The Effect of Overweight and Obesity on Liver Biochemical Markers in Children and Adolescents

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The effect of overweight and obesity on liver biochemical markers in children and adolescents

Short title: Liver enzymes in children and adolescents

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Abstract

**Background:** Elevated plasma concentrations of liver enzymes are routinely used as markers of liver injury in adults and children. Currently, the age- and sex-specific effects of adiposity on pediatric liver enzyme concentrations are unclear.

**Methods:** We included participants from two cohorts of Danish children and adolescents: 1,858 from a population-based cohort, and 2,155 with overweight or obesity, aged between six and 18 years. Age- and sex-specific percentile curves were calculated for fasting plasma concentrations of alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), gamma-glutamyltransferase (GGT), bilirubin, and alkaline phosphatase (ALP) in both cohorts. Hepatic fat content was assessed by proton magnetic resonance spectroscopy in 458 participants.

**Results:** Concentrations of ALT, AST, LDH, and ALP decreased with age in both girls and boys, while GGT and bilirubin were comparable across age groups in girls and increased slightly with age in boys. Children and adolescents with overweight or obesity exhibited higher concentrations of ALT in all age groups. Concentrations of ALT, and to a lesser degree GGT, increased with age in boys with overweight or obesity. Optimal ALT cut-points for diagnosing hepatic steatosis (liver fat content > 5%) was 24.5 U/L for girls (sensitivity: 55.6%, specificity: 84.0%), and 34.5 U/L for boys (sensitivity: 83.7%, specificity: 68.2%).

**Conclusions:** Pediatric normal values of liver enzymes vary with both age and sex. Overweight and obesity is associated with elevated biochemical markers of liver damage. These findings emphasize the need for prevention and treatment of overweight and obesity in children and adolescents.
Précis

We investigated concentrations of fasting plasma liver biochemical markers in a population-based cohort, and a cohort with overweight and obesity. We found them affected by overweight and obesity.
**Introduction**

The prevalence of obesity in children and adolescents has increased worldwide in the past decades. Overweight and obesity in children increase the risk of developing diseases linked to the metabolic syndrome, including non-alcoholic fatty liver disease (NAFLD). NAFLD is among the most common comorbidities in children with obesity, with reported prevalence rates of 30-70%. NAFLD covers a spectrum of disease activity in the absence of a high alcohol consumption, beginning with its main characteristic, accumulation of fat in the liver (hepatic steatosis). Hepatic steatosis may cause inflammation and subsequent liver cell damage (steatohepatitis), progress to fibrosis and scarring, and potentially result in end-stage liver disease as childhood onset cirrhosis. Furthermore, hepatocellular carcinomas may occur, even in the absence of cirrhosis. It is important to detect liver damage, both at an early age and at an early stage of NAFLD, in order to avoid irreversible disease progression to an irreversible stage. Liver biopsy is the gold standard for assessing liver damage, but the invasiveness and risks associated with this procedure makes it unsuitable for routine clinical screening and monitoring.

In daily clinical practice, plasma concentrations of liver enzymes and bilirubin are routinely used as a marker of liver injury. The most frequently measured biochemical markers include alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), gamma-glutamyltransferase (GGT), bilirubin, and alkaline phosphatase (ALP). Plasma concentrations of ALT are commonly used to screen for NAFLD in children with overweight or obesity worldwide. However, the specific screening methodology, including cut-off values and whether additional biochemical markers are also evaluated, varies between countries and studies.

Assessing biochemical markers in a pediatric cohort requires not only consideration of age, but also of sex, growth, and pubertal development. It is essential that reference values are...
defined from a healthy, large, and representative population recruited outside the hospital setting\textsuperscript{21}. This establishes reliable cut-off values, which are able to identify liver disease in children and adolescents with overweight or obesity\textsuperscript{22,23}.

In the present study, we provide age- and sex-specific reference values for ALT, AST, GGT, LDH, bilirubin, and ALP in a population-based cohort of Danish children and adolescents aged 6-18 years. Furthermore, we examine how the values in a large cohort of children and adolescents with overweight or obesity compare with this reference. In addition we assess the hepatic fat content by proton magnetic resonance spectroscopy (\textsuperscript{1}H-MRS) in a sub-sample of the cohort with overweight or obesity and the population-based cohort, and calculate optimal cut-offs of plasma ALT concentrations for identifying hepatic steatosis.
Materials and Methods

Study populations

Two cohorts aged 0.5-26.5 years were invited to participate in the present study: 1) A population-based cohort of children and adolescents, recruited from schools across 11 municipalities in Zealand, Denmark and 2) a cohort of children and adolescents with overweight or obesity recruited at The Children’s Obesity Clinic, Department of Pediatrics, Copenhagen University Hospital Holbæk, Denmark. Informed written consent was obtained from all of the participants above 18 years, and from the parents for participants younger than 18 years. The Ethics Committee of Region Zealand, Denmark (protocol no SJ-104) and the Danish Data Protection Agency approved the study. Phenotyping was performed by trained medical professionals, and included measurements of height and weight, a clinical examination, and a fasting venous blood sample. An extensive questionnaire was completed at home for the population-based cohort prior to the visit and at the hospital for the cohort with overweight or obesity.

Population-based cohort

The population-based cohort was recruited from October 2010 to February 2015 (N = 2,898). The exclusion criteria for this study were: 1) age younger than 6.0 or older than 18.0 years (N = 240), 2) no available data on liver biochemical markers (N = 56), 3) more than 30 days between anthropometrics and blood sampling (N = 94), 4) intake of medication known to influence liver enzymes (N = 10), 5) a self-reported alcohol intake >60 g alcohol per week (N = 43), 6) overweight or obesity (BMI > 90th percentile) (N = 463), and 7) underweight (BMI < 10th percentile) (N = 134).

Cohort of children and adolescents with overweight or obesity
The cohort of children and adolescents with overweight or obesity was recruited from January 2009 to April 2018 (N = 2,846) \textsuperscript{24,27}, and all exhibited a BMI above the 90\textsuperscript{th} percentile according to Danish BMI charts (corresponding to a BMI standard deviation score (SDS) > 1.28). Exclusion criteria were similar to those used in the population-based cohort, resulting in the following exclusion numbers: 1) age (N = 181); 2) missing data (N = 12); 3) more than 30 days between anthropometrics and blood samples (N = 456); 4) medication (N = 33), and 5) alcohol intake (N = 9).

**Anthropometric measurements**

Body weight was measured in light clothing and without shoes to the nearest 100 g on a BC-418 Segmental Body Composition Analyzer (Tanita, Tokyo, Japan). Height was measured to the nearest 1 mm by a stadiometer. BMI SDS was calculated using the LMS method according to Danish BMI charts \textsuperscript{27}.

**Biochemical analyses**

Blood samples were obtained by venipuncture of the antecubital vein from 7 am to 9 am after a fast of at least 8 hours. Analysis of plasma concentrations of ALT, AST, LDH, GGT, bilirubin, and ALP were processed immediately in the laboratory of Copenhagen University Hospital Holbæk, Denmark. All analyses were performed on a Cobas\textsuperscript{®}6000 (Roche diagnostics, Mannheim, Germany) until May 15th 2013, and on a Dimension Vista\textsuperscript{®}1500 (Siemens Healthcare, Erlangen, Germany) using enzymatic colorimetric method from May 16\textsuperscript{th} 2013 onwards.

**Pubertal data**
Pubertal staging was performed according to the criteria of Marshall and Tanner (on the basis of breast development in girls and testicular volume in boys)\textsuperscript{28}. For participants from the population-based cohort, Tanner stage was self-reported using picture pattern recognition. A pediatrician examined the participants from the cohort with overweight and obesity at the first visit to the clinic. Self-reported Tanner-staging has been found adequate for distinguishing between pre- and post-pubertal developmental stage\textsuperscript{29}. Accordingly, participants from both cohorts were grouped into either pre- or post-pubertal based on the Tanner staging (1 = pre-pubertal, 2-5 = post-pubertal)\textsuperscript{29}.

\textbf{\textsuperscript{1}H MRS evaluation}

Liver fat content was measured on a subset of both the population-based cohort, and the cohort with overweight or obesity on a 3T Achieva MR imaging system (Philips Medical Systems, Best, the Netherlands). The spectroscopy voxel (11mm x 11mm x 11mm) was placed in the right liver lobe, avoiding major vessels and bile ducts. The acquired spectra were fitted to obtain their areas by an experienced senior MR physicist using a standard post-processing protocol at the MR imaging system. Details of the applied MR methodology have previously been described\textsuperscript{30,31}. We defined hepatic steatosis using two different cutoffs of liver fat content: 1) above 5%, a routinely used cutoff that is based on the upper normal limit of liver fat content in lean adults; and 2) above 1.5%, a cut-off we recently found to more accurately represent the upper normal limit of liver fat content in children\textsuperscript{31}.

\textbf{Statistical analyses}

Statistical analyses were performed using R statistical software (v.3.5.0). Age- and sex-specific percentiles and percentile curves were calculated using the Generalized Additive Models for...
Location Scale and Shape (GAMLSS) package, using the Box–Cox transformation distribution family. To examine effects of age on biochemical markers of liver damage, we grouped the participants into three groups: 6–9, 10–13, and 14–18 years of age. As most of the biochemical parameters were non-normally distributed, a Wilcoxon rank sum tests was used to compare differences between age groups. To compare the accuracy of ALT as a diagnostic tool for identifying individuals with and without hepatic steatosis, receiver operating characteristic (ROC) analyses and area under the curve (AUC) were calculated using the R package “pROC” 32. The optimal cut-off for classifying steatosis was determined by Youden’s index.
Results

Study population

From the population-based cohort, 1,858 (1,096 girls) normal weight children and adolescents with a median age of 11.6 years and 10.6 years for girls and boys respectively, were included in the study. From the cohort with overweight or obesity, 2,155 (1,158 girls) children and adolescents with a median age of 11.6 years and 11.8 years for girls and boys, respectively, were included in the study. The number of girls and boys in each 1-year age stratum in each cohort are shown in Supplementary Table S1, and the distribution of BMI SDS stratified by age, is shown in Supplemental Figure 1. Age- and sex-specific percentiles for ALT, AST, LDH, GGT, bilirubin, and ALP for the population-based cohort are shown in Supplemental Tables S2 and S3. A small number of participants had missing values for each biochemical parameter (range < 3% to 17%). Children and adolescents with overweight or obesity (N = 463) or underweight (N = 134) in the population-based cohort were excluded from the main analysis, to ensure that the reference values were based solely on healthy normal-weight participants. However, including these participants did not majorly change any of the reference values reported in Supplemental Tables S2 and S3 (data not shown).

Anthropometric parameters and percentile curves of liver biochemical markers

Median and interquartile ranges for the six biochemical markers, stratified by age group, sex, and cohort, as well as anthropometric parameters of the cohort are shown in Table 1. Population-based cohort

The percentile curves for ALT, AST, LDH, GGT, bilirubin, and ALP are shown stratified by age and sex for the population-based cohort in the left panels (A) of Figures 1-4. The ALT percentile curves showed a small decrease with age in girls, but not in boys. The median ALT concentration in
girls aged 6–9, 10–13 and 14–18 years were 21 U/L, 18 U/L, and 18 U/L, respectively, and 21 U/L, 19 U/L, and 20 U/L in boys, respectively. Increasing BMI-SDS was associated with increasing ALT in boys (P < 0.001) but not in girls (P = 0.885) from the population based cohort. The AST percentile curves showed significant decreases with age in both girls and boys. Median AST concentration in girls aged 6–9, 10–13, and 14–18 years were 31 U/L, 25 U/L, and 20 U/L, respectively, and 32 U/L, 27 U/L, and 25 U/L in boys, respectively. The percentile curves for LDH concentrations in girls and boys showed a decrease with higher age. The median LDH concentration in girls aged 6–9, 10–13, and 14–18 years were 228 U/L, 201 U/L, and 161 U/L, respectively. The corresponding values in boys were 233 U/L, 212 U/L, and 184 U/L, respectively. The percentile curves for GGT concentrations showed no changes with age in neither girls nor boys. Median concentrations in girls and boys aged 6–9, 10–13, and 14–18 years were 17 μmol/L, 16 μmol/L, and 16 μmol/L, and 17 μmol/L, 18 μmol/L, and 18 μmol/L, respectively. The bilirubin percentile curves showed no change with age in girls, but a modest increase with age in boys. Median concentrations in girls aged 6–9, 10–13, and 14–18 years were 7 μmol/L, 7 μmol/L, and 8 μmol/L. The corresponding values in boys were 6 μmol/L, 7 μmol/L, and 9 μmol/L. The percentile curves for ALP showed a complex pattern in the population cohort, with a modest increase until reaching a peak at ages 11–12 years in girls and 13–14 years in boys, followed by a sharp decline in both sexes. Median concentrations in girls and boys aged 6–9, 10–13 and, 14–18 years were 262 U/L, 275 U/L, and 101 U/L and 247 U/L, 271 U/L, and 233 U/L, respectively.

We calculated standard deviation scores for ALT and AST for each child from the population-based cohort. There were 27 girls and 16 boys with ALT-SDS above the 97.5th percentile, and 26 girls and 14 boys with AST-SDS above the 97.5th percentile. Of these, 8 girls and 6 boys had both ALT-SDS and AST-SDS over the 97.5th percentile (Supplemental Table S4). The 27 girls with high ALT alone and the 26 girls with high AST alone were similar to the rest of the
girls in the population-based cohort, whereas the girls with both high ALT and AST had slightly
lower BMI-SDS. Compared to the rest of the boys, the 16 boys with high ALT and the 14 boys with
high AST, as well as the six boys with high ALT and AST were older, taller, had larger waist and
hip circumferences and had a higher BMI-SDS.

Cohort with overweight or obesity

The percentile curves for ALT, AST, LDH, GGT, bilirubin, and ALP are shown stratified by age
and sex for the cohort with overweight or obesity in the right panels (B) of Figures 1-4.

Compared to the population-based cohort, participants in the cohort with overweight and obesity
exhibited higher concentrations of ALT in all age groups and in both sexes (6–9, 10–13, and 14–18
years of age, P < 0.001 for all comparisons, Table 1). In contrast to the pattern observed in the
population-based cohort, there was a marked increase in ALT with increasing age among children
and adolescents with overweight or obesity, which was most pronounced in boys. At 6–9, 10–13,
and 14–18 years of age, boys with overweight or obesity exhibited median ALT concentrations of
22 U/L, 23 U/L, and 29 U/L, respectively. We examined whether differences in abdominal
adiposity might play a role in the seemingly stronger effect seen in boys. There was a strong direct
correlation between age and waist/hip ratio (a proxy of abdominal obesity) in boys (P < 0.003), but
an inverse association in girls (P < 0.001), regardless of adjustment for BMI-SDS. Increasing BMI-
SDS was strongly associated with increasing ALT in both girls and boys with overweight or obesity
(P < 0.001, both). The percentile curve patterns for AST in the population-based cohort and the
cohort with overweight or obesity were overall comparable. However, in girls and boys below 10
years of age, there was a modest decrease in AST in the cohort with overweight or obesity
compared to the population-based cohort (P < 0.001). In contrast, girls with overweight or obesity
older than 13 years of age exhibited a small increase in AST compared to similar aged girls from
the population-based cohort (P = 0.02). Concentrations of LDH were overall higher in the cohort with overweight and obesity than in the population-based cohort, for all ages, and in both sexes (P < 0.001). The LDH percentile curves showed a robust decrease with age in the cohort with overweight and obesity, comparable to what was observed in the population-based cohort. Compared to the population-based cohort, there was a modest age-associated increase in GGT in girls and boys with overweight or obesity, which was most pronounced in boys. Girls and boys with overweight or obesity who were older than 13 years had significantly higher median concentrations of GGT (17 U/L and 20 U/L, respectively) compared to participants of the same age in the population-based cohort. The percentile curves for bilirubin and ALP were not significantly different in the population-based and the cohort with overweight or obesity, except for ALP in the youngest age group for both girls and boys (P < 0.001) 

**Effects of puberty**

Data on puberty stage was available in 78.5% and 58.0% of girls and boys, respectively, in the population-based cohort, and in 85.5% and 74.7% of girls and boys in the cohort with overweight or obesity. Compared to pre-puberty, puberty was associated with, 2 U/L lower ALT (P = 0.005), 2 U/L lower AST (P = 0.003), 1.6 U/L lower GGT (P = 0.001), and 96 U/L higher ALP (P < 0.001) in girls from the population-based cohort. Only ALP was influenced by puberty in boys from the population-based cohort (28 U/L higher ALP, P = 0.017). In the cohort with overweight or obesity, puberty was associated with 4 U/L lower ALT (P = 0.024), 10 U/L lower LDH (P = 0.007), 3 U/L lower GGT (P = 0.001), and 28 U/L higher ALP (P < 0.001) in girls. In boys with overweight and obesity puberty was associated with 4 U/L lower ALT (P = 0.036), 3 U/L lower GGT (P = 0.008), and 30 U/L higher ALP in boys (P < 0.001). All associations were adjusted for age and BMI-SDS.
Hepatic steatosis measured by $^1$H-MRS

Combined from both cohorts, hepatic $^1$H-MRS data within 30 days of blood sampling was available on 458 children and adolescents (248 girls). We defined hepatic steatosis as a liver fat content of $>5\%$.\textsuperscript{34,35} Using this definition, 25 girls (9.9\%) and 44 boys (21\%) exhibited hepatic steatosis.

Children and adolescents with hepatic steatosis had a higher degree of adiposity than those without steatosis (Supplemental Table S5).\textsuperscript{33} We used ROC-curves and AUC to assess the ability of elevated plasma ALT to identify children and adolescents with hepatic steatosis (Figure 5). For both girls and boys combined, the optimal ALT cut-off was 31.5 U/L, which yielded an AUC of 76.6\%, a sensitivity of 80.7\% and a specificity of 65.2\% for correctly identifying those with hepatic steatosis. The corresponding cut-off in girls was 24.5 U/L (sensitivity: 55.6\%, specificity: 84.0\%, AUC: 71.8\%), and 34.5 U/L in boys (sensitivity: 83.7\%, specificity: 68.2\%, AUC: 79.1\%).

We also assessed the ability of ALT to identify hepatic steatosis as defined by liver fat content above 1.5\% (Supplemental Figure S2), a cut-off we recently found to more accurately identify steatosis in children.\textsuperscript{31} Using the 1.5\% definition, 68 girls (27.4\%) and 83 boys (39.5\%) exhibited hepatic steatosis. Here we found the optimal ALT cut-off to be 25.5 U/L with an AUC of 72.2\% a sensitivity of 64.2\% and a specificity of 70.9\% for both boys and girls combined. The corresponding cut-off in girls was 24.5 U/L (sensitivity: 61.7\%, specificity: 75.0\% AUC: 70.3\%), and 31.5 U/L in boys (sensitivity: 83.5\%, specificity: 54.2\% AUC: 73.0\%).
This study provides age- and sex-specific reference values for plasma concentrations of ALT, AST, LDH, GGT, bilirubin, and ALP from a large population-based cohort of children and adolescents aged between 6 and 18 years. In comparison to these, children and adolescents with overweight or obesity exhibited higher concentrations of ALT in all age groups, even among the youngest children below 10 years of age. The increase was most pronounced in boys above 13 years of age.

Previous studies have reported pediatric reference values for some of the liver biochemical markers analyzed in this study. Bussler et al. provided age- and sex-specific percentile curves for ALT, AST, and GGT in a cohort of 3,131 healthy and normal weight German girls and boys aged 11 months to 16 years. Overall, the percentile curves for ALT, AST, and GGT in the German study were similar to those observed in the population-based cohort in the present study. For example, the median ALT concentration was approximately 20 U/L in boys and girls above six years of age in both studies. The upper limit of normal ALT, defined as the 97th percentile, ranged from 24 U/L to 32 U/L in girls, and from 30 U/L to 38 U/L in boys in the study by Bussler et al. These data are overall comparable with the 97.5th percentiles reported in the present study. Li et al. found that concentrations of ALT and AST declined with age among 1,394 healthy children from China aged two to 14 years, in agreement with the patterns observed in the present study and others. The concentrations of ALP decline during childhood, increase during puberty, and decrease again after puberty, reflecting changes in bone growth during childhood and puberty. The patterns observed for ALP concentrations in the present study are similar to those reported in previous studies.

Percentile curves for biochemical markers of liver damage have not previously been reported from a large cohort of children with overweight or obesity. However, several studies have reported that children with obesity have increased biochemical markers of liver damage. In
agreement with these previous studies, we found that children and adolescents with overweight or obesity had elevated concentrations of ALT, the most liver-specific of the biochemical markers assessed in the present study. The other biochemical markers of liver damage were less influenced by adiposity in our study.

The increased plasma concentrations of ALT in girls and boys with overweight or obesity might reflect the presence of NAFLD or non-alcoholic steatohepatitis in many of these children. We found increased BMI-SDS to be associated with higher ALT in children with overweight and obesity, and among boys (but not girls) from the population-based cohort, observations that are consistent with the well-established causal link between obesity and NAFLD. The age-related increase in ALT in the cohort with overweight and obesity was more pronounced in boys than in girls. It is possible that hormonal (e.g. testosterone) or behavioral (e.g. alcohol intake) changes in boys explains the age-associated increase in ALT concentrations, and that these effects are amplified by overweight and obesity in the oldest age group of the boys. Another explanation could be that boys with overweight or obesity tend to accumulate more visceral fat compared with girls with overweight or obesity, who are more prone to store excess fat subcutaneously. Visceral fat is a stronger risk factor for the development of NAFLD than is subcutaneous fat.

Hepatic steatosis defined by a liver fat content of > 5% was confirmed by $^1$H-MRS in 9.9% of the girls and in 21% of the boys with available measurements. These prevalences are lower than those reported in a recent study of 408 children with obesity and liver fat content assessed by $^1$H-MRS (22.6 % for girls and 29.4 % for boys). The lower prevalence in our study is likely the result of having included children with normal weight in the sub-cohort. We found that cutoff values of 24.5 U/L and 34.5 U/L in girls and boys, respectively, were optimal for classifying hepatic steatosis. These cut-off values were lower than those reported by Yu et al. (30 U/L in girls and 42 U/L in boys), but nevertheless yielded comparable AUC (0.79 and 0.72 in boys and girls,
respectively, in our study versus 0.81 and 0.75 in Yu et al\textsuperscript{34}). The differences in optimal cut-offs are likely owing to differences in cohort characteristics.

There are several limitations to our study that should be considered. We did not screen for viral infections that might affect liver enzyme levels, such as hepatitis C. However, the prevalence of hepatitis C is less than 0.4% in the Danish general population\textsuperscript{23}. Alcohol consumption was self-reported, which is likely to suffer from some degree of misreporting. Another limitation is that we did not quantify liver fat content on all the participants. It is therefore possible that some children in the population-based cohort had asymptomatic fatty liver disease affecting their liver enzyme concentration. The modest number of participants in some of the 1-year age strata is another potential limitation. The percentile values for groups with less than 40 individuals (e.g. boys and girls with overweight or obesity aged 16-18) should therefore be interpreted with caution.

Blood samples were drawn within 30 days of the $^{1}$H-MRS, but not necessarily on the same day as the $^{1}$H-MRS was performed. Thus, concentrations of liver biochemical markers might have changed for some of the participants in the 30 day timeframe from blood sampling to the $^{1}$H-MRS assessment. Finally, even though the biochemical markers examined here are commonly used to evaluate liver damage, some of them are not entirely liver specific. For example, elevated AST may reflect muscle, heart, or pancreas injury, and LDH is also a marker of hemolysis and tissue breakdown in general.

Our study also has certain strengths that should be noted. The population-based cohort was based on a large, homogeneous cohort of children, and the sample size of our cohort is comparable to, or larger than, previous studies aimed at establishing pediatric reference values. We were also able to exclude individuals with diseases or intake of medication known to influence plasma liver enzymes. Furthermore, we accounted for alcohol intake by excluding individuals who
consumed more than 60 grams of alcohol per week. Other strengths are that blood samples were collected in a standardized manner and analyzed in the same laboratory, minimizing inter-individual variation caused by non-biological factors.

In conclusion, this study provides age- and sex-specific reference values for plasma concentrations of ALT, AST, LDH, GGT, bilirubin, and ALP derived from a population-based cohort of Danish children and adolescents aged six to 18 years. We found that age and sex influence the patterns of these biochemical markers throughout childhood and adolescence. Obesity in children and adolescents is associated with elevated biochemical markers of liver damage, likely reflecting the effects of non-alcoholic fatty liver disease. These findings emphasize the need for both efficient preventive and treatment strategies in pediatric populations with overweight and obesity.
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References


Figure and Table legends

Figure 1. Percentile curves for alanine aminotransferase (ALT), aspartate aminotransferase (AST) lactate dehydrogenase (LDH), in girls from the population-based cohort (left panels) and the cohort with overweight or obesity (right panels). Upper and lower black lines represent the 2.5th and 97.5th percentile, dark grey lines are medians, and grey lines are the 5th, 25th, 75th, and 95th percentiles.

Figure 2. Percentile curves for gamma-glutamyltransferase (GGT), bilirubin and alkaline phosphatase (ALP) in girls from the population-based cohort (left panels) and the cohort with overweight or obesity (right panels). Upper and lower black lines represent the 2.5th and 97.5th percentile, dark grey lines are medians, and grey lines are the 5th, 25th, 75th, and 95th percentiles.

Figure 3. Percentile curves for alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) in boys from the population-based cohort (left panels) and the cohort with overweight or obesity (right panels). Upper and lower black lines represent the 2.5th and 97.5th percentile, dark grey lines are medians, and grey lines are the 5th, 25th, 75th, and 95th percentiles.

Figure 4. Percentile curves for gamma-glutamyltransferase (GGT), bilirubin and alkaline phosphatase (ALP) in boys from the population-based cohort (left panels) and the cohort with overweight or obesity (right panels). Upper and lower black lines represent the 2.5th and 97.5th percentile, dark grey lines are medians, and grey lines are the 5th, 25th, 75th, and 95th percentiles.
Figure 5. Receiver operating characteristics (ROC) curves for ALT as a diagnostic tool for hepatic steatosis of > 5% in children and adolescents. (A) Boys and girls combined. The area under the curve (AUC) was 76.6 %, and the optimal cutoff of ALT 31.5 U/L identified hepatic steatosis with a sensitivity of 80.7 % and a specificity of 65.2 %. (B) Boys alone. The AUC was 79.1 %, the optimal cutoff of ALT of 34.5 U/L identified hepatic steatosis with a sensitivity of 83.7 % and a specificity of 68.2 %. (C) Girls alone. The AUC was 71.8 %, and an optimal cutoff of ALT of 24.5 U/L identified hepatic steatosis with a sensitivity of 55.6 % and a specificity of 84.0 %.

Table 1: Comparison of anthropometrics and liver biochemical markers between the population cohort and the cohort with overweight or obesity. Values are medians and interquartile ranges. Abbreviations: ALT: alanine aminotransferase, AST: aspartate aminotransferase, LDH: lactate dehydrogenase, GGT: gammaglutamyltransferase, ALP: alkaline phosphatase, BMI SDS: Body mass index standard deviation score. P-values are by Wilcoxon rank sum test.
<table>
<thead>
<tr>
<th></th>
<th>6–9 years</th>
<th>10–13 years</th>
<th>14–18 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Population based</td>
<td>Cohort with</td>
<td>Population based</td>
</tr>
<tr>
<td></td>
<td>cohort</td>
<td>overweight and</td>
<td>cohort</td>
</tr>
<tr>
<td></td>
<td></td>
<td>obesity</td>
<td></td>
</tr>
<tr>
<td><strong>P</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Height, cm</strong></td>
<td>131 [124-138]</td>
<td>136 [131-142]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Weight, kg</strong></td>
<td>27 [23-30]</td>
<td>42 [37-48]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Waist, cm</strong></td>
<td>58 [55-60]</td>
<td>78 [73-84]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Hip, cm</strong></td>
<td>68 [64-71]</td>
<td>82 [78-88]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>BMI kg/m²</strong></td>
<td>16 [15-17]</td>
<td>23 [21-25]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>BMI-SDS</strong></td>
<td>0.01 [-0.53 - 0.58]</td>
<td>3 [2-3]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>LDH, U/L</strong></td>
<td>228 [208-254]</td>
<td>251 [230-274]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Bilirubin, µmol/L</strong></td>
<td>7 [6-9]</td>
<td>7.00 [5-9]</td>
<td>0.111</td>
</tr>
<tr>
<td><strong>ALP, U/L</strong></td>
<td>262 [223-303]</td>
<td>280 [238-325]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Boys</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Height, cm</strong></td>
<td>131 [125-137]</td>
<td>139 [132-144]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Weight, kg</strong></td>
<td>27 [24-30]</td>
<td>45 [39-53]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Hip, cm</strong></td>
<td>58 [56-61]</td>
<td>81 [75-87]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>BMI kg/m²</strong></td>
<td>16 [15-17]</td>
<td>24 [22-26]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>BMI-SDS</strong></td>
<td>0.03 [-0.41-0.54]</td>
<td>3 [3-4]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Bilirubin, µmol/L</strong></td>
<td>6 [5-8]</td>
<td>6 [5-8]</td>
<td>0.101</td>
</tr>
</tbody>
</table>
Figure 1

Population cohort (Girls) (A)

Cohort with overweight or obesity (Girls) (B)
Figure 2

Population cohort (Girls) (A)

Cohort with overweight or obesity (Girls) (B)
Figure 3

Population cohort (Boys) (A)

Cohort with overweight or obesity (Boys) (B)
Figure 4

Population cohort (Boys)

Cohort with overweight or obesity (Boys)
Figure 5

A

31.5 (80.7%, 65.2%)

AUC: 76.6%

Specificity (%)

B

34.5 (83.7%, 68.2%)

AUC: 79.1%

Specificity (%)

C

24.5 (55.6%, 84.0%)

AUC: 71.8%

Specificity (%)

Sensitivity (%)