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RESEARCH ARTICLE

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Revision of serum ALT upper limits of normal facilitates assessment of mild liver injury in obese children with non-alcoholic fatty liver disease

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Abstract

Background: The serum alanine aminotransferase (ALT) level is a critical parameter for evaluating liver injury in non-alcoholic fatty liver disease (NAFLD). However, the currently accepted upper limits of normal (ULN) for serum ALT (ULN-ALT) are debated, as they may be excessively high.

Methods: A total of 1638 children aged 6-16 years, comprising 507 children with normal BMI (500 healthy children and 7 children with NAFLD), 199 overweight children, and 932 obese children, were included in the analysis. We re-evaluated the ULN-ALT in 500 healthy Chinese children using the 95th percentiles of serum ALT levels as revised ULN-ALT. Fatty liver was identified by ultrasound examination.

Results: Significant positive correlations between serum ALT levels and body mass index (BMI) were detected in overweight boys ($r = .399$, $P < .001$), obese boys ($r = .398$, $P < .001$), and obese girls ($r = .392$, $P < .001$). The prevalence percentages of NAFLD were 93.6%, 75.8%, and 37.9% in obese boys with serum ALT levels of >50, 25-50, and ≤25 U/L and were 81.6%, 67.9%, and 20.6% in obese girls with serum ALT levels of >40, 20-40, and ≤20 U/L, respectively.

Conclusion: Serum ALT levels significantly correlated with abnormal BMI values in children, suggesting a rigorous BMI threshold is needed to establish the cutoffs for

serum ULN-ALT in children. Besides, the revised serum ULN-ALT can uncover mild liver injury in obese children with NAFLD.

KEYWORDS
alanine aminotransferase, body mass index, non-alcoholic fatty liver disease, upper limit of normal

1 | INTRODUCTION

Childhood obesity has become an alarming public health issue worldwide.[1,2] The prevalence of non-alcoholic fatty liver disease (NAFLD) in children parallels the dramatic rise in childhood obesity.[3,4] The accumulation of fat in the liver could lead to liver damage, which may progress to non-alcoholic steatohepatitis, cirrhosis, and end-stage liver disease.[5] However, children with NAFLD are usually asymptomatic and have no physical signs of chronic liver disease, which is a significant challenge for clinical diagnosis.[6,7] Liver biopsy is the current gold standard for the definitive diagnosis of NAFLD. As an invasive technique, liver biopsy is generally unacceptable in children. Moreover, the method is not entirely unreliable, owing to sampling heterogeneity.[8-10] At present, ultrasonography is the main approach for diagnosing fatty liver disease; however, it is not commonly applied to children for their compliance, limiting the early detection of steatohepatitis.[11,12]

Serum alanine aminotransferase (ALT) is a widely used indicator for the detection of liver damage in clinical practice.[13-15] An appropriate upper limit of normal for serum ALT (serum ULN-ALT) is needed to assess the abnormal liver function of patients.[16] Previous studies have demonstrated that the serum ALT level is valuable for screening fatty liver disease in children, and elevated serum ALT levels are essential for evaluating the progression of NAFLD.[17-19] However, the currently accepted serum ULN-ALT (50 U/L for boys, and 40 U/L for girls) for Chinese children has been fiercely debated. An inaccurate set of ULNs may underestimate mild liver injury and lead to misdiagnosis of the natural course of hepatitis B virus (HBV) infection.[20-22]

In the present study, using a database of liver function chemistry of healthy children, we re-evaluated the serum ULN-ALT in Chinese children and analyzed the correlation between BMI and ALT levels. In addition, we compared the sensitivity of the revised and current ULNs in assessing the fatty liver. Our study may be helpful to uncover mild liver injuries in children with NAFLD.

2 | MATERIALS AND METHODS

2.1 | Data collection and healthy children for evaluating serum ALT parameters

Data were collected from the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University. Children aged 6-16 years between the July 2016 and June 2018 were included in the study. The data were primarily collected from a database of health check-ups, which were mainly required by schools and/or parents. If the child had multiple checks, only the data set for the first check-up was included.

To ensure working with a primarily healthy and normal BMI cohort, we carried out several steps of exclusion (Figure 1). Healthy children were excluded if they had any of the following conditions: (a) an abnormal body mass index (BMI; outside the normal range of the 5th-85th percentiles),[23] (b) positive for HBV surface antigen (HBsAg), (c) positive for hepatitis C virus (HCV) antibodies, (d) abnormal blood routine tests, and (e) abnormal abdominal ultrasonography.

2.2 | Overweight and obese children

The definitions of "overweight" and "obese" based on BMI for children shift throughout childhood for both sexes; changes in healthy BMI are not linear during childhood.[24,25] In this study, the national BMI reference established and proposed by the Working Group on Obesity in China (WGOC) was used to screen for overweight and obese children[26]; this reference corresponded with the Eastern Asia ethnic characteristics of body fat growth. BMI values in the 85th percentile and 95th percentile were used as thresholds for "overweight" and "obese" children, consistent with the recommendations of Barlow and the Expert Committee.[23] Children with overweight and obese statuses were also excluded if they had common causes of liver disease, such as HBV, HCV, and autoimmune hepatitis. BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). To standardize BMI values at different ages and genders, BMI grades were calculated as BMI grade = (BMI – overweight BMI)/(obese BMI – overweight BMI) + 1. The cutoffs for overweight and obese classifications were derived from the national BMI reference established by WGOC.

2.3 | Detection of serum alanine aminotransferase

Serum ALT levels were determined using the continuous monitoring assay (Aptio Automation; Siemens ADVIA2400 Automatic Biochemical Analyzer). The test parameters were set according to the manufacturer's protocol. The linear range of the assay for serum ALT was 0-1500 U/L, and the reagents were all used within the valid period. During the observation period, two levels of indoor quality

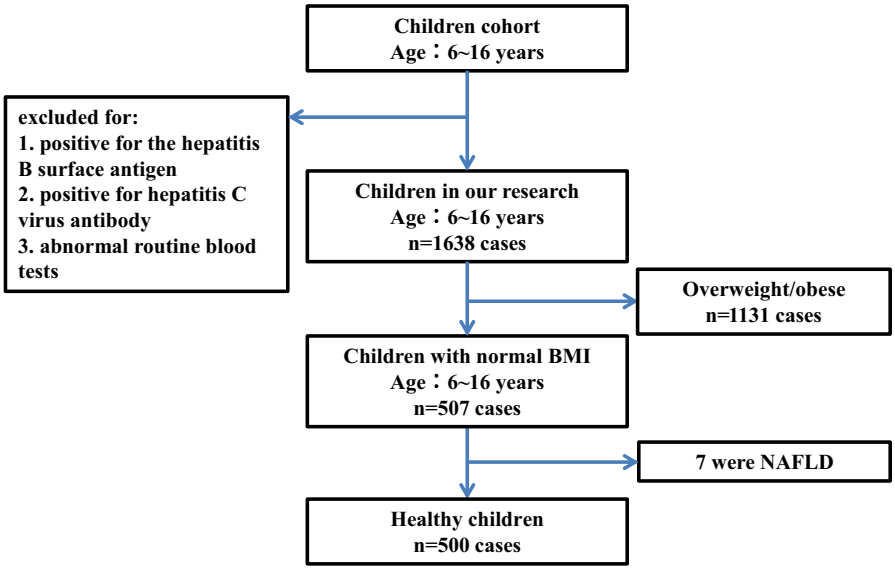

FIGURE 1 Flow diagram of screening for healthy children excluding overweight/obese or NAFLD. The reference population were composed of children participated in the health check-up. The criterion of include and exclude were shown as in the flowchart

control were performed every day, and the horizontal deviations of the two observation items were within 2 standard deviations.

2.4 | Classification of non-NAFLD and NAFLD based on the ultrasound examination

Children without known liver disease or significant alcohol use were defined as having NAFLD if their liver imaging is consistent with the diagnostic criteria of fatty liver.[27,28] An ultrasound examination of the liver was performed for 1638 children in the supine position. The diagnosis of hepatic steatosis was made according to criteria proposed by Saverymuttu et al based on a comparison of the hepatic and renal parenchyma, considering the dorsal attenuation, penetration of the diaphragm, and ability to assess liver vessels.[29]

2.5 | Statistical analysis and ethics

Under standard practice, the serum ULN-ALT was defined as the 95th percentile of the distribution of values. Based on the data type, normality, and variance, the non-parametric Mann-Whitney *U* test was selected to evaluate differences in the levels of serum ALT. The Spearman rank correlation coefficient analysis was performed to determine the association between serum ALT levels and BMI. Chi-squared tests were used to assess differences in the prevalence of NAFLD. The ability to predict NAFLD by serum ALT was measured by the area under the ROC curve (AUC), sensitivity, and specificity. All statistical analyses were based on two-sided hypothesis tests with a significance level of $P < .05$ using SPSS V19.0 (SPSS).

The study design was in accordance with the ethical guidelines of the Declaration of Helsinki and was approved by the Ethics Committees of the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University. As a retrospective analysis of existing administrative and clinical data, the requirement to obtain informed patient consent was waived by the Ethics Committees.

3 | RESULTS

3.1 | Characteristics of children according to gender and BMI-for-age

A total of 1638 children aged 6-16 years were recruited. Children under the age of 6 years were not included owing to the rare occurrence of fatty liver disease in this population.[30] The characteristics of the study cohort are summarized in Table 1. These children included 1222 boys (74.6%) and 416 girls (25.4%); 932 (56.9%) were obese group, 199 (12.1%) were overweight group, and 507 (30.6%) with normal BMI were control group. The definitions of "overweight" and "obese" based on gender and BMI-for-age are described in the materials and methods. There were no significant differences in age among control, overweight, and obese groups for both sexes (Table 1). However, overweight and obese groups were far more likely to have increased serum ALT levels compared to control group ($P < .001$). In addition, the serum ALT level was significantly higher in obese group than in overweight group in both boys and girls ($P < .001$).

3.2 | Correlation between serum ALT levels and BMI in children

Serum ALT levels were significantly related to BMI in boys in the overweight ($r = .399$, $P < .001$) and obese groups($r = .398$, $P < .001$) (Figure 2C, E). Girls in the obese group also showed a significant positive correlation between serum ALT levels and BMI ($r = .392$, $P < .001$) (Figure 2F). However, no such association was observed in control group (Figure 2A, B) and in overweight girls (Figure 2D).

TABLE 1 Demographics and laboratory profiles of children

	Control group	Overweight group	Obese group
Boy (n)	338	168	716
Age (y)	9 (6 ~ 16)	11 (6 ~ 15)	10 (6 ~ 16)
BMI (kg/m^2)	16.09 (9.79 ~ 21.21)	21.18 (16.62 ~ 26.47)	25.76 (17.74 ~ 38.14)
ALT (U/L)	13 (5 ~ 220)	17.5 (7 ~ 152)***	30 (3 ~ 294)***
Girl (n)	169	31	216
Age (y)	8 (6 ~ 15)	9 (6 ~ 16)	9 (6 ~ 16)
BMI (kg/m^2)	15.70 (12.56 ~ 20.83)	18.99 (16.23 ~ 26.30)	25.05 (18.11 ~ 41.8)
ALT (U/L)	12 (5 ~ 35)	12 (7 ~ 74)***	21 (5 ~ 238)***

Note: Values are medians (range) or numbers. Non-parametric Mann-Whitney *U* tests were used to evaluate differences.

***P < .001 for the comparison between the overweight/obese group and control.

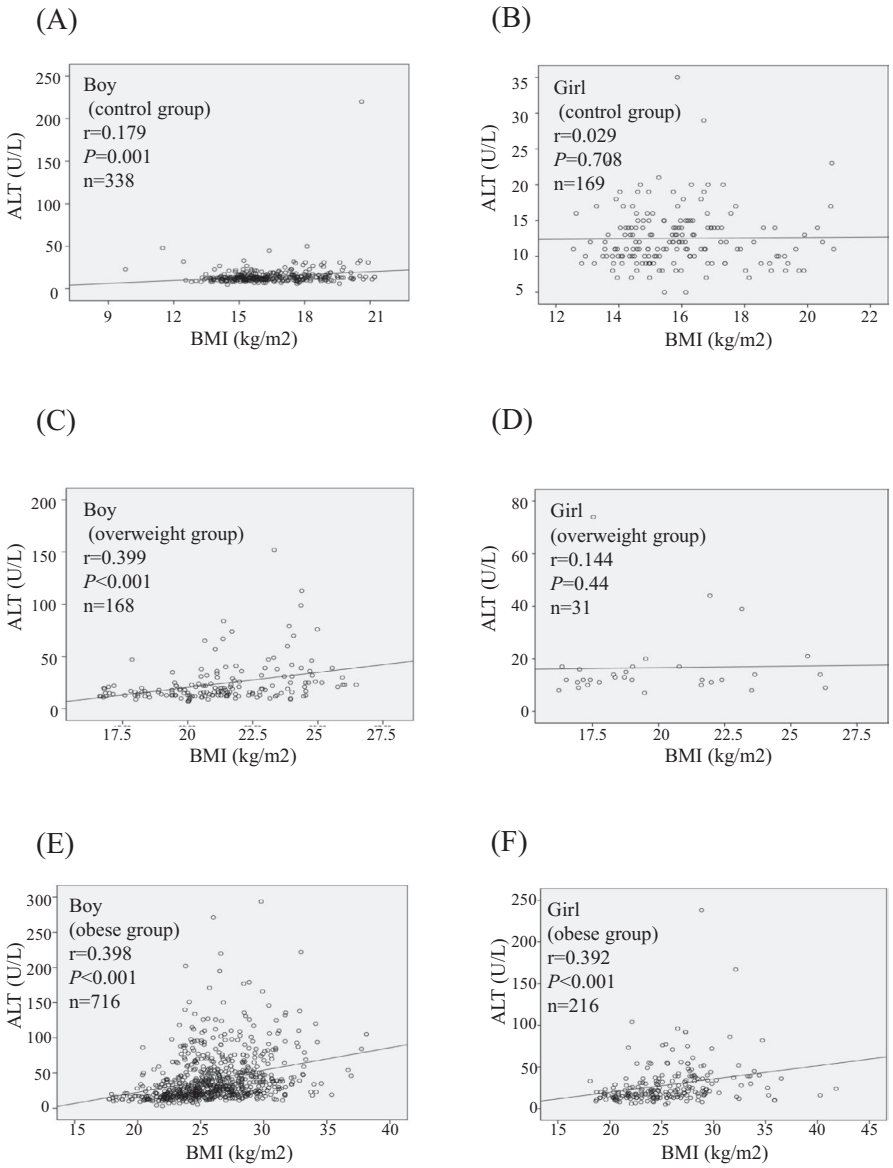

FIGURE 2 Correlation between serum ALT and BMI in children. Scatter plot showed associations between serum ALT levels and BMI in (A, B) control, (C, D) overweight, and (E, F) obese groups. Correlation coefficients (*r*) were analyzed using Spearman tests

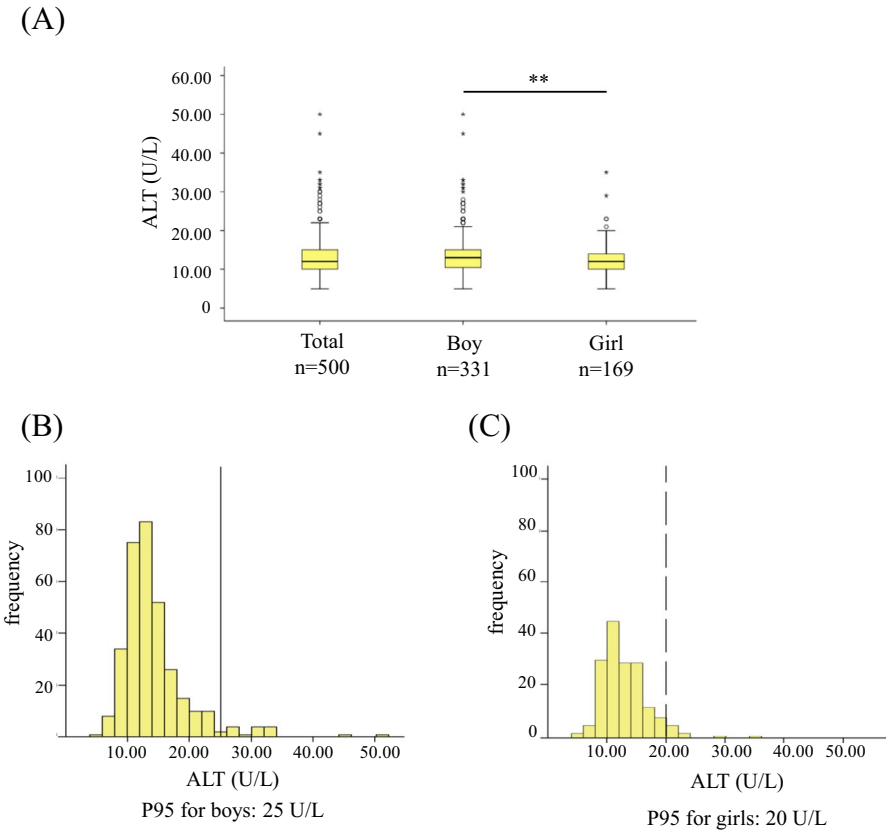

FIGURE 3 Determination of the 95th percentiles of serum ALT levels in healthy children. A, Distributions of serum ALT levels in boys and girls; median serum ALT levels are labeled. B, The 95th percentile of normal serum ALT in boys is 25 U/L, indicated by a solid line. C, The 95th percentile of normal serum ALT in girls is 20 U/L, indicated by a dashed line. Differences in serum ALT levels were analyzed using Mann-Whitney *U* tests. $^{**}P < .01$

These results indicated that significant positive correlations between serum ALT levels and BMI were specific to obese children as well as overweight boys($r > .3$, $P < .001$).

3.3 | Determination of the 95th percentiles of serum ALT levels in healthy children

After excluding 7 with NAFLD, 500 healthy children were screened out from the control group, including 331 boys (66.2%) and 169 girls (33.8%). The mean ages were 9.1 ± 2.4 years (median, 9 years) for boys and 8.4 ± 2.4 years (median, 8 years) for girls. The mean BMI values were 16.35 ± 1.80 kg/m^2 (median, 16.05 kg/m^2) for boys and 15.82 ± 1.80 kg/m^2 (median, 15.70 kg/m^2) for girls. The serum ALT levels in boys were significantly higher than those in girls ($P < .01$), and the median serum ALT levels were 13 U/L in boys and 12 U/L in girls (Figure 3A). The 95th percentiles of serum ALT levels in boys and girls were 25 U/L and 20 U/L (Figure 3B, C). To distinguish these thresholds from the current accepted serum ULN-ALT (50 U/L for boys and 40 U/L for girls), we defined our calculated gender-specific values as the "revised serum ULN-ALT" (25 U/L for boys and 20 U/L for girls).

3.4 | Assessment of NAFLD in children by using the revised and current serum ULN-ALT

The prevalence of NAFLD was 93.6%, 75.8%, and 37.9%, respectively, in obese boys with serum ALT levels of >50 U/L (n = 187), 25-50 U/L (n = 244), and ≤25 U/L (n = 285); they were 81.6%, 67.9%, and 20.6% in obese girls with serum ALT levels of >40 U/L (n = 38), 20-40 U/L (n = 81), and ≤20 U/L (n = 97), respectively (Figure 4A, B). It is worth noting that 185 of 244 obese boys (75.8%) and 55 of 81 obese girls (67.9%) who had normal serum ALT levels according to the current serum ULN-ATL but levels exceeding the revised serum ULN-ALT had NAFLD. These results indicated that our revised serum ULN-ALT could increase detection rate of mild liver injury in obese children with NAFLD.

To explore an optimal serum ALT cutoff point for assessing NAFLD, we obtain the point that represents the largest sum of sensitivity and specificity, based on ROC analyses (Figure 4C, D). Our data showed that the threshold of serum ALT for NAFLD is 22 U/L in boys and 20 U/L in girls. The AUC (95% CI) for NAFLD is 0.894 (0.876, 0.912) in boys and 0.893 (0.859, 0.928) in girls. Our revised pediatric ULNs (boy: sensitivity, 74.8%; specificity, 85.4%; positive predictive value, 79.8%; negative predictive value, 81.5%; girl: sensitivity, 80.7%; specificity, 85.3%; positive predictive value, 66.2%; negative predictive value 92.6%) had a better assessment than current ULNs (boy: sensitivity, 35.2%; specificity, 99.8%; positive predictive value, 93.0%; negative predictive value, 66.2%; girl: sensitivity, 29.4%; specificity, 97.4%; positive predictive value, 80.0%; negative predictive value 79.5%) (Table 2).

4 | DISCUSSION

Setting an appropriate ULN-ALT for children is still a challenging issue.[31] Inappropriate reference intervals might affect clinical

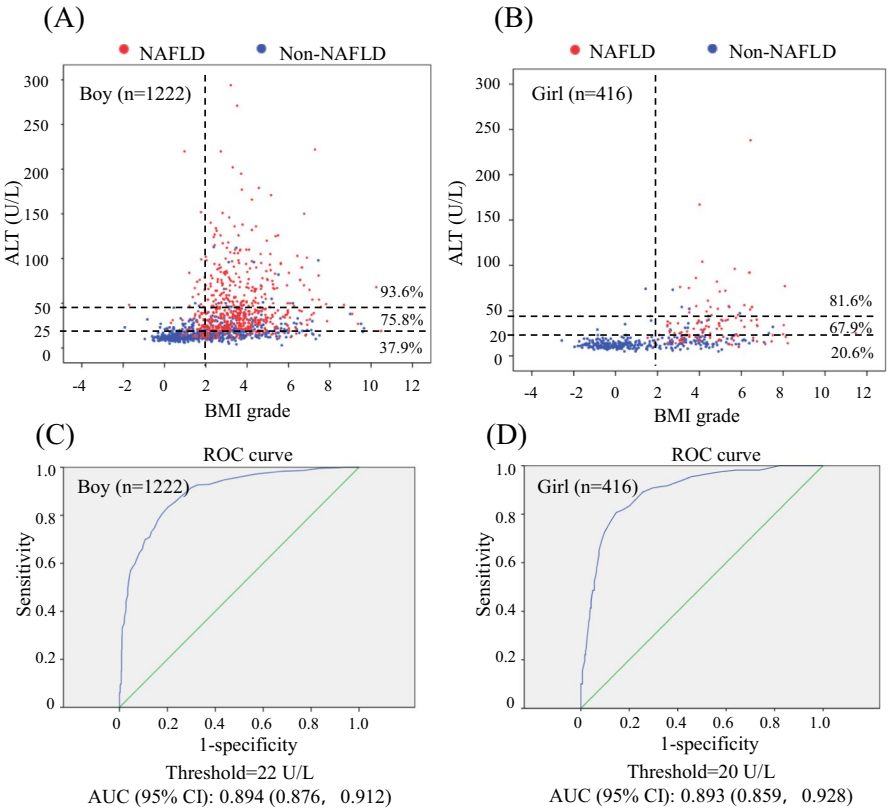

FIGURE 4 Assessment of NAFLD in children by using the revised and current serum ULN-ALT. Blue and red points in the scatter diagram indicate the levels of serum ALT and BMI grades in (A) boys with non-NAFLD and NAFLD; B, girls with non-NAFLD and NAFLD. C, The threshold of serum ALT is 22 U/L, and area under the ROC curve (AUC) is 0.894 in boys; D, the threshold of serum ALT is 20 U/L, and AUC is 0.893 in girls. BMI grades were calculated as BMI grade = (BMI − overweight BMI)/(obese BMI − overweight BMI) + 1. The cutoffs for overweight and obese classifications were derived from a national BMI reference established by the Working Group on Obesity in China (WGOC). The current serum ULN-ALT values were 50 U/L for boys and 40 U/L for girls, and the revised serum ULN-ALT values were 25 U/L for boys and 20 U/L for girls, shown as horizontal dashed lines. Obese children (BMI grade > 2) could be divided to three groups by using the revised and current ULNs for ALT. The detection rates of NAFLD in different groups are labeled

TABLE 2 Comparison of current and revised serum ULN-ALT to predict NAFLD in children

Cutoffs of ALT (U/L)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Boy (n = 1222)				
25	74.8	85.4	79.8	81.5
50	35.2	99.8	93.0	66.2
Girl (n = 416)				
20	80.7	85.3	66.2	92.6
40	29.4	97.4	80.0	79.5

decisions and lead to potential adverse effects, including delayed diagnosis, misdiagnosis, patient risk, and inadequate treatment. However, most laboratories still interpret pediatric laboratory test results based on inappropriate reference ranges for liver markers that are established in adults.[32] In the present study, we re-evaluated the serum ULN-ALT in healthy Chinese children based on physical examination data. We found that serum ALT levels and BMI were strongly correlated in obese children, and the revised serum ULN-ALT values were more sensitive in the assessment of NAFLD.

Consistently with previous reports of elevated serum ALT levels in children with abnormal BMI values,[9] obese group showed stronger correlations between serum ALT levels and BMI than control group (Figure 2), indicating an association between serum ALT elevation and hepatic steatosis in childhood obesity. In addition, several relevant reports have shown that the rate of serum ALT

elevation in obese children from the United States, Mexico, Spain, the Netherlands, and Isfahan were close to 25%,[9,18,33-35] whereas the rate of serum ALT elevation in obese children from Israel was 60.3%.[36] Despite the different criteria for serum ALT elevation and ethnic differences among these previous studies, these findings support the close relationship between elevated serum ALT and childhood obesity.

Based on a metabolically healthy and liver disease-free pediatric population with normal BMI, we re-evaluated the serum ULN-ALT in Chinese children. The revised serum ULN-ALT (25 U/L for boys and 20 U/L for girls) was significantly lower than the current cutoff values. It may facilitate early detection of mild liver injury in obese children. Our revised ULNs are close to the values reported for American children by Schwimmer et al (25.8 U/L for boys and 22.1 U/L for girls).[37] However, our revised ULNs were lower than pediatric ULNs in Korea (33 U/L for boys and 25 U/L for girls)[38] and Iran (30 U/L for boys and 21 U/L for girls).[39] Apart from racial factors, this difference can probably be explained by differences in the selection of the reference population. Our study excluded NAFLD, HBV, and HCV, whereas the Korean pediatric research only ruled out individuals with HBV[38] and the Iran pediatric study ruled out individuals with HBV and HCV.[39] Owing to the sample size and geographical limitations, a national effort to standardize ULNs for ALT levels in Chinese children is needed.

Besides, using the revised serum ULN-ALT, we revealed that for an elevated serum ALT (25-50 U/L for boys and 20-40 U/L for girls), the risk of NAFLD exceeds 60% in obese boys and girls. We did not confirm fatty liver by histology because liver biopsy in these children is not ethically acceptable nor practicable. However, similar findings have been reported by Molleston et al,[40] who found that in comparison with children with normal serum ALT, children with the elevated serum ALT (26-50 U/L for boys and 23-44 U/L for girls) had significantly higher rates of steatosis (50% vs 24%) and fibrosis (54% vs 12%). Furthermore, it has been reported that 11% of overweight or obese children with elevated serum ALT have advanced fibrosis.[41] These findings suggested that children with mild liver injury may require clinical attention and our revision of the ULNs may have a substantial impact on the screening of childhood NAFLD, facilitating early detection of mild liver injury in obese children with NAFLD.

To assess the value of revised pediatric ULN-ALT in the evaluation of NAFLD, we analyzed the sensitivity, specificity, positive, and negative predictive values of current and revised ULNs-ALT in the assessment of NAFLD. The sensitivity and negative predictive value could be improved by using the revised ULN-ALT. Meanwhile, based on ROC analyses, we obtain an optimal serum ALT cutoff point for assessing NAFLD, (22 U/L for boys, 20 U/L for girls), which is close to our revised serum ULN-ALT (25 U/L for boys and 20 U/L for girls).

The main limitation of our study is diagnosis of NAFLD based only on ultrasound. Liver biopsy is the current standard to define the presence and severity of NAFLD under newly published NASPGHAN guidelines (March 2017).[11] Ultrasound examination is a primarily non-invasive screening method for NAFLD diagnosis. However, it has not sensitivity enough for the children who have lower degrees of steatosis.[42] Computed tomography (CT), magnetic resonance imaging, and spectroscopy (MRI and MRS) are more sensitive for assessing steatosis than ultrasound, but it was not widely used in the health check-ups. We defined NAFLD based only on ultrasound examination due to the limitation of retrospective analysis, which is a deficiency of the study. In this retrospective study, we totally collected 500 cases. Although a larger size of cases would help improve the accuracy of cutoffs for normal serum ALT in children, this case number is sufficient for conducting the statistical analysis.

In conclusion, based on a retrospective analysis, we redefined the serum ULN-ALT in Chinese children and evaluated its value in the assessment of NAFLD. Although elevated serum ALT levels are not sufficient to diagnose NAFLD,[43] our findings emphasize the importance of revising the reference range for serum ALT in children, which may help to uncover early abnormal serum ALT levels in childhood fatty liver disease. Besides, serum ALT levels significantly correlated with abnormal BMI values in children, suggesting a rigorous BMI threshold is needed to establish the cutoffs for serum ULN-ALT in children.

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