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# Adjuvant effects of vitamin A and vitamin D supplementation on treatment of children with attention-deficit/hyperactivity disorder: a study protocol for a randomised, double-blinded, placebo-controlled, multicentric trial in China



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# Adjuvant effects of vitamin A and vitamin D supplementation on treatment of children with attention-deficit/hyperactivity disorder: a study protocol for a randomised, double-blinded, placebo-controlled, multicentric trial in China

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**WHO Trial Registration Data Set**

**WHO Trial Registration Data Set**

| Data category | Information |
|---|---|
| Primary Registry and Trial Identifying Number | ClinicalTrials.gov: NCT04284059 |
| Date of Registration in Primary Registry | February 25, 2020 |
| Secondary Identifying Numbers | the Institutional Review Board of Children's Hospital of Chongqing Medical University, China: (2019) IRB (STUDY) NO.262 |
| Source(s) of Monetary or Material Support | Chongqing Municipal Education Commission (grant number: CYS19199) |
| Primary Sponsor | Li Chen |

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| Secondary Sponsor(s) | Ping Zhou |
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| Public Title | Adjuvant Effects of Vitamin A and Vitamin D Supplementation on Treatment of Children with ADHD |
| Scientific Title | Adjuvant Effects of Vitamin A and Vitamin D Supplementation on Treatment of Children with ADHD: A Randomised, Double Blind, Placebo-controlled, Multicentric Trial. |
| Countries of Recruitment | China |
| Health Condition(s) or Problem(s) Studied | Attention-deficit/hyperactivity disorder (ADHD), deficiency or insufficiency in vitamin A and vitamin D, vitamin supplementation, ADHD symptoms |
| Intervention(s) | Active comparator: |

| | |
|---|---|
| | Vitamin AD group: Vitamin AD capsule, 3 capsules/time, once a day, for 8 weeks (Vitamin A 2000 IU/capsule and vitamin D 700 IU/capsule)<br>Vitamin D group: Vitamin D capsule, 3 capsules/time, once a day, for 8 weeks (Vitamin D 700 IU/capsule)<br>Placebo comparator: Placebo capsule, 3 capsules/time, once a day, for 8 weeks (composed of oily liquids without active ingredients) |
| Key Inclusion and Exclusion Criteria | Inclusion criteria<br>1) Diagnosis of ADHD, 2) Vitamin A (≤ 1.05 μmol/L) and vitamin D (≤ 50 nmol/L), 3) Aged 6–12 years, 4) IQ ≥ 70, 5) Receiving 18–54 mg/day methylphenidate (trade name Concerta) once a day (began with 18 mg/day for a week and titrated gradually to the optimum dose not more than 54 mg/day) , 6) Obtained informed consent<br>Exclusion criteria<br>1) Use of anticonvulsant or hydrocortisone, 2) Current or past history of other neurological disorders or mental illnesses, 3) History of other metabolic disorders, 4) Use of vitamins or vitamin-containing products 3 months before the study |
| Study Type | Interventional<br>Allocation: randomised<br>Intervention model: parallel assignment<br>Masking: triple (participant, care provider, investigator)<br>Primary purpose: treatment |

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|---|---|
| | Phase 4 |
| Date of First Enrolment | March 11, 2021 (Planned) |
| Sample Size | 504 |
| Recruitment Status | Recruiting |
| Primary Outcome(s) | Outcome Name: attention-deficit/hyperactivity disorder symptoms<br>Metric/method of measurement: Vanderbilt parent assessment scale and Vanderbilt teacher assessment scale, Questionnaire – Children with Difficulties<br>Timepoint: 4 and 8 weeks following treatment |
| Key Secondary Outcomes | Outcome Name: serum concentration of vitamin A and vitamin D<br>Metric/method of measurement: high performance liquid chromatography<br>Timepoint: 8 weeks following end of treatment |
| Ethics Review | Status: Approved (Approval Number: (2019) IRB(STUDY)NO.262)<br>Date of approval: November 29, 2019<br>Name and contact details of Ethics committee(s):<br>Board Name: Institutional Review Board of Children's Hospital of Chongqing Medical University, China<br>Phone: (86)023-63622754<br>Email: joyzhaojuan@126.com<br>Address:<br>No136, 2nd Zhongshan Rd, Yuzhong District, Chongqing, China. |

<table>
<tr><td>Completion date</td><td>May 30, 2022</td></tr>
<tr><td>Summary Results</td><td>1) Date of posting of results summaries: August 30, 2022<br>2) Date of the first journal publication of results: NR<br>3) URL hyperlink(s) related to results and publications: NR<br>4) Baseline Characteristics: demographics (age, sex, height, weight, blood pressure and heart rate), serum concentration of vitamin A and vitamin D, supplementation of vitamin A/D products or vitamin A/D-containing products.<br>Clinical data: diagnosis of ADHD, disease classification, current treatment, and comorbid conditions.<br>5) Participant flow:
<table>
<tr><td></td><td colspan="4">STUDY PERIOD</td></tr>
<tr><td></td><td>Enrolment</td><td>Allocation</td><td colspan="2">Post-allocation</td><td>Close-out</td></tr>
<tr><td>TIMEPOINT</td><td>0</td><td>0</td><td>4th week</td><td>8th week</td><td>8th week</td></tr>
<tr><td>ENROLMENT:</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Eligibility screen</td><td>X</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Informed consent</td><td>X</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Allocation</td><td></td><td>X</td><td></td><td></td><td></td></tr>
<tr><td>INTERVENTIONS:</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Vitamin AD group</td><td></td><td>◆——</td><td>——</td><td>——◆</td><td></td></tr>
</table>
</td></tr>
</table>

| | | | | | | |
|---|---|---|---|---|---|---|
| | *Vitamin D group* | | ◆——— | | ———◆ | |
| | *Placebo group* | | ◆——— | | ———◆ | |
| | ASSESSMENTS: | | | | | |
| | *ADHD symptom severity* | X | X | X | X | X |
| | *Serum vitamin A concentration* | X | X | | X | X |
| | *Serum vitamin D concentration* | X | X | | X | X |

6) Adverse events: worsening of ADHD symptoms, vitamin A poisoning includes toxicity to the liver, visual impairment, bone and muscle pain..

7) Outcome measures: NR (the results are not accessible at present)

8) URL link to protocol file(s) with version and date: NR

9) Summary: This study aims to explore the effect of vitamin supplementation on symptoms of children with ADHD by administering patients with capsules of vitamin A and D, vitamin D alone, or placebo. Through this prospective study, we hope to report findings that will supplement and improve the current ADHD clinical guidelines.

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|---|---|
| IPD sharing statement | Plan to Share IPD: No |

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|---|---|
| | Data is not open to public during the study. The data that support the findings of this study are available from the corresponding author upon reasonable request. |

# Abstract

## Introduction

Approximately 7.2% of children in the world suffer from attention-deficit/hyperactivity disorder (ADHD). Due to the availability of the osmotic-release oral-system methylphenidate, ADHD currently has a remission rate of up to 30.72%. Nevertheless, it has been reported that patients with ADHD tend to exhibit vitamin A and vitamin D deficiency, which may aggravate the symptoms of ADHD. This study aims to determine the effect of vitamin A and vitamin D supplementation as adjunctive therapy to methylphenidate on the symptoms of ADHD.

## Methods and analysis

This is a parallel, prospective, interventional multicentric study. Patients will be enrolled from the southern, central, and northern parts of China. A target of 504 patients will be followed for 8 weeks. They will be allocated into 3 groups (vitamin AD, vitamin D, placebo) and administered the interventions accordingly. Data on changes in the symptoms of ADHD, as well as changes in the serum concentrations of vitamin A and vitamin D will be recorded. Both responders and non-responders based on the sociodemographic and clinical data will also be described to mitigate selection bias.

## Ethics and dissemination

This study is performed in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Children's Hospital of Chongqing Medical University, China (Approval Number: (2019) IRB (STUDY) NO.262). The results of the trial will be reported in peer-reviewed scientific journals and academic conferences regardless of the outcomes.

## Trial registration number

NCT04284059.

**Strengths and limitations of this study**

- Designed as a multi-centre study across China, thereby increasing the generalisability of the study results.
- First trial to examine vitamin A plus vitamin D supplementation on ADHD.
- Classification of ADHD will elucidate differential effects of vitamins A and D on ADHD subtypes and provide evidence regarding vitamin A and vitamin D supplementation in patients with ADHD.
- The effects of vitamin A are unclear as the effect of vitamin A alone on ADHD was not investigated.
- Methylphenidate may mask the effects of vitamin A and vitamin D owing to its strong and numerous effects.

## Background

Attention-deficit/hyperactivity disorder (ADHD) is a childhood-onset neurodevelopmental disorder with a worldwide prevalence of 7.2%[1]. It is characterized by developmentally inappropriate levels of inattention and hyperactivity or impulsivity which can profoundly affect children's academic achievement, social interactions, and self-esteem[2]. The dopamine hypothesis is thought to be the main biological mechanism underlying ADHD. Methylphenidate and amphetamine, which are both stimulants, are the first-line pharmacological agents for the treatment of ADHD[1]. A study involving 757 children with ADHD aged 6–18 years reported a 30.72% remission rate and a 16.38% recovery rate with osmotic-release oral-system methylphenidate[3]. This treatment method increases the activity of central dopamine and norepinephrine in the cortex and striatum, which are involved in executive and attentional function regulation[4]. However, it is necessary for patients with ADHD to adjust the dose of the stimulants or be co-administered nonstimulants for therapeutic

effect. In addition, it has been reported that children with ADHD suffer from deficiencies of various nutrients (vitamin D, folate, etc.) at a higher rate than their non-ADHD counterparts[5-7], and such nutritional deficiencies may exacerbate the symptoms of ADHD. Therefore, it is essential to find an effective adjuvant therapy with minimal side effects to maximize the effect of ADHD therapy.

Vitamin A, which is an anti-oxidant, plays an essential role in neuroplasticity via its active metabolite retinoic acid (RA)[8-10]. RA acts as a transcriptional regulator in the corpus striatum to regulate dopamine metabolism[11]. The concentration of 5-hydroxytryptamine, which is involved in the mechanism of ADHD along with dopamine[12], is also influenced by vitamin A[13]. Nevertheless, a research study noted that vitamin A deficiency (VAD) as a public health problem affects 5.16% , while marginal VAD (MVAD) affects 24.29% of Chinese children aged 12 years and under[14]. There has not been any accessible epidemiologic investigations about vitamin A deficiency among ADHD patients to date. However, as these individuals are particularly susceptible to VAD, more research is warranted to help this population[15]. In May 2019, our group investigated 31 outpatients with ADHD aged 6–12 years, 22 of whom were diagnosed with VAD or MVAD. Despite the limited size, this study suggested that patients with ADHD tend to have lower serum concentration of retinol, which determines a patient's vitamin A status, compared with normal children of the same age (70.97% vs. 5.16% and 24.29%).

Vitamin D is an essential fat-soluble vitamin for calcium homeostasis and bone metabolism[16]. It has been reported that retinoid X receptor influences the activity of vitamin D receptor[17-19]. Vitamin D is also involved in prompting the development and maturation of dopaminergic

neurons[20-22], which may play a potential role in the ADHD pathologies[23]. Vitamin D deficiency is highly prevalent in all age groups globally[16]. Serum 25-hydroxyvitamin D (25(OH)D) concentration, which measures a patient's vitamin D status, is significantly lower in patients with ADHD than in heathy controls[24]. Furthermore, a meta-analysis of five case-control studies demonstrated that lower vitamin D status is significantly related to the likelihood of ADHD (odds ratio: 2.57; 95% CI: 1.09–6.04; $I^2 = 84.3\%$)[5].

Aside from the exiting data, a prospective study assessing the adjuvant effects of vitamin A and vitamin D administered with methylation in the ADHD population will be performed simultaneously, under the hypothesis that vitamin A and vitamin D could enhance the effects of therapy on ADHD symptoms. Previous studies conducted by Mohammadpour N, Dehbokri N, Elshorbagy HH, et al. have found that ADHD symptoms were significantly relieved under vitamin D supplementation[25-27]. Experiments conducted on rats revealed that high vitamin A intake during pregnancy has long-lasting programming effects on the dopamine system of the offspring[28]. Basic research found that vitamin A influences vitamin D by binding to acceptors of vitamin D in vivo [29]. Based on these findings, identifying the effects of vitamin A and vitamin D on the therapy of ADHD is highly essential, as ADHD patients may be excellent candidates for vitamin A and vitamin D combination therapy.

## Study objectives

### General objective

To our knowledge, the combined effect of vitamin A and vitamin D on ADHD treatment has never been reported. Using the current treatment regimen involving methylphenidate, the present study

aims to verify the effect of vitamin D and assess the joint effect of vitamin A and vitamin D on the symptom changes of ADHD.

**Specific objectives**

1. To verify the effect of vitamin D in addition to methylphenidate on the subtypes of ADHD with a larger sample size.
2. To explore whether co-administration of vitamin A with vitamin D enhances, suppresses, or does not affect symptomatic relief of ADHD seen with vitamin D supplementation alone, and how this effect differs between ADHD subtypes. .

# Methods and analysis

**Study setting**

This multicentre, parallel, prospective, interventional study will be performed from October to May of the next year at the Children's Hospital of Chongqing Medical University, Qilu Hospital of Shandong University, and the First Hospital of Jilin University, which are located in the south, central and north regions of China, respectively. The Children's Hospital of Chongqing Medical University will act as the leading organization.

**Patient and public involvement**

The patients diagnosed with ADHD, who show deficiency or insufficiency in vitamin A (≤ 1.05 μmol/ L) and vitamin D (≤ 50 nmol/L) will be informed about the study and contacted for their informed consent if they are willing to be involved in the study. Enrolment of patients will last for 2 months. To collect the medical history of children with suspected ADHD, the junior developmental behaviour specialists will initially screen for the related symptoms in the children, and then evaluate them using the Wechsler Intelligence Scale, Vanderbilt assessment scale and

Questionnaire – Children with Difficulties (QCD). Next, parental and patient interviews will be further evaluated by developmental behaviour specialists at the associate level or above, and diagnoses will be made based on the results of a comprehensive analysis of clinical manifestations , Vanderbilt scales and QCD. The participants will be stratified by gender and randomly assigned in a double-blind fashion, at a ratio of 1:1:1, to the vitamin AD supplementation group, vitamin D supplementation group or the placebo group. Sealed, numbered, opaque envelopes containing computer-generated random numbers, which are associated with corresponding interventions, will be used. Vitamin AD supplementation group will be administrated vitamin AD capsules (3 capsules/time, once a day for 8 weeks), which contain vitamin A (2000 IU/capsule) and vitamin D (700 IU/capsule). Vitamin D supplementation group will be administrated vitamin D capsules (700 IU/capsule, 3 capsules/time, once a day for 8 weeks). The placebo capsules given to the placebo group (3 capsules/time, once a day for 8 weeks), consists of oily liquids which do not contain vitamin A and vitamin D, and were produced by Shandong DYNE Marine Biopharmaceutical Co., Ltd in China. Placebo, vitamin AD and vitamin D capsules are identical in appearance and odour to guarantee blinding. These patients will be followed up at weeks 4 and 8 following the addition of the adjunctive therapy to methylphenidate to evaluate changes in ADHD symptoms. The serum concentration of retinol and 25(OH)D will be measured at week 8. Accordingly, the placebo group and vitamin D group will be prescribed vitamin A and vitamin D as retinol and 25 (OH)D concentration after the study. The results of the study will be disseminated to the involved patients and peer-reviewed scientific journals in the form of scientific articles. However, the personal information about the patients will not be made publicly accessible and this information will be deleted after the study.

The trial design is summarized in Figure 1.

**Primary outcomes**

We will use the Vanderbilt parent assessment scale and Vanderbilt teacher assessment scale to estimate the symptoms of various ADHD subtypes (predominantly inattentive, predominantly hyperactivity/impulsive, and combined) at baseline. We will use the Vanderbilt parent follow-up scales and Vanderbilt teacher follow-up scales to estimate the changes in ADHD symptoms at weeks 4 and 8, respectively. Moreover, we will assess problems in the daily life of children at particular times of the day by Questionnaire - Children with Difficulties (QCD).

**Secondary outcomes**

The serum concentrations of vitamin A and vitamin D will be measured through high performance liquid chromatography using peripheral blood.

**Criteria**

**Inclusion criteria**

1) Diagnosis of ADHD based on the diagnostic criteria of Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5); 2) Deficiency or insufficiency in vitamin A (≤ 1.05 μmol/L) and vitamin D (≤ 50 nmol/L); 3) Aged 6–12 years; 4) Intelligence quotient (IQ)≥ 70; 5) Receiving methylphenidate (trade name Concerta) 18–54 mg/day once a day (began with 18 mg/day for a week and titrated gradually to the optimum dose not more than 54 mg/day).

**Exclusion criteria**

1) Inconsistent or changing dose of methylphenidate during the participation period; 2) Use of anticonvulsant drugs or hydrocortisone; 3) Current or past history of other neurological

disorders or mental illnesses, such as convulsions, anxiety and depression; 4) History of metabolic disorders such as cholestasis, liver dysfunction, pancreatic insufficiency, measles, diarrhoea, respiratory illness, severe inflammation, malnutrition, etc.; 5) Use of vitamins and vitamin-containing products 3 months before the study; 6) IQ < 70; 7) Serum concentration of vitamin A > 1.05 μmol/L and/or vitamin D > 50 nmol/L.

**Exit and termination criteria**

The study will be terminated if 1) the ADHD symptoms worsen, or side effects such as vitamin A poisoning, including toxicity to the liver, visual impairment, bone and muscle pain, appear during the study period; 2) The subjects drop out for other reasons.

**Recruitment**

Any patient meeting the inclusion criteria will be informed about the study and give informed consent at their personal discretion. Basic information including past medical history of the patient, family history, including any neuropsychiatric disorders, such as epilepsy, depression, ASD and ADHD, congenital diseases and metabolic diseases in his or her family, as well as results of physical examinations will be recorded. Enrolment of patients is expected to last for 2 months.

**Instruments**

**Sociodemographic and clinical data**

The sociodemographic data comes from the child's primary caregiver, detailing child's name, gender, date of birth, height, weight, blood pressure, heart rate, and supplementation of vitamin A/D products or vitamin A/D-containing products. Clinical data will be ascertained from the medical records, including information about the DSM-5 diagnosis, disease classification, current treatment, and comorbid conditions. All collected data will be double-checked.

**Evaluation methods and tools**

*Chinese version of Vanderbilt Assessment Scales[30, 31]*

The initial assessment scales are designed to measure the severity of ADHD symptoms for children aged 6 to 12. There are two versions available: parent assessment scale and teacher assessment scale. Each version consists of 3 components: symptom assessment, performance impairment and comorbid diseases. The symptom assessment screens for symptoms related to inattentive (items 1–9) and hyperactive (items 10–18) ADHD. Scores of 2 or 3 in each question of symptom assessment part (scored 0 to 3) reflect often-occurring or very often-occurring behaviours. The performance measures in the scale are located in items 49–56 and 36– 43 of the parent and teacher assessment scales, respectively. Scores of 4 or 5 in the performance measures (scored 1 to 5) mean somewhat of a problem or problematic. Items 19–48 in the parent assessment scale and items 19– 35 in the teacher assessment scale are used to screen for co-morbid diseases—oppositional-defiant disorder, conduct disorder and anxiety/depression, and scores of 2 or 3 are considered positive. To meet the DSM-5 criteria for ADHD diagnosis, one must score a 2 or 3 on 6 out of either the inattentive 9 or hyperactive 9 core symptoms or both, and score a 4 or 5 on any of the performance questions. Higher scores indicate a worse outcome. Symptom assessment combined with performance measures are divided into three subtypes: predominantly inattentive subtype, predominantly hyperactivity/impulsive subtype and combined type. The score criteria are detailed in Table 1.

Table 1. The score criteria for ADHD subtypes and comorbidities using the Vanderbilt assessment scales[30]

| | Vanderbilt parent assessment scale | Vanderbilt teacher assessment scale |
|---|---|---|

| | | Symptom section | Performance section | Symptom section | Performance section |
|---|---|---|---|---|---|
| Predominantly inattentive subtype | | At least 6 of questions 1–9 must score a 2 or 3. | At least 1 of questions 49–56 must score a 4 or 5. | At least 6 of questions 1–9 must score a 2 or 3. | At least 1 of questions 36–43 must score a 4 or 5. |
| Predominantly hyperactivity/impulsive subtype | | At least 6 of questions 10–18 must score a 2 or 3. | At least 1 of questions 49–56 must score a 4 or 5. | At least 6 of questions 10–18 must score a 2 or 3. | At least 1 of questions 36–43 must score a 4 or 5. |
| Combined type | | At least 6 of questions 1–9 and 6 of questions 10–18 must score a 2 or 3. | At least 1 of questions 49–56 must score a 4 or 5. | At least 6 of questions 1–9 and 6 of questions 10–18 must score a 2 or 3. | At least 1 of questions 36–43 must score a 4 or 5. |
| Comorbidities | Oppositional-Defiant Disorder | At least 4 of questions 19– 26 must score a 2 or 3. | A score of 4 or 5 on any of questions 49–56. | At least 3 of questions 19–28 must score a 2 or 3. | A score of 4 or 5 on any of questions 36–43. |

| | | | | | |
|---|---|---|---|---|---|
| | Conduct Disorder | At least 3 of questions 27–41 must score a 2 or 3. | A score of 4 or 5 on any of questions 49–56. | At least 3 of questions 19–28 must score a 2 or 3. | A score of 4 or 5 on any of questions 36–43. |
| | Anxiety/Depression | At least 3 of questions 42–48 must score a 2 or 3. | A score of 4 or 5 on any of questions 49–56. | At least 3 of questions 29–35 items must score a 2 or 3. | A score of 4 or 5 on any of questions 36–43. |

With the same assessment items on symptom (items 1–18) and performance (items 19–26) as the initial scales, the parent and teacher follow-up scales have added a side-effect reporting scale that can be applied to evaluate and monitor the occurrence of adverse effects to prescribed medications, if any, such as headache, stomach ache, change of appetite, extreme sadness or unusual crying and so on. The scoring criteria evaluated at weeks 4 and 8 are as follows: 1) Calculating total symptom score for questions 1–18; 2) Calculating average performance score for questions 19–26. Higher scores indicate worse outcome.

*Chinese-Wechsler Intelligence Scale for Children (C-WISC)[32]*

C-WISC is revised from the Wechsler Intelligence Scale for Children based on the Chinese cultural background, and tests the individual intelligence for children aged from 6 to 16. It is composed of

11 subtests: information, sort, arithmetic, comprehension, digit span, and vocabulary for verbal intelligence; coding, picture completion, block design, picture arrangement, mazes, and object assembly for performance intelligence. The C-WISC raw total score obtained by the sum of verbal and performance scores is transformed to IQ, including verbal IQ, nonverbal IQ, and full scale IQ, based on an algorithm. An IQ less than 70 is considered as abnormal.

*The Chinese version of Questionnaire – Children with Difficulties (QCD)[33]*

The QCD assesses problems in the daily life of children aged 6–18 years at a particular time of the day, including in the morning or evening, during or after school, and overall difficulties over the entire day and night. The Chinese version of QCD has been shown to have good validity and reliability. Filled in by the parents, the scale consists of 20 questions with respect to ADHD-related difficulties. Each question is scored on a four-point scale: 0 = completely disagree, 1 = somewhat (partially) agree, 2 = mostly agree, and 3 = completely agree. A score of 30–35 is considered the cut-off range for functional impairment and a score of less than 30 indicates functional impairment (highest score possible: 57). Lower scores indicate lower life function and more difficulty in the children's daily activities[33].

*Determination of vitamin A and vitamin D status*

The serum concentrations of retinol and 25(OH)D are measured by high performance liquid chromatography from 2 mL of venous blood. Vitamin A status is categorized as follows: < 0.35 μmol/L is considered very deficient, 0.35–0.70 μmol/L deficient, 0.70–1.05 μmol/L marginal, and > 1.05 μmol/L adequate. The values of serum vitamin D level are classified into 4 categories: < 30

nmol/L is regarded as deficiency, 30–50 nmol/L insufficiency, 50–250 nmol/L normal, and > 250 nmol/L toxic.

**Sample size**

Based on an alpha of 0.05, power of 80%, and a dropout rate of 10%, we adopted an ANOVA F-Test by using the PASS software 2020 to evaluate the sample size. This study is a randomised double-blind controlled trial. Intervention groups are vitamin AD group and vitamin D group, while the control is the placebo group. The primary outcome index is changes in ADHD symptoms as evaluated by Vanderbilt assessment scales at weeks 4 and 8 compared with that at baseline. In the study conducted by Mohammadpour N et al. [25], where the score generated using Conner's Parent Rating Scale (CPRS) was considered the main outcome, the mean ± standard deviation (SD) of ADHD index in CPRS was 55.84 ± 10.20 for the vitamin D + methylphenidate group (n = 25), and 56.79 ± 9.60 for the placebo + methylphenidate group (n = 29). The Vanderbilt assessment scale is considered as effective as the CPRS in assessing the changes of ADHD symptoms [31]. Based on the hypothesis described above, vitamin A, along with vitamin D, promotes the improvement of ADHD symptoms. We cautiously presume that the mean score ± SD for vitamin AD + methylphenidate group is lower than that of vitamin D + methylphenidate group, while the control group scores the highest using the Vanderbilt assessment scales, with a score of 54.00 ± 9.88 for the vitamin AD + methylphenidate group, 55.84 ± 10.20 for the vitamin D + methylphenidate group, and 56.79 ± 9.60 for the control group. The number of subjects to be enrolled in the study is 504.

**Statistical analysis**

All data will be analysed using the Statistical Package for the Social Sciences version 19. The normality of variables will be assessed by Kolmogorov Smirnov test. F test and Kruskal-Wallis test

will be carried out for the comparison of parametric and nonparametric variables between groups, respectively. Paired t-test and Wilcoxon signed-rank test will be used to investigate within-group differences. Confounding factors will be adjusted by the analysis of covariance.

**Bias control**

To achieve masking to prevent bias, the participants and care providers are unaware of which group they are enrolled in. Furthermore, the clinicians' roles are limited to recruiting the patients, informing the patients about the study, and then stratifying the patients by gender and randomly assigning the patients in a 1:1:1 ratio to group A, group B or group C. The intervention assignments are designated by computer-generated random numbers, which are concealed in numbered, sealed, opaque envelopes. The drugs will be dispensed by a third party, who is responsible for taking notes about the patients' basic information and medication records. After the study, the third party will give the notes to one of investigators who is not in the trial group for unblinding, and then to the outcomes assessor to complete statistical analysis, to the clinicians to provide compensatory therapy for the patients. In addition, we will describe both responders and non-responders on sociodemographic and clinical data in detail to mitigate the selection bias. Furthermore, to decrease the rate of missed follow-ups, we will contact the patients' guardians to inform them regarding adherence to the treatment regimen by Wechat (a digital communication platform), E-mail, or telephone.

## Discussion

To our knowledge, this is the first trial to examine vitamin A plus vitamin D supplementation in ADHD. Based on the known theoretic foundation-vitamin A binding to the vitamin D receptors to influence the metabolism of vitamin D, the study is expected to provide more substantial findings

regarding the potential use of vitamin A and vitamin D in addition to methylphenidate in cases of ADHD complicated by vitamin A and vitamin D deficiency, and to provide supporting data to supplement and help revise the current ADHD clinical guidelines.

As the study will be carried out in the southern, central, and northern parts of China, regional differences will be minimized. This study design not only verifies the effect of vitamin D on the treatment of ADHD using a larger sample size[25-27], but also explores whether vitamin A along with vitamin D is effective in the treatment of ADHD. At the same time, the classification of ADHD will be conducted to further elucidate the effects of vitamin A and vitamin D on ADHD and to lay a foundation to explore the mechanism underlying this condition. Although the sample size is calculated by referring to the CPRS scale rather than the Vanderbilt assessment scales, our study proposes a much larger sample size than previous studies in the literature, reducing selection bias as much as possible. There are still some limitations in our study. We will not be administering vitamin A alone as our intervention due to the restrictions on pharmaceutical production, which may pose limitations in determining the exact effect of vitamin A. Considering ethical conditions, we will be enrolling all patients with deficiency or insufficiency in vitamin A and vitamin D and administer methylphenidate along with these vitamins to improve patient adherence. As a result, we cannot conclude the effect of vitamin A or vitamin D on ADHD patients with normal serum concentrations of vitamin A and vitamin D. Furthermore, methylphenidate may mask the effects of vitamin A and vitamin D owing to its strong and numerous effects. However, these restrictions are not the Achilles' heel of this study, and the topics of the study remains to be further investigated, as

the mechanism of action of vitamins A and D on ADHD should be explored based on its promise shown in current literature.

## Ethics and dissemination

This study was approved by the Institutional Review Board of Children's Hospital of Chongqing Medical University, China (Approval Number: (2019) IRB (STUDY) NO.262).The patients participated in the study will sign the informed consent (obtained from the guardian and patients). The participants, care providers and investigators will be masked in the clinical trial. And the drugs will be dispensed by a third party. Patient's name will be abbreviated and the research data will be assigned a code. The authorization from parents on the patient's health information remains valid until the study is completed. After that, investigators will delete private information from the study record. The results of the study will be disseminated in form of academic conferences or publication in peer view of journals.

## Acknowledgements

We wish to thank Dr. Mark Lee Wolraich for authorization to use of Vanderbilt assessment scales, Shandong DYNE Marine Biopharmaceutical Co., Ltd in China for offering the vitamins and placebos for free and Beijing Harmony Health Medical Diagnostics Co.,Ltd for the technology support to measure concentrations of vitamin A and vitamin D, as well as the study participants, their families and clinicians for their contribution.

## Authors' contributions

PZ designed the study and wrote the paper. LC designed the study and conducted the writing. MW conducted the writing and gave some advices and supports on the study. AC, TL, QC, FJ, BL, YL,

TY, JC gave some advices and supports on the study. CL and BP gave statistical advices. LZ and XL revised the manuscript. All Authors read and approved the final manuscript.

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## Competing interests

None declared.

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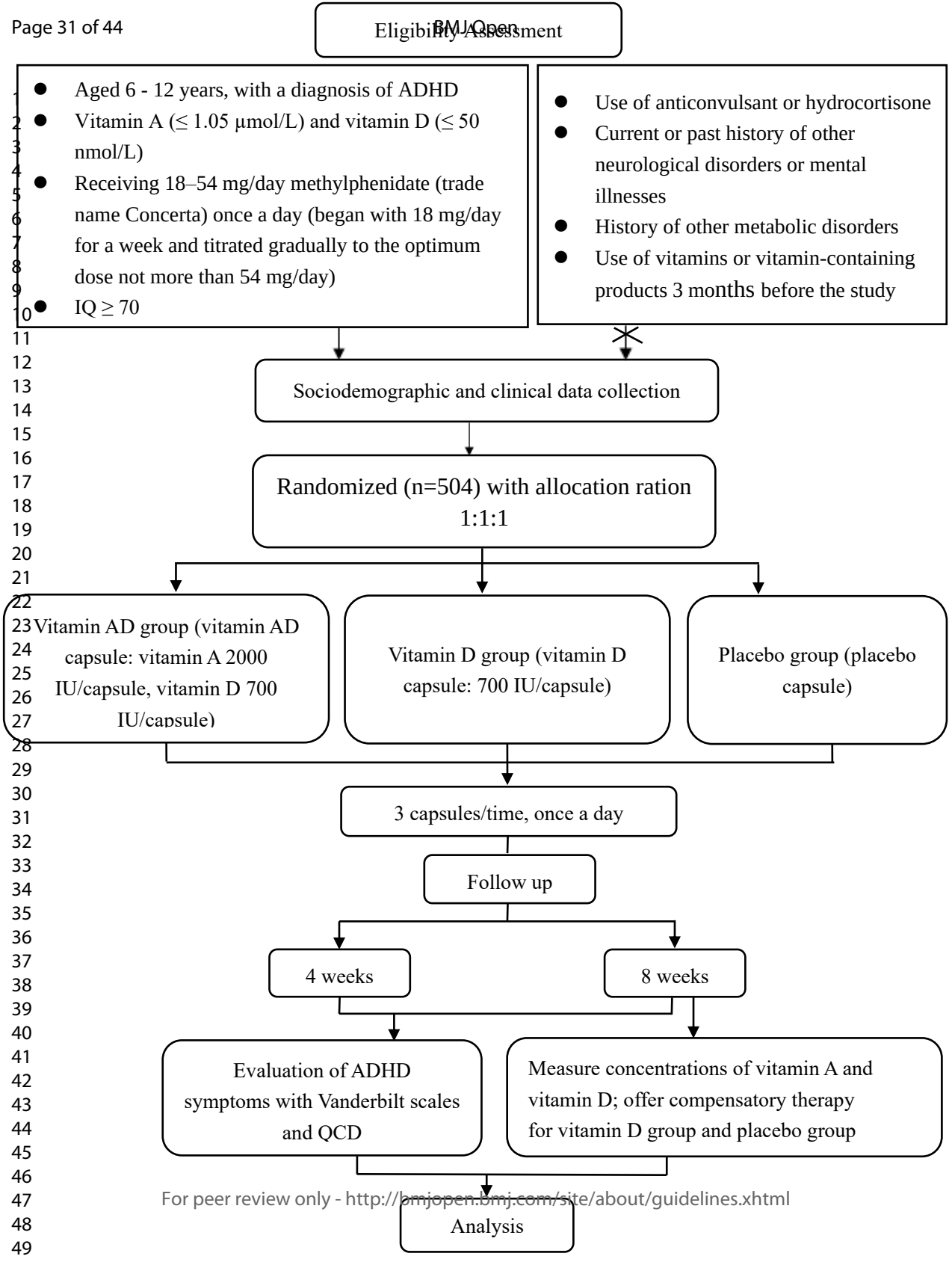
# Figure Legends

**Figure. 1 Flow diagram of the study protocol.**

ADHD, Attention-deficit/hyperactivity disorder; QCD, Questionnaire – Children with Difficulties.




SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

| Section/item | Item No | Description | Addressed on page number |
|---|---|---|---|
| **Administrative information** | | | |
| Title | 1 | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym | P1 |
| Trial registration | 2a | Trial identifier and registry name. If not yet registered, name of intended registry | P2, P10 |
| | 2b | All items from the World Health Organization Trial Registration Data Set | P2-8 |
| Protocol version | 3 | Date and version identifier | P1 (Each page) |
| Funding | 4 | Sources and types of financial, material, and other support | P24 |
| Roles and responsibilities | 5a | Names, affiliations, and roles of protocol contributors | P1, P24 |
| | 5b | Name and contact information for the trial sponsor | P3 |

| | | |
|---|---|---|
| 5c | Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities | P24 (Not indicated in the manuscript according to the format of the magazine. This paper reflects only the authors' views, and the Chongqing Municipal Education Commission is not liable for any use of the information contained in the protocol. It also played no role in the study design, writing of the protocol or decision to publish the paper.) |

| | | | |
|---|---|---|---|
| | 5d | Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) | P24 (Dr. Mark Lee Wolraich is responsible for monitoring data and quality control and he is independent from the sponsor and competing interests. Because the data of trial is doubled checked by corresponding investigators and it is a double-blinded trial, everyone played an important role are masked in the trial. Every step will be recorded in notebook and there aren't any data monitoring committee.) |
| **Introduction** | | | |
| Background and rationale | 6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention | P10-12 |
| | 6b | Explanation for choice of comparators | P13 |
| Objectives | 7 | Specific objectives or hypotheses | P12 |
| Trial design | 8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) | P4, P12-14 |

**Methods: Participants, interventions, and outcomes**

| | | | |
|---|---|---|---|
| Study setting | 9 | Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained | P13 |
| Eligibility criteria | 10 | Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) | P15-16 (P12, There aren't definite inclusion and exclusion criteria for study centres. They are located in the north, central and south of China with different latitudes and longitudes and with pediatric practice certificate.) |
| Interventions | 11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered | P13-14 and the figure 1 (Supplementary file) |
| | 11b | Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) | P16 |
| | 11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) | P22 |
| | 11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial | P16 |
| Outcomes | 12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended | P15-21 |

| | | | |
|---|---|---|---|
| Participant timeline | 13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) | P13-14 and figure 1 (Supplemental file) |
| Sample size | 14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations | P21 |
| Recruitment | 15 | Strategies for achieving adequate participant enrolment to reach target sample size | P16 |
| **Methods: Assignment of interventions (for controlled trials)** | | | |
| Allocation: | | | |
| Sequence generation | 16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions | P14 |
| Allocation concealment mechanism | 16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned | P14, P22 |
| Implementation | 16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | P21-23 |
| Blinding (masking) | 17a | Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how | P22 |

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| | 17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial | NR (There aren't detailed information about this item in the protocol. Actually, once the patients withdraw from the trial, his/her allocated intervention will be revealed and he/she will be administrated with corresponding compensatory treatment. At the same time, the new random number will be generated.) |
| **Methods: Data collection, management, and analysis** | | | |
| Data collection methods | 18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol | P16-21 |
| | 18b | Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols | P22 |
| Data management | 19 | Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol | P16, P22 |
| Statistical methods | 20a | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol | P21-22 |

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|---|---|---|---|
| | 20b | Methods for any additional analyses (eg, subgroup and adjusted analyses) | P21-22 |
| | 20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) | P21-22 |
| **Methods: Monitoring** | | | |
| Data monitoring | 21a | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed | NR (Dr. Mark Lee Wolraich is responsible for monitoring data and quality control and he is independent from the sponsor and competing interests. Because the data of trial is doubled checked by corresponding investigators and it is a double-blinded trial, everyone played an important role are masked in the trial. Every step will be recorded in notebook and there aren't any data monitoring committee.) |

| | | |
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| 21b | Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial | NR (Because the patients participated in the trial only last for 2 months and they don't withdraw from the trial until they meet the exit criterion. However, these data about the patients can be found in the inspection of the IRB and the administrative department at a higher level. They have access to these interim results and make the final decision to terminate the trial. The investigators won't conduct the interim analyses until the process of unblinding.) |

| | | | |
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| Harms | 22 | Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct | P19 (The medical records will contain the side effects of the drugs or other unintended effects of the trial and we will analyse them in the final statistical analysis.) |
| Auditing | 23 | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | P24 (Dr. Mark Lee Wolraich is responsible for this with unscheduled visits (at least 3 times during the trial), including on-the-spot audit and online meetings, and the process of on-the-spot audit is independent from investigators and sponsor. As for the online meeting, he will randomly select a few patients and audit trial conduct via internet connection.) |

**Ethics and dissemination**

| | | | |
|---|---|---|---|
| Research ethics approval | 24 | Plans for seeking research ethics committee/institutional review board (REC/IRB) approval | P5, P9, P24 |
| Protocol amendments | 25 | Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) | NR (Once we have any protocol modifications, we will record these in the clinicaltrial.gov and inform these to the IRB, journals and regulators.) |
| Consent or assent | 26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) | P22 (The clinicians will inform the potential participated patients about the trial and obtain the informed consent.) |
| | 26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable | NR(There aren't any ancillary studies.) |
| Confidentiality | 27 | How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial | P22 |
| Declaration of interests | 28 | Financial and other competing interests for principal investigators for the overall trial and each study site | NR (P25. The principal investigators and each study site declare that they have no competing interests.) |

| | | | |
|---|---|---|---|
| Access to data | 29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators | P22 (The data only open to the investigators as well as supervision department according to the corresponding laws and they will be deleted after the study.) |
| Ancillary and post-trial care | 30 | Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation | P7, P16 |
| Dissemination policy | 31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions | P9, P14, P24 |

| | | |
|---|---|---|
| 31b | Authorship eligibility guidelines and any intended use of professional writers | NR (The authorship is based on the Authorship in the "Transparency in authors' contributions and responsibilities to promote integrity in scientific publication". The results will be published on a journal and all the authors will be ranked according to their contribution level. We have asked help from the English editing service.) |

| | | | |
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| | 31c | Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code | NR (The protocol is open to everyone. However, the participant-level dataset, and statistical code are confidential during the study. The results will be published on the journal and accessible from the corresponding author upon reasonable request.) |
| **Appendices** | | | |
| Informed consent materials | 32 | Model consent form and other related documentation given to participants and authorised surrogates | NR.(In the protocol, there aren't detailed information about consent form. However, we have uploaded informed consent as supplementary.) |

| | | | |
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| Biological specimens | 33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable | NR(The specimens during the current trial will be centrifuged and measured as soon as possible after they are taken from the patients in the clinical laboratory. The specimens will be processed according to corresponding laws. In addition, there aren't any ancillary studies.) |

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

# Adjuvant effects of vitamin A and vitamin D supplementation on treatment of children with attention-deficit/hyperactivity disorder: a study protocol for a randomised, double-blinded, placebo-controlled, multicentric trial in China



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# Adjuvant effects of vitamin A and vitamin D supplementation on treatment of children with attention-deficit/hyperactivity disorder: a study protocol for a randomised, double-blinded, placebo-controlled, multicentric trial in China

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# Abstract

## Introduction

Approximately 7.2% of children in the world suffer from attention-deficit/hyperactivity disorder (ADHD). Due to the availability of the osmotic-release oral-system methylphenidate, ADHD currently has a remission rate of up to 30.72%. Nevertheless, it has been reported that patients with ADHD tend to exhibit vitamin A and vitamin D deficiency, which may aggravate the symptoms of ADHD. This study aims to determine the effect of vitamin A and vitamin D supplementation as adjunctive therapy to methylphenidate on the symptoms of ADHD.

## Methods and analysis

This is a parallel, prospective, interventional multicentric study. Patients will be enrolled from the southern, central, and northern parts of China. A target of 504 patients will be followed for 8 weeks. They will be allocated into 3 groups (vitamin AD, vitamin D, placebo) and administered the interventions accordingly. Data on changes in the symptoms of ADHD, as well as changes in the serum concentrations of vitamin A and vitamin D will be recorded. Both responders and non-responders based on the sociodemographic and clinical data will also be described to mitigate selection bias.

## Ethics and dissemination

This study is performed in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Children's Hospital of Chongqing Medical University, China (Approval Number: (2019) IRB (STUDY) NO.262). The results of the trial will be reported in peer-reviewed scientific journals and academic conferences regardless of the outcomes.

## Trial registration number

NCT04284059.

**Strengths and limitations of this study**

- Designed as a multi-centre study across China, thereby increasing the generalisability of the study results.
- First trial to examine vitamin A plus vitamin D supplementation on ADHD.
- Classification of ADHD will elucidate differential effects of vitamins A and D on ADHD subtypes and provide evidence regarding vitamin A and vitamin D supplementation in patients with ADHD.
- The effects of vitamin A are unclear as the effect of vitamin A alone on ADHD was not investigated.
- Methylphenidate may mask the effects of vitamin A and vitamin D owing to its strong and numerous effects.

## Background

Attention-deficit/hyperactivity disorder (ADHD) is a childhood-onset neurodevelopmental disorder with a worldwide prevalence of 7.2%[1]. It is characterized by developmentally inappropriate levels of inattention and hyperactivity or impulsivity which can profoundly affect children's academic achievement, social interactions, and self-esteem[2]. The dopamine hypothesis is thought to be the main biological mechanism underlying ADHD. Methylphenidate and amphetamine, which are both stimulants, are the first-line pharmacological agents for the treatment of ADHD[1]. A study involving 757 children with ADHD aged 6–18 years reported a 30.72% remission rate and a 16.38% recovery rate with osmotic-release oral-system methylphenidate[3]. This treatment method increases the activity of central dopamine and norepinephrine in the cortex and striatum, which are involved in executive and attentional function regulation[4]. However, it is necessary for patients with ADHD to adjust the dose of the stimulants or be co-administered nonstimulants for therapeutic

effect. In addition, it has been reported that children with ADHD suffer from deficiencies of various nutrients (vitamin D, folate, etc.) at a higher rate than their non-ADHD counterparts[5-7], and such nutritional deficiencies may exacerbate the symptoms of ADHD. Therefore, it is essential to find an effective adjuvant therapy with minimal side effects to maximize the effect of ADHD therapy.

Vitamin A, which is an anti-oxidant, plays an essential role in neuroplasticity via its active metabolite retinoic acid (RA)[8-10]. RA acts as a transcriptional regulator in the corpus striatum to regulate dopamine metabolism[11]. The concentration of 5-hydroxytryptamine, which is involved in the mechanism of ADHD along with dopamine[12], is also influenced by vitamin A[13]. Nevertheless, a research study noted that vitamin A deficiency (VAD) as a public health problem affects 5.16% , while marginal VAD (MVAD) affects 24.29% of Chinese children aged 12 years and under[14]. There has not been any accessible epidemiologic investigations about vitamin A deficiency among ADHD patients to date. However, as these individuals are particularly susceptible to VAD, more research is warranted to help this population[15]. In May 2019, our group investigated 31 outpatients with ADHD aged 6–12 years, 22 of whom were diagnosed with VAD or MVAD. Despite the limited size, this study suggested that patients with ADHD tend to have lower serum concentration of retinol, which determines a patient's vitamin A status, compared with normal children of the same age (70.97% vs. 5.16% and 24.29%).

Vitamin D is an essential fat-soluble vitamin for calcium homeostasis and bone metabolism[16]. It has been reported that retinoid X receptor influences the activity of vitamin D receptor[17-19]. Vitamin D is also involved in prompting the development and maturation of dopaminergic

neurons[20-22], which may play a potential role in the ADHD pathologies[23]. Vitamin D deficiency is highly prevalent in all age groups globally[16]. Serum 25-hydroxyvitamin D (25(OH)D) concentration, which measures a patient's vitamin D status, is significantly lower in patients with ADHD than in heathy controls[24]. Furthermore, a meta-analysis of five case-control studies demonstrated that lower vitamin D status is significantly related to the likelihood of ADHD (odds ratio: 2.57; 95% CI: 1.09–6.04; $I^2 = 84.3\%$)[5].

Aside from the exiting data, a prospective study assessing the adjuvant effects of vitamin A and vitamin D administered with methylation in the ADHD population will be performed simultaneously, under the hypothesis that vitamin A and vitamin D could enhance the effects of therapy on ADHD symptoms. Previous studies conducted by Mohammadpour N, Dehbokri N, Elshorbagy HH, et al. have found that ADHD symptoms were significantly relieved under vitamin D supplementation[25-27]. Experiments conducted on rats revealed that high vitamin A intake during pregnancy has long-lasting programming effects on the dopamine system of the offspring[28]. Basic research found that vitamin A influences vitamin D by binding to acceptors of vitamin D in vivo [29]. Based on these findings, identifying the effects of vitamin A and vitamin D on the therapy of ADHD is highly essential, as ADHD patients may be excellent candidates for vitamin A and vitamin D combination therapy.

## Study objectives

### General objective

To our knowledge, the combined effect of vitamin A and vitamin D on ADHD treatment has never been reported. Using the current treatment regimen involving methylphenidate, the present study

aims to verify the effect of vitamin D and assess the joint effect of vitamin A and vitamin D on the symptom changes of ADHD.

**Specific objectives**

1. To verify the effect of vitamin D in addition to methylphenidate on the subtypes of ADHD with a larger sample size.
2. To explore whether co-administration of vitamin A with vitamin D enhances, suppresses, or does not affect symptomatic relief of ADHD seen with vitamin D supplementation alone, and how this effect differs between ADHD subtypes. .

# Methods and analysis

**Study setting**

This multicentre, parallel, prospective, interventional study will be performed from February to May of the next year at the Children's Hospital of Chongqing Medical University, Qilu Hospital of Shandong University, and the First Hospital of Jilin University, which are located in the south, central and north regions of China, respectively. The Children's Hospital of Chongqing Medical University will act as the leading organization.

**Patient and public involvement**

The patients diagnosed with ADHD, who show deficiency or insufficiency in vitamin A (≤ 1.05 μmol/ L) and vitamin D (≤ 50 nmol/L) will be informed about the study and contacted for their informed consent if they are willing to be involved in the study. Enrolment of patients will last for 2 months. To collect the medical history of children with suspected ADHD, the junior developmental behaviour specialists will initially screen for the related symptoms in the children, and then evaluate them using the Wechsler Intelligence Scale, Vanderbilt assessment scale and

Questionnaire – Children with Difficulties (QCD). Next, parental and patient interviews will be further evaluated by developmental behaviour specialists at the associate level or above, and diagnoses will be made based on the results of a comprehensive analysis of clinical manifestations , Vanderbilt scales and QCD. The participants will be randomly assigned in a double-blind fashion, at a ratio of 1:1:1, to the vitamin AD supplementation group, vitamin D supplementation group or the placebo group. Sealed, numbered, opaque envelopes containing computer-generated random numbers, which are associated with corresponding interventions, will be used. Vitamin AD supplementation group will be administrated vitamin AD capsules (3 capsules/time, once a day for 8 weeks), which contain vitamin A (2000 IU/capsule) and vitamin D (700 IU/capsule). Vitamin D supplementation group will be administrated vitamin D capsules (400 IU/capsule, 6 capsules/time, once a day for 2 weeks, then change to 5 capsules/time, once a day for 6 weeks). The placebo capsules given to the placebo group (3 capsules/time, once a day for 8 weeks), consists of oily liquids which do not contain vitamin A and vitamin D, and were produced in strict accordance with China's drug management and packaging requirements for placebo by Shandong DYNE Marine Biopharmaceutical Co., Ltd in China. Placebo, vitamin AD and vitamin D capsules are identical in appearance and odour to guarantee blinding. The medicine is dispensed by the staff who was not involved in the process of evaluation, diagnosis and treatment. These patients will be followed up at weeks 4 and 8 following the addition of the adjunctive therapy to methylphenidate to evaluate changes in ADHD symptoms. The serum concentration of retinol and 25(OH)D will be measured at week 8. Accordingly, the placebo group and vitamin D group will be prescribed vitamin A and vitamin D as retinol and 25 (OH)D concentration after the study. The results of the study will be disseminated to the involved patients and peer-reviewed scientific journals in the form of scientific

articles. However, the personal information about the patients will not be made publicly accessible and this information will be deleted after the study.

The trial design is summarized in Figure 1.

**Primary outcomes**

We will use the Vanderbilt parent assessment scale and Vanderbilt teacher assessment scale to estimate the symptoms of various ADHD subtypes (predominantly inattentive, predominantly hyperactivity/impulsive, and combined) at baseline. We will use the Vanderbilt parent follow-up scales and Vanderbilt teacher follow-up scales to estimate the changes in ADHD symptoms at weeks 4 and 8, respectively. Moreover, we will assess problems in the daily life of children at particular times of the day by Questionnaire - Children with Difficulties (QCD).

**Secondary outcomes**

The serum concentrations of vitamin A and vitamin D will be measured through high performance liquid chromatography using peripheral blood.

**Criteria**

**Inclusion criteria**

1) Diagnosis of ADHD based on the diagnostic criteria of Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5); 2) Deficiency or insufficiency in vitamin A (≤ 1.05 μmol/L) and vitamin D (≤ 50 nmol/L); 3) Aged 6–12 years; 4) Intelligence quotient (IQ)≥ 70; 5) Receiving methylphenidate (trade name Concerta) 18–54 mg/day once a day (began with 18 mg/day for a week and titrated gradually to the optimum dose not more than 54 mg/day).

**Exclusion criteria**

1) Inconsistent or changing dose of methylphenidate during the participation period; 2) Use of anticonvulsant drugs or hydrocortisone; 3) Current or past history of other neurological disorders or mental illnesses, such as convulsions, anxiety and depression; 4) History of metabolic disorders such as cholestasis, liver dysfunction, pancreatic insufficiency, measles, diarrhoea, respiratory illness, severe inflammation, malnutrition, etc.; 5) Use of vitamins and vitamin-containing products 3 months before the study; 6) IQ < 70; 7) Serum concentration of vitamin A > 1.05 μmol/L and/or vitamin D > 50 nmol/L.

**Exit and termination criteria**

The study will be terminated if 1) the ADHD symptoms worsen, or side effects such as vitamin A poisoning, including toxicity to the liver, visual impairment, bone and muscle pain, appear during the study period; 2) The subjects drop out for other reasons.

**Recruitment**

Any patient meeting the inclusion criteria will be informed about the study and give informed consent at their personal discretion. Basic information including past medical history of the patient, family history, including any neuropsychiatric disorders, such as epilepsy, depression, ASD and ADHD, congenital diseases and metabolic diseases in his or her family, as well as results of physical examinations will be recorded. Enrolment of patients is expected to last for 2 months.

**Instruments**

**Sociodemographic and clinical data**

The sociodemographic data comes from the child's primary caregiver, detailing child's name, gender, date of birth, height, weight, blood pressure, heart rate, and supplementation of vitamin A/D products or vitamin A/D-containing products. Clinical data will be ascertained from the medical

records, including information about the DSM-5 diagnosis, disease classification, current treatment, and comorbid conditions. All collected data will be double-checked.

**Evaluation methods and tools**

*Chinese version of Vanderbilt Assessment Scales[30, 31]*

The initial assessment scales are designed to measure the severity of ADHD symptoms for children aged 6 to 12. There are two versions available: parent assessment scale and teacher assessment scale. Each version consists of 3 components: symptom assessment, performance impairment and comorbid diseases. The symptom assessment screens for symptoms related to inattentive (items 1–9) and hyperactive (items 10–18) ADHD. Scores of 2 or 3 in each question of symptom assessment part (scored 0 to 3) reflect often-occurring or very often-occurring behaviours. The performance measures in the scale are located in items 49–56 and 36– 43 of the parent and teacher assessment scales, respectively. Scores of 4 or 5 in the performance measures (scored 1 to 5) mean somewhat of a problem or problematic. Items 19–48 in the parent assessment scale and items 19– 35 in the teacher assessment scale are used to screen for co-morbid diseases—oppositional-defiant disorder, conduct disorder and anxiety/depression, and scores of 2 or 3 are considered positive. To meet the DSM-5 criteria for ADHD diagnosis, one must score a 2 or 3 on 6 out of either the inattentive 9 or hyperactive 9 core symptoms or both, and score a 4 or 5 on any of the performance questions. Higher scores indicate a worse outcome. Symptom assessment combined with performance measures are divided into three subtypes: predominantly inattentive subtype, predominantly hyperactivity/impulsive subtype and combined type. The score criteria are detailed in Table 1.

Table 1. The score criteria for ADHD subtypes and comorbidities using the Vanderbilt assessment scales[30]

| | | Vanderbilt parent assessment scale | | Vanderbilt teacher assessment scale | |
|---|---|---|---|---|---|
| | | Symptom section | Performance section | Symptom section | Performance section |
| Predominantly inattentive subtype | | At least 6 of questions 1–9 must score a 2 or 3. | At least 1 of questions 49–56 must score a 4 or 5. | At least 6 of questions 1–9 must score a 2 or 3. | At least 1 of questions 36–43 must score a 4 or 5. |
| Predominantly hyperactivity/impulsive subtype | | At least 6 of questions 10–18 must score a 2 or 3. | At least 1 of questions 49–56 must score a 4 or 5. | At least 6 of questions 10–18 must score a 2 or 3. | At least 1 of questions 36–43 must score a 4 or 5. |
| Combined type | | At least 6 of questions 1–9 and 6 of questions 10–18 must score a 2 or 3. | At least 1 of questions 49–56 must score a 4 or 5. | At least 6 of questions 1–9 and 6 of questions 10–18 must score a 2 or 3. | At least 1 of questions 36–43 must score a 4 or 5. |
| Comorbidities | Oppositional-Defiant Disorder | At least 4 of questions 19– 26 must score a 2 or 3. | A score of 4 or 5 on any of questions 49–56. | At least 3 of questions 19–28 must score a 2 or 3. | A score of 4 or 5 on any of questions 36–43. |

| | | | | | |
|---|---|---|---|---|---|
| | Conduct Disorder | At least 3 of questions 27–41 must score a 2 or 3. | A score of 4 or 5 on any of questions 49–56. | At least 3 of questions 19–28 must score a 2 or 3. | A score of 4 or 5 on any of questions 36–43. |
| | Anxiety/Depression | At least 3 of questions 42–48 must score a 2 or 3. | A score of 4 or 5 on any of questions 49–56. | At least 3 of questions 29–35 items must score a 2 or 3. | A score of 4 or 5 on any of questions 36–43. |

With the same assessment items on symptom (items 1–18) and performance (items 19–26) as the initial scales, the parent and teacher follow-up scales have added a side-effect reporting scale that can be applied to evaluate and monitor the occurrence of adverse effects to prescribed medications, if any, such as headache, stomach ache, change of appetite, extreme sadness or unusual crying and so on. The scoring criteria evaluated at weeks 4 and 8 are as follows: 1) Calculating total symptom score for questions 1–18; 2) Calculating average performance score for questions 19–26. Higher scores indicate worse outcome.

*Chinese-Wechsler Intelligence Scale for Children (C-WISC)[32]*

C-WISC is revised from the Wechsler Intelligence Scale for Children based on the Chinese cultural background, and tests the individual intelligence for children aged from 6 to 16. It is composed of

11 subtests: information, sort, arithmetic, comprehension, digit span, and vocabulary for verbal intelligence; coding, picture completion, block design, picture arrangement, mazes, and object assembly for performance intelligence. The C-WISC raw total score obtained by the sum of verbal and performance scores is transformed to IQ, including verbal IQ, nonverbal IQ, and full scale IQ, based on an algorithm. An IQ less than 70 is considered as abnormal.

*The Chinese version of Questionnaire – Children with Difficulties (QCD)[33]*

The QCD assesses problems in the daily life of children aged 6–18 years at a particular time of the day, including in the morning or evening, during or after school, and overall difficulties over the entire day and night. The Chinese version of QCD has been shown to have good validity and reliability. Filled in by the parents, the scale consists of 20 questions with respect to ADHD-related difficulties. Each question is scored on a four-point scale: 0 = completely disagree, 1 = somewhat (partially) agree, 2 = mostly agree, and 3 = completely agree. A score of 30–35 is considered the cut-off range for functional impairment and a score of less than 30 indicates functional impairment (highest score possible: 57). Lower scores indicate lower life function and more difficulty in the children's daily activities[33].

*Determination of vitamin A and vitamin D status*

The serum concentrations of retinol and 25(OH)D are measured by high performance liquid chromatography from 2 mL of venous blood. Vitamin A status is categorized as follows: < 0.35 μmol/L is considered very deficient, 0.35–0.70 μmol/L deficient, 0.70–1.05 μmol/L marginal, and > 1.05 μmol/L adequate. The values of serum vitamin D level are classified into 4 categories: < 30

nmol/L is regarded as deficiency, 30–50 nmol/L insufficiency, 50–250 nmol/L normal, and > 250 nmol/L toxic.

**Sample size**

Based on an alpha of 0.05, power of 80%, and a dropout rate of 10%, we adopted an ANOVA F-Test by using the PASS software 2020 to evaluate the sample size. This study is a randomised double-blind controlled trial. Intervention groups are vitamin AD group and vitamin D group, while the control is the placebo group. The primary outcome index is changes in ADHD symptoms as evaluated by Vanderbilt assessment scales at weeks 4 and 8 compared with that at baseline. In the study conducted by Mohammadpour N et al. [25], where the score generated using Conner's Parent Rating Scale (CPRS) was considered the main outcome, the mean ± standard deviation (SD) of ADHD index in CPRS was 55.84 ± 10.20 for the vitamin D + methylphenidate group (n = 25), and 56.79 ± 9.60 for the placebo + methylphenidate group (n = 29). The Vanderbilt assessment scale is considered as effective as the CPRS in assessing the changes of ADHD symptoms [31]. Based on the hypothesis described above, vitamin A, along with vitamin D, promotes the improvement of ADHD symptoms. We cautiously presume that the mean score ± SD for vitamin AD + methylphenidate group is lower than that of vitamin D + methylphenidate group, while the control group scores the highest using the Vanderbilt assessment scales, with a score of 54.00 ± 9.88 for the vitamin AD + methylphenidate group, 55.84 ± 10.20 for the vitamin D + methylphenidate group, and 56.79 ± 9.60 for the control group. The number of subjects to be enrolled in the study is 504.

**Statistical analysis**

All data will be analysed using the Statistical Package for the Social Sciences version 19. The normality of variables will be assessed by Kolmogorov Smirnov test. F test and Kruskal-Wallis test

will be carried out for the comparison of parametric and nonparametric variables between groups, respectively. Paired t-test and Wilcoxon signed-rank test will be used to investigate within-group differences. Confounding factors will be adjusted by the analysis of covariance.

**Bias control**

To achieve masking to prevent bias, the participants and care providers are unaware of which group they are enrolled in. Furthermore, the clinicians' roles are limited to recruiting the patients, informing the patients about the study, and then randomly assigning the patients in a 1:1:1 ratio to group A, group B or group C. The intervention assignments are designated by computer-generated random numbers, which are concealed in numbered, sealed, opaque envelopes. The drugs will be dispensed by the staff, who is responsible for taking notes about the patients' basic information and medication records and not involved in the process of evaluation, diagnosis and treatment. After the study, the staff will give the unblinded results to outcomes assessor to complete statistical analysis, to the clinicians to provide compensatory therapy for the patients. In addition, we will describe both responders and non-responders on sociodemographic and clinical data in detail to mitigate the selection bias. Furthermore, to decrease the rate of missed follow-ups, we will contact the patients' guardians to inform them regarding adherence to the treatment regimen by Wechat (a digital communication platform), E-mail, or telephone.

## Discussion

To our knowledge, this is the first trial to examine vitamin A plus vitamin D supplementation in ADHD. Based on the known theoretic foundation-vitamin A binding to the vitamin D receptors to influence the metabolism of vitamin D, the study is expected to provide more substantial findings regarding the potential use of vitamin A and vitamin D in addition to methylphenidate in cases of

ADHD complicated by vitamin A and vitamin D deficiency, and to provide supporting data to supplement and help revise the current ADHD clinical guidelines.

As the study will be carried out in the southern, central, and northern parts of China, regional differences will be minimized. This study design not only verifies the effect of vitamin D on the treatment of ADHD using a larger sample size[25-27], but also explores whether vitamin A along with vitamin D is effective in the treatment of ADHD. In terms of dosage, the dose of vitamin D -2100IU- isn't higher than the previous study, neither the reported 3000 IU/day of vitamin D lasting for 12 weeks in the study of Dr. Hatem Hamed Elshorbagy [27], nor 50,000 IU/week of vitamin D lasting for 6 weeks in the study of Dr. Nadia Dehbokri[26], and is as similar as 2000IU/day lasting for 8 weeks from Nakisa Mohammadpour[25]. Additionally, according to the category criterion of WHO, China is still a country with mild VA deficiency. Data from the Chinese Dietary Reference Intakes shows the UL (tolerable upper intake levels) of vitamin A in children above 4 years old is 6600IU/day [34]. Furthermore, the Nelson textbook of pediatrics 21st edition showed that 'Chronic daily intakes of 15,000 μg and 6,000 μg can be toxic in adults and children, respectively'[35]. And the reported chronic toxic dose in Chinese pediatrics textbook is 50,000 IU/day-100,000 IU/day for children, more than 6 months [36]. Considering the proportion of vitamin A and D dosage forms in China, we chose 6000IU/day of vitamin A during 3 months observation period in our study, which is lower than UL and chronic toxic dose. It's safe dose. Apart from the fact that all the patients are diagnosed, treated alone and they all take medicine separately at home, the staff dispensing the drugs does not participate in the patient's diagnosis and treatment process. Therefore, the results of the study are not be biased even though the oral amount of vitamin D capsules is different from that of

the other groups. Moreover, the classification of ADHD will be conducted to further elucidate the effects of vitamin A and vitamin D on ADHD and to lay a foundation to explore the mechanism underlying this condition. Although the sample size is calculated by referring to the CPRS scale rather than the Vanderbilt assessment scales, our study proposes a much larger sample size than previous studies in the literature, reducing selection bias as much as possible. There are still some limitations in our study. We will not be administering vitamin A alone as our intervention due to the restrictions on pharmaceutical production, which may pose limitations in determining the exact effect of vitamin A. Considering ethical conditions, we will be enrolling all patients with deficiency or insufficiency in vitamin A and vitamin D and administer methylphenidate along with these vitamins to improve patient adherence. As a result, we cannot conclude the effect of vitamin A or vitamin D on ADHD patients with normal serum concentrations of vitamin A and vitamin D. Furthermore, methylphenidate may mask the effects of vitamin A and vitamin D owing to its strong and numerous effects. However, these restrictions are not the Achilles' heel of this study, and the topics of the study remains to be further investigated, as the mechanism of action of vitamins A and D on ADHD should be explored based on its promise shown in current literature.

## Ethics and dissemination

This study was approved by the Institutional Review Board of Children's Hospital of Chongqing Medical University, China (Approval Number: (2019) IRB (STUDY) NO.262).The patients participated in the study will sign the informed consent (obtained from the guardian and patients). The participants, care providers and investigators will be masked in the clinical trial. And the drugs will be dispensed by a staff not involved in the process of diagnosis and treatment. The authorization from parents on the patient's health information remains valid until the study is completed. After

that, investigators will delete private information from the study record. The results of the study will be disseminated in form of academic conferences or publication in peer view of journals.

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## Authors' contributions

PZ designed the study and wrote the paper. LC designed the study and conducted the writing. MW conducted the writing and gave some advices and supports on the study. AC, TL, QC, FJ, BL, YL, TY, JC gave some advices and supports on the study. CL and BP gave statistical advices. LZ and XL revised the manuscript. All Authors read and approved the final manuscript.

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## Competing interests

None declared.

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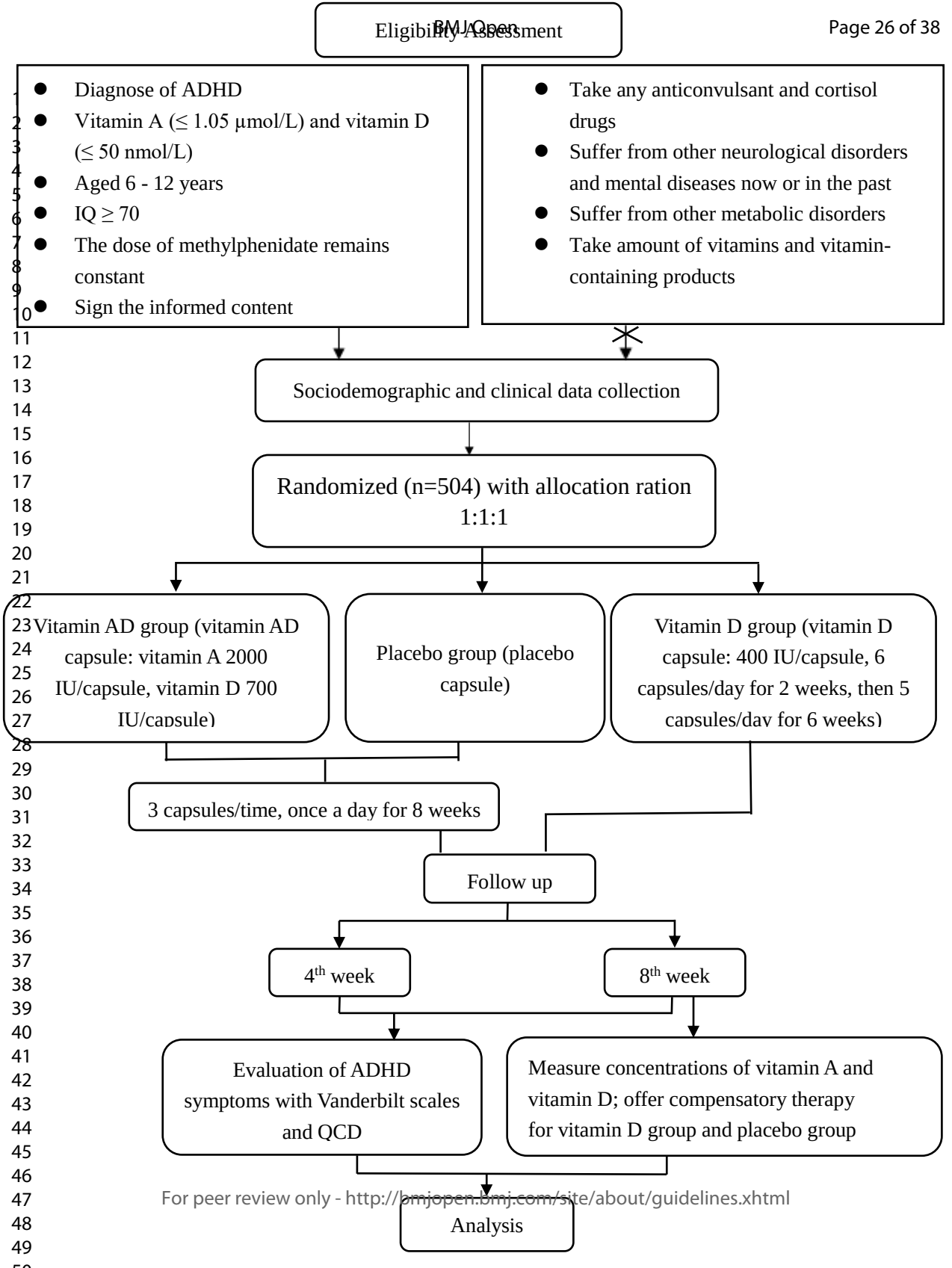
## Figure Legends

**Figure. 1 Flow diagram of the study protocol.**

ADHD, Attention-deficit/hyperactivity disorder; QCD, Questionnaire – Children with Difficulties.



SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

| Section/item | Item No | Description | Addressed on page number |
|---|---|---|---|
| **Administrative information** | | | |
| Title | 1 | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym | P1 |
| Trial registration | 2a | Trial identifier and registry name. If not yet registered, name of intended registry | P2, P10 |
| | 2b | All items from the World Health Organization Trial Registration Data Set | P2-8 |
| Protocol version | 3 | Date and version identifier | P1 (Each page) |
| Funding | 4 | Sources and types of financial, material, and other support | P24 |
| Roles and responsibilities | 5a | Names, affiliations, and roles of protocol contributors | P1, P24 |
| | 5b | Name and contact information for the trial sponsor | P3 |

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|---|---|---|
| 5c | Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities | P24 (Not indicated in the manuscript according to the format of the magazine. This paper reflects only the authors' views, and the Chongqing Municipal Education Commission is not liable for any use of the information contained in the protocol. It also played no role in the study design, writing of the protocol or decision to publish the paper.) |

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|---|---|---|---|
| | 5d | Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) | P24 (Dr. Mark Lee Wolraich is responsible for monitoring data and quality control and he is independent from the sponsor and competing interests. Because the data of trial is doubled checked by corresponding investigators and it is a double-blinded trial, everyone played an important role are masked in the trial. Every step will be recorded in notebook and there aren't any data monitoring committee.) |
| **Introduction** | | | |
| Background and rationale | 6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention | P10-12 |
| | 6b | Explanation for choice of comparators | P13 |
| Objectives | 7 | Specific objectives or hypotheses | P12 |
| Trial design | 8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) | P4, P12-14 |

**Methods: Participants, interventions, and outcomes**

| | | | |
|---|---|---|---|
| Study setting | 9 | Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained | P13 |
| Eligibility criteria | 10 | Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) | P15-16 (P12, There aren't definite inclusion and exclusion criteria for study centres. They are located in the north, central and south of China with different latitudes and longitudes and with pediatric practice certificate.) |
| Interventions | 11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered | P13-14 and the figure 1 (Supplementary file) |
| | 11b | Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) | P16 |
| | 11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) | P22 |
| | 11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial | P16 |
| Outcomes | 12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended | P15-21 |

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|---|---|---|---|
| Participant timeline | 13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) | P13-14 and figure 1 (Supplemental file) |
| Sample size | 14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations | P21 |
| Recruitment | 15 | Strategies for achieving adequate participant enrolment to reach target sample size | P16 |
| **Methods: Assignment of interventions (for controlled trials)** | | | |
| Allocation: | | | |
| Sequence generation | 16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions | P14 |
| Allocation concealment mechanism | 16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned | P14, P22 |
| Implementation | 16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | P21-23 |
| Blinding (masking) | 17a | Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how | P22 |

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| | 17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial | NR (There aren't detailed information about this item in the protocol. Actually, once the patients withdraw from the trial, his/her allocated intervention will be revealed and he/she will be administrated with corresponding compensatory treatment. At the same time, the new random number will be generated.) |
| **Methods: Data collection, management, and analysis** | | | |
| Data collection methods | 18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol | P16-21 |
| | 18b | Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols | P22 |
| Data management | 19 | Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol | P16, P22 |
| Statistical methods | 20a | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol | P21-22 |

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| | 20b | Methods for any additional analyses (eg, subgroup and adjusted analyses) | P21-22 |
| | 20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) | P21-22 |
| **Methods: Monitoring** | | | |
| Data monitoring | 21a | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed | NR (Dr. Mark Lee Wolraich is responsible for monitoring data and quality control and he is independent from the sponsor and competing interests. Because the data of trial is doubled checked by corresponding investigators and it is a double-blinded trial, everyone played an important role are masked in the trial. Every step will be recorded in notebook and there aren't any data monitoring committee.) |

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| 21b | Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial | NR (Because the patients participated in the trial only last for 2 months and they don't withdraw from the trial until they meet the exit criterion. However, these data about the patients can be found in the inspection of the IRB and the administrative department at a higher level. They have access to these interim results and make the final decision to terminate the trial. The investigators won't conduct the interim analyses until the process of unblinding.) |

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| Harms | 22 | Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct | P19 (The medical records will contain the side effects of the drugs or other unintended effects of the trial and we will analyse them in the final statistical analysis.) |
| Auditing | 23 | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | P24 (Dr. Mark Lee Wolraich is responsible for this with unscheduled visits (at least 3 times during the trial), including on-the-spot audit and online meetings, and the process of on-the-spot audit is independent from investigators and sponsor. As for the online meeting, he will randomly select a few patients and audit trial conduct via internet connection.) |

**Ethics and dissemination**

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| Research ethics approval | 24 | Plans for seeking research ethics committee/institutional review board (REC/IRB) approval | P5, P9, P24 |
| Protocol amendments | 25 | Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) | NR (Once we have any protocol modifications, we will record these in the clinicaltrial.gov and inform these to the IRB, journals and regulators.) |
| Consent or assent | 26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) | P22 (The clinicians will inform the potential participated patients about the trial and obtain the informed consent.) |
| | 26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable | NR(There aren't any ancillary studies.) |
| Confidentiality | 27 | How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial | P22 |
| Declaration of interests | 28 | Financial and other competing interests for principal investigators for the overall trial and each study site | NR (P25. The principal investigators and each study site declare that they have no competing interests.) |

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| Access to data | 29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators | P22 (The data only open to the investigators as well as supervision department according to the corresponding laws and they will be deleted after the study.) |
| Ancillary and post-trial care | 30 | Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation | P7, P16 |
| Dissemination policy | 31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions | P9, P14, P24 |

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| 31b | Authorship eligibility guidelines and any intended use of professional writers | NR (The authorship is based on the Authorship in the "Transparency in authors' contributions and responsibilities to promote integrity in scientific publication". The results will be published on a journal and all the authors will be ranked according to their contribution level. We have asked help from the English editing service.) |

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| | 31c | Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code | NR (The protocol is open to everyone. However, the participant-level dataset, and statistical code are confidential during the study. The results will be published on the journal and accessible from the corresponding author upon reasonable request.) |
| **Appendices** | | | |
| Informed consent materials | 32 | Model consent form and other related documentation given to participants and authorised surrogates | NR.(In the protocol, there aren't detailed information about consent form. However, we have uploaded informed consent as supplementary.) |

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| Biological specimens | 33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable | NR(The specimens during the current trial will be centrifuged and measured as soon as possible after they are taken from the patients in the clinical laboratory. The specimens will be processed according to corresponding laws. In addition, there aren't any ancillary studies.) |

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.