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# Establishing reference intervals for ALT, AST, UR, Cr, and UA in apparently healthy Chinese adolescents

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ABSTRACT

*Background:* The current child-specific reference intervals (RIs) are inadequate or even unavailable for many analyses in China. Many of the RIs used in Chinese laboratories were derived from Chinese adult standards or from foreign studies. The aim of this study was to establish specific RIs for alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea (UR), creatinine (Cr) and uric acid (UA) for apparently healthy Chinese adolescents.

*Methods:* Overall, 1682 apparently healthy adolescents were enrolled. Serum ALT, AST, UR, Cr and UA were measured by an ARCHITECT C-8000 automated chemistry analyzer. The 2.5th and 97.5th percentile RIs were determined using non-parametric methods.

*Results:* The established reference intervals for ALT, AST, UR, CR and UA were 7.5–42.8 U/L, 12.8–40.2 U/L, 3.12–6.38 mmol/L, 42.7–91.2 μmol/L, and 180.2–409.6 μmol/L in boys and 6.5–32.8 U/L, 10.4–32.5 U/L, 3.05–6.47 mmol/L, 40.2–88.8 μmol/L and 176.5–394.0 μmol/L in girls, respectively. The median and upper and lower limits for the RIs of ALT, AST, Cr and UA were higher in boys than they were in girls ($P < 0.05$).

*Conclusion:* RIs based on adult criteria are not applicable to adolescents. It was necessary to establish specific, accurate and suitable RIs for Chinese adolescents. We have established reference intervals of ALT, AST, UR, Cr and UA that are defined specifically for Chinese adolescents and are appropriate for universal use among Chinese laboratories.

## 1. Introduction

Reference intervals (RIs) are widely used in the process of making medical diagnoses, therapeutic management decisions, and other physiological assessments. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea (UR), creatinine (Cr), and uric acid (UA) are commonly used chemistry analytes that are widely applied in routine clinical diagnosis, treatment, prognosis, and evaluation. They reflect the current condition of a patient's liver and kidney functions, hematologic disorders, and muscular disorders [1–3]. Therefore, it is necessary to establish liver and kidney function test reference intervals that are specific for the Chinese population and can be universally applied in most hospitals across China.

To date, there are few reports on RIs for the Chinese adult population [4–7], and there have been few large population-based adolescent studies of RIs that are appropriate for laboratory applications in China [8]. In fact, the development of physiology and hormones in adolescents can lead to changes in the laboratory test values [9]. Because of the importance of establishing pediatric RIs, some large-scale projects were conducted around the world [10–13]. Therefore, it is necessary to establish RIs for serum ALT, AST, UR, Cr, and UA for adolescents in our region to offer better guidance for the medical diagnosis and treatment of diseases in adolescents.

In this study, we enrolled 1682 apparently healthy Chinese children from 12 to 18 years old, in accordance with the C28-A3 guidelines from the Clinical and Laboratory Standards Institute (CLSI) [14], to establish gender-specific RIs for ALT, AST, UR, Cr, and UA for Chinese adolescents.

## 2. Methods

### 2.1. Ethics approval

This clinical research was approved by the ethics committee of the Second Xiangya Hospital of Central South University.

*Abbreviations:* CALIPER, Canadian laboratory initiative on pediatric reference intervals; CLSI, Clinical and laboratory standards institute

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**Table 1**
Exclusion criteria according to BMI in different age groups.

| Years | Boys | | Girls | |
|---|---|---|---|---|
| | BMI(kg/m$^2$) | BMI(kg/m$^2$) | BMI(kg/m$^2$) | BMI(kg/m$^2$) |
| 12~ | ≥25.0 | ≤14.4 | ≥24.5 | ≤14.1 |
| 13~ | ≥25.3 | ≤14.8 | ≥24.9 | ≤14.6 |
| 14~ | ≥26.1 | ≤15.3 | ≥25.2 | ≤15.3 |
| 15~ | ≥26.4 | ≤15.8 | ≥25.3 | ≤16.0 |
| 16~ | ≥26.6 | ≤16.2 | ≥25.5 | ≤16.4 |
| 17–18 | ≥27.4 | ≤16.6 | ≥25.8 | ≤16.6 |

BMI: Body mass index (kg/m$^2$).

### 2.2. Subject selection

According to the (CLSI) C28-A3 guidelines [14], we collected a total of 3000 children aged 12–18 years old between January 2015 and December 2016 at the Health Management Center of the Second Xiangya Hospital of Central South University. All subjects had undergone weight, height, axillary temperature, pulse rate, and respiratory rate examinations by trained nurses and physicians. Subjects were included in the study if they were found to be eligible. The exclusion criteria were as follows: age over 12–18 years and systolic blood pressure over 90-120 mm Hg. The body mass index (BMI) of the subjects was determined according the exclusion criteria of Table 1. Subjects with total cholesterol (TC) ≥6.22 mmol/L, triglyceride (TG) ≥2.26 mmol/L, fasting blood sugar (FBS) ≥7.0 mmol/L, hemoglobin (HGB) < 110 g/L were excluded from the study. Study subjects who were on any medical treatment or who were found to be positive for human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), history of heart attack, liver or kidney disease or any infection were excluded from the study.

### 2.3. Sample collection and laboratory method

Participants were informed that over the three days prior to sample collection, they should maintain their living and diet habits as usual but should avoid alcohol intake during the last 24 h. After overnight fasting for 10 to 14 h, the subjects were asked to drink no water 1 h before collection and not to smoke half an hour before collection. Then, 5 mL of blood was collected in gel-separator vacuum test tubes (Hubei jinxing Tech co. Ltd. Hubei, China) from 8 AM to 10 AM by well-trained nurses. All samples were kept at room temperature for 30 min until clotted. All blood samples were centrifuged to separate the serum at 1200*g* for 10 min within 2 h after collection, and serum was analyzed within 2 h after separation.

Serum samples were measured for ALT (NADH method, without pyridoxal-5′-phosphate), AST (NADH method, without pyridoxal-5′-phosphate), UR (enzymatic methods), Cr (enzymatic methods) and UA (enzymatic methods) on an Architect C-8000 (Abbott laboratories, USA) automated chemistry analyzer. All reagents except quality control were provided by Abbott Laboratories. The two levels of quality control (Unassayed Advance Chemistry Control level 1 and Unassayed Advance Chemistry level 2) were run every day according to the recommendation of Abbott Laboratory. We reviewed the quality control results and acceptance criteria after changing the reagent lot (Supplementary Table 1). The reference materials GBW09174 and GBW09177 were determined (Supplementary Table 2). The laboratory underwent performance testing by the National Center for Clinical Laboratories three times per year, and five specimens were tested each time (Supplementary Table 3). Precision and accuracy were determined according to the document EP 15A [15], as recommended by CLSI. Precision was expressed as the total coefficient of variation (CV), and accuracy was expressed as the percent bias between the mean of the test results and the target value for each analysis. The estimated total CV and the percent bias were < 1/3CLIA′88.

### 2.4. Statistical analysis and processing

All statistical data were analyzed by the SPSS19.0 software according to CLSI C28-A3document [14]. The data were analyzed by a one-sample Kolmogorov-Smirnov normality test, with $P > 0.05$, if the data indicated normal distribution. If the data were normally distributed, the RIs were expressed as $\bar{\chi} \pm 1.96SD$. For a non-normal distribution, a non-parametric test was used to calculate the RIs, and the values were expressed between the 2.5th and 97.5th percentiles. A single-sample *t*-test and single-factor variance analysis were used to compare the differences in analytes in different groups.

For outlier exclusion, Dixon-Reed's outlier method was used [14,16,17]. Outliers were removed at a ratio of $D/R \geq 1/3$, where D is the absolute difference between an extreme observation (large or small) and the next largest (or smallest) observation and R is the range of all observations. If the observed value of D was equal to or greater than one-third of the range R, the extreme observation would be rejected.

## 3. Results

After screening and removing the outliers, 1682 apparently healthy children (boys 850, girls 832) were enrolled as RIs in this study. Table 2 shows the main characteristics of the participants in this study.

### 3.1. RIs of ALT, AST, UR, Cr and UA for adolescents

Table 3 shows the 95% upper and lower limits RIs for ALT, AST, UR, Cr and UA for both genders. Using the non-parametric method (2.5th and 97.5th percentiles), the upper and lower limits were determined. The 2.5th and 97.5th percentile RIs for ALT, AST, UR, CR and UA in healthy adolescents were 6.5–41.5 U/L, 11.6–39.4 U/L, 3.09–6.38 mmol/L, 40.5–90.0 μmol/L and 178.3–405.1 μmol/L, respectively. Median values were also calculated.

### 3.2. Gender-dependent RIs

The gender-dependent RIs of apparently healthy children are shown in Tables 4 and 5. ALT, AST, Cr and UA had statistically significant differences between genders ($P < 0.05$), but UR had no statistically significant difference between genders ($P > 0.05$). The median and RI upper and lower limits of ALT, AST, Cr and UA were higher in boys than they were in girls ($P < 0.05$). There were no differences between 97.5 percentile RIs, but the 2.5percentile RIs for urea, creatinine and uric acid in boys increased with age. The ALT, AST, UR, Cr and UA reference range for healthy adolescents was established according to their gender.

### 3.3. Comparison of reference intervals

Compared with the reference intervals obtained from this study, the reference intervals from different sources (Asians (Korea), Australians, and Americans (Canada), which are currently used in China) are shown in Table 6. The RIs of ALT, AST, UR, Cr, and UA showed great differences from the different sources. Compared to other studies conducted around the world, the RIs of ALT, AST, UR, Cr and UA for Chinese adolescents are closer to those of Koreans than to those of non-Asians.

## 4. Discussion

In China, the age range of 12–18 is the age of middle-school students and is a critical period for the body growth from childhood to adulthood. According to the statistics of the sixth national population census from the National Bureau of Statistics of China, conducted in 2010, nearly 16.60% of the population in China was classified as children [21]. Because children are a special group of people, the levels of