844 | 0.199 | 0.073 |  |
|  | Maternal exposure to ETS | 0.697 | 0.164 | 0.125 |  |

PTR refers to the ratio of vitamin or metal concentrations between maternal and cord serum.
PTR: placental transport ratio; VA-PTR: PTR of vitamin A.

**Table 5.** Multiple regression analysis of independent variables in relation to IQ domain scores (n=97)

| Dependent variable | Independent variable | Regression coefficient (β) |  | $p$-value | $R^2/R^2_{adj}$ |
|---|---|---|---|---|---|
|  |  | Parameter estimate | Standardized estimate |  |  |
| VIQ | Maternal age | 1.87 | 0.350 | 0.004 | 0.142/0.093 |
|  | Maternal education level | 7.93 | 0.262 | 0.031 |  |
|  | VE-PTR | 13.3 | 0.227 | 0.052 |  |
|  | Cord serum Cd level (μg/L) | -10.5 | -0.271 | 0.025 |  |
|  | Hg-PTR | 3.58 | 0.180 | 0.136 |  |
|  | Maternal exposure to ETS | -6.79 | -0.223 | 0.065 |  |
| PIQ | Maternal education level | 13.4 | 0.413 | <0.0001 | 0.130/0.098 |
|  | VA-PTR | 10.0 | 0.212 | 0.066 |  |
|  | VE-PTR | 23.8 | 0.379 | 0.002 |  |
|  | Pb-PTR | -7.74 | -0.232 | 0.054 |  |
| FSIQ | Maternal age | 1.11 | 0.215 | 0.068 | 0.168/0.117 |
|  | Maternal education level | 9.96 | 0.339 | 0.004 |  |
|  | VA-PTR | 9.82 | 0.231 | 0.041 |  |
|  | VE-PTR | 15.0 | 0.264 | 0.025 |  |
|  | Cd-PTR | -0.618 | -0.170 | 0.142 |  |
|  | Maternal exposure to ETS | -6.41 | -0.217 | 0.063 |  |

PTR refers to the ratio of vitamin or metal concentrations between maternal and cord serum.
VIQ, PIQ, and FSIQ: verbal, performance, and full-scale intelligence quotient, respectively; VA-PTR, VE-PTR, Cd-PTR, Hg-PTR, and Pb-PTR: placental transport ratio of vitamin A, vitamin E, cadmium, mercury, and lead, respectively.

PIQ (β=-7.74; $p$=0.054). High Cd-PTR did not have a significant association with FSIQ (β=-0.61764; $p$=0.142).

### ***Logistic regression analysis for the relationship of IQ with exposure to antioxidant vitamins and heavy metals***

The results of logistic regression analysis are shown in Table 6. In the logistic multiple variable model, low IQ was defined as a score of less than 90 for the IQ test. Maternal exposure to environmental tobacco smoke (ETS) was associated with an increased prevalence of low VIQ (OR=4.60; 95% CI, 1.04-20.4; $p$=0.044) and low PIQ (OR=4.89; 95% CI, 1.22-19.6; $p$=0.025). High maternal age was a protective factor against low VIQ (OR=0.661; 95% CI, 0.500-0.875; $p$=0.004) and low FSIQ (OR=0.700; 95% CI, 0.512-0.957; $p$=0.025). A 1% increase in VE-PTR was associated with protection against low PIQ (OR=0.025; 95% CI, 0.001-0.572; $p$=0.021) and low FSIQ (OR<0.001; 95% CI, <0.001-0.028; $p$=0.004). In addition, increased maternal education and economic level were protective factors against low FSIQ (maternal education level: OR=0.038; 95% CI, 0.003-0.458; $p$=0.010, economic level of family: OR=0.047; 95% CI, 0.004-0.579; $p$=0.017).

## DISCUSSION

Early hypotheses[9,18] suggested that environmental factors, particularly maternal malnutrition during pregnancy and critical periods of neonatal development, could have a long-term impact on the health of the offspring in later life. There has been great interest in potential interactions between biochemical cofactors, including vitamins and heavy metal ions, and IQ. Previous work has shown that IQ was associated positively with dietary intake of vitamin C[19] and folate,[19,20] but was impaired by heavy metals

**Table 6.** Logistic multiple regression analysis of IQ anomaly (n=97)

| IQ anomaly | Independent variable | OR | *p*-value | 95% CI |
|---|---|---|---|---|
| Low VIQ | Maternal age | 0.661 | 0.004 | (0.500, 0.875) |
| | Maternal exposure to ETS | 4.60 | 0.044 | (1.04, 20.4) |
| Low PIQ | Maternal exposure to ETS | 4.89 | 0.025 | (1.22, 19.6) |
| | VE-PTR | 0.025 | 0.021 | (0.001, 0.572) |
| Low FSIQ | Maternal age | 0.700 | 0.025 | (0.512, 0.957) |
| | Maternal education level | 0.038 | 0.010 | (0.003, 0.458) |
| | Economic level of family | 0.047 | 0.017 | (0.004, 0.579) |
| | Child's head circumference at 5 years | 0.534 | 0.064 | (0.0275, 1.04) |
| | VE-PTR | <0.001 | 0.004 | (<0.001, 0.028) |
| | Cord serum Cd level (μmol/L) | 9.42 | 0.074 | (0.804, 110) |

PTR refers to the ratio of vitamin or metal concentrations between maternal and cord serum.
OR: odds ratio; IQ: intelligence quotient; VIQ, PIQ, and FSIQ: verbal, performance, and full-scale intelligence quotient, respectively; VE-PTR: placental transport ratio of vitamin E.

such as lead.[21, 22] This is consistent with this study, which IQ was positively with vitamins and negatively with heavy metals. Moreover, some studies have indicated that pre- or postnatal nutritional status may be associated with adult diseases, such as obesity, cancer, cardiovascular disease, metabolic syndrome, neurodegeneration, and cognitive impairment, among others.[9,23,24] Oxidative stress damage has been associated with vulnerable regions of the neurodegenerative brain and alterations that are important in the pathological mechanisms of disease.[25-27] Interactions may exist between the antioxidant vitamins A, E, and C and heavy metals, such as lead, cadmium, and mercury.[28,29] Moreover, maternal oxidative stress and the level of heavy metals during pregnancy have been shown to play important roles in the etiopathogenesis of poor birth outcomes.[10,30,31]

Previous studies have sought to determine whether vitamin and heavy metal levels at birth could impact individual neurodevelopment during infancy.[28,32,33] In this 5-year cohort study, we investigated whether antioxidant vitamin and heavy metal status during early life was associated with growth and IQ development in 97 maternal-neonatal pairs. The subjects included in the analysis did not differ ($p<0.05$) from those who were not included, except that the children in the inclusion group had a higher average vitamin C PTR (Wilcoxon rank sum test, $p=0.017$). Zhang et al found that a moderately high maternal vitamin A intake combined with neonatal vitamin A and retinoic acid supplementation benefitted early bone development.[34]

In this study, we observed a significant correlation between VA-PTR and birth weight and between VA-PTR and head circumference at 5 years of age. We also observed this same correlation previously.[29] After adjusting for confounding factors, including prenatal ETS exposure, maternal education level, and gestational age, a higher VE-PTR was positively correlated with PIQ and FSIQ. Similarly, VA-PTR was positively associated with FSIQ, whereas VE-PTR was a protective factor against low PIQ. In the relatively ordinary score range, vitamin E was a protective factor against low IQ, and vitamin A was associated with higher IQ, but was not a protective factor.

Cord serum level and PTR of vitamins are indices of maternal-neonatal nutritional status. Here we observed a higher PTR for vitamin A than what was reported in a prior investigation.[35] Placental transport efficiency indicates fetoplacental signalling of fetal nutrient demand.[36] It is worth noting that the neonatal vitamin levels in our cohort were maintained in a relatively limited range (data not shown) despite the marked swings in maternal vitamin levels that were observed. The stability of neonatal vitamin levels indicates that the placenta plays an important role in regulating vitamin transportation from mothers to baby.

Interestingly, PTRs of antioxidant vitamins correlated significantly with the growth and IQ levels of children in our study, but cord serum antioxidant vitamin levels were not. Christian et al found that prenatal supplementation with micronutrients, including vitamin E, was not associated with the intelligence development of school-aged children.[37] Our study suggests that the development of children is not only associated with cord vitamin status, but also with the needs of the fetus. Cord serum vitamin levels and vitamin PTRs are different ways of reflecting the vitamin status between mothers and neonates. The PTR was calculated as the ratio of newborn to maternal serum concentration, which more accurately indicates the fetoplacental signalling of fetal nutrient demand.[36] On the other hand, our data suggest that there was no significant correlation between vitamin C status during pregnancy and the intelligence development of children.

It was reported previously that prenatal lead, mercury, and cadmium exposures have significant effects on the intelligence development of children.[13,14,38] In the present study, there was a significant negative correlation between cord blood cadmium levels and VIQ, and an insignificant negative correlation between the PTR of lead and PIQ. This discrepancy could be due to the limited number of samples analyzed. In 2008, Lederman et al reported that cord blood mercury influenced IQ development. Although we did not find the same correlation in the present study, the cord blood mercury concentration observed in the Lederman study was double that of ours.

Some studies have reported that pregnancy after age 50 is a risk factor for the mother and child.[39,40] In the present study, maternal age was a protective factor against low VIQ and FSIQ. The average maternal age of this cohort was 25±3 years (range: 21-32 years). Therefore, we hypothesize that intelligence development and physical growth increased with both advancing maternal age until around the early 30 s, after which it might fall.

There are some limitations in this study. Firstly, the subjects of this study were recruited from hospitals which contributed to the influence of the characteristics of the women visiting the clinic for prenatal care on the results of this study. Secondly, the subjects' sample size was relatively small. A large-scale prospective study would be needed.

## *Conclusion*

We observed that VA-PTR was associated positively with physical growth in a cohort of children in Tongliang. Increased VA-PTR and VE-PTR values at delivery were beneficial to the intelligence development of children. A high cord blood cadmium concentration was harmful to the intelligence development of children at 5 years of age. Maternal ETS was associated with an increased prevalence of low VIQ and low PIQ, whereas high maternal age was a protective factor against low VIQ and low FSIQ.

## AUTHOR DISCLOSURES

The authors declare that there are no conflicts of interest.

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Original Article

# Relationship between perinatal antioxidant vitamin and heavy metal levels and the growth and cognitive development of children at 5 years of age

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# 围产期抗氧化维生素和重金属水平与5岁儿童生长和认知发展之间的关系

为了评估出生前维生素和重金属暴露对出生后生长发育的影响。我们测定了产妇血清和脐带血中抗氧化维生素（A、E和C）的含量。本研究共测量了97名5岁儿童的全量表智商、操作智商和语言智商。其中维生素A的胎盘转运率（PTR）与全量表智商成正相关（$p$=0.041），维生素E的胎盘转运率与操作智商（$p$=0.002）和全量表智商（$p$=0.025）评分成正相关。脐血镉水平与5岁儿童的语言智商呈负相关（$p$=0.025）。较高的维生素E胎盘转运率是操作智商（OR=0.025；$p$=0.021）和全量表智商（OR<0.001；$p$=0.004）的保护因素。高龄产妇是语言智商（OR=0.661；95% CI=0.500-0.875；$p$=0.004）和全量表智商（OR=0.700；95% CI=0.512-0.957；$p$=0.025）的保护因素。母亲拥有较高的受教育水平（OR=0.038；95% CI=0.003-0.458；$p$=0.010）和经济水平（OR=0.047；95% CI=0.004-0.579；$p$=0.017）是全量表智商的保护因素。维生素A的转运率可以预测出生后的生长发育。维生素A和维生素E转运率可以预测5岁儿童的智力测试水平。脐血中镉的水平越高可能对出生后的智力造成负面影响。

**关键词：抗氧化维生素、重金属、智商、脐血、孩子**