Pediatric Percentiles for Transient Elastography Measurements - Effects of Age, Sex, Weight Status and Pubertal Stage

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Index of abbreviations

BMI	Body Mass Index
CAP	Controlled Attenuation Parameter
CT	Computed Tomography
LSM	Liver Stiffness Measurement
MRI	Magnetic Resonance Imaging
NAFLD	Non-Alcoholic Fatty Liver Disease
OACCU	Overall Accuracy
OCCC	Overall Concordance Correlation Coefficient
OPREC	Overall Precision
SDS	Standard Deviation Score
TE	Transient Elastography
TS	Tanner Stage

Introduction

Relevance of the topic

Childhood obesity is a growing health issue worldwide and has reached pandemic dimensions (1). As a consequence, diseases that are related to obesity show a rising prevalence as well. Non-alcoholic fatty liver disease (NAFLD) is one of them, having become the most common liver disease in children and adolescents. A recent meta-analysis showed a mean prevalence of NAFLD of 7.6% in children with normal weight worldwide. The prevalence of NAFLD in children with obesity was reported distinctly higher, reaching a percentage of 34.2 (2).

NAFLD in children has a complex pathogenesis composed of prenatal factors, such as maternal obesity during pregnancy and gestational diabetes, as well as postnatal factors such as childhood obesity, hyperinsulinemia and insulin resistance, to name only some of them (3). Also a genetic component is part of the pathogenesis (4). Children with NAFLD are often asymptomatic (5). When they show symptoms, these are mostly unspecific like, e. g., irritability, fatigue, headache, trouble concentrating and muscle aches or cramps (6). NAFLD is, furthermore, associated with an increased risk of cardiovascular disease, type 2 diabetes and increased mortality at adult age (7). Moreover, it can lead to liver fibrosis and cirrhosis and increases the risk of developing hepatocellular carcinoma (3,8). Thus, it is apparent that NAFLD poses a significant risk - if not threat - to the health of a considerable proportion of the global population, besides leading to several secondary problems and large follow-up costs for individuals as well as societies as a whole.

However, detected at early stages, when there is no irreversible damage to the liver, NAFLD is treatable. Currently, the treatment of NAFLD consists of lifestyle modification to improve diet and increase physical activity (9). In addition, four different drugs (Anti-LPS antibody IMM-124e (Trial Number NCT03042767), Metreleptin (Trial Number NCT02654977), Cysteamine bitartrate (Trial Number NCT01529268) and Losartan (Trial Number NCT03467217)) are currently undergoing, respectively underwent, phase 2 studies (7). With regards to adults, far more drugs are being tested (10). This development makes it presumable that, alongside lifestyle intervention, it will be possible to treat NAFLD in pediatric patients pharmacologically in the near future.

Therefore, to detect NAFLD in pediatric patients reliably and at an early stage, when there is still time for therapeutic intervention, seems imperative.

Until today, the histopathological examination of a liver biopsy is the gold standard to diagnose NAFLD. Liver biopsy is an invasive procedure with concomitant risks like infection, bleeding or mispuncture. Moreover, there is the risk of misdiagnosing due to sampling bias (11) and children often need a general anesthesia for the procedure, which, again, entails risks for the children. Taken together, liver biopsy in children is, for good reasons, performed reluctantly, and especially not suitable for screening purposes. Hence, non-invasive, yet still reliable tools to diagnose NAFLD are urgently needed.

In order to identify these above-mentioned non-invasive tools, several studies have examined and evaluated various diagnosing approaches, most of them with rather disappointing results: For instance, a less invasive method is the examination of blood samples. The most commonly used serum marker for screening purposes is the liver enzyme alanine aminotransferase (ALT). Yet, research has shown that there are neither reliable cutoffs, nor has this method proven to be very sensitive and specific in diagnosing NAFLD (7). Kwon et al. investigated several, more specific biomarkers (P1NP, IL-6 or M2BPGi) and came to the conclusion that, combined with basic laboratory parameters, these could be used for screening purposes as well as for follow-ups (12). However, the population they investigated consisted of only 60 participants, implying an obvious lack of generalizability. It is, thus, necessary to validate this outcome in a larger cohort. In another study, Maffeis et al. investigated on scores to detect NAFLD based on anthropometric data combined with laboratory parameters (e.g. adiponectin and the liver enzyme ALT), but they do also state that their results need validation due to the small cohort they investigated (13). Koot et al. investigated the diagnostic accuracy of existing scores (NAFLD liver fat score (NAFLD score), Fatty Liver Index (FLI), Hepatic Steatosis Index (HSI), paediatric formula (ped-NAFLD score)) in a cohort of 119 obese children and came to the conclusion that the accuracy of these scores is insufficient (14).

Further on, also completely non-invasive tools have been examined: The two imaging tools MRI and CT have proven to be reliable in detecting NAFLD (7,15), but both bear their own disadvantages. CT must not be used regularly in pediatric population due to its radiation. MRI on the other hand, which works without radiation, is expensive and not widely available, making it unsuitable for regular use or for screening purposes. Regular ultrasound also works without radiation and is an inexpensive and widely available tool, yet it is strongly observer

dependent (16) and various studies have shown that regular ultrasound is neither sensitive nor specific in diagnosing NAFLD (15), respectively steatosis (17) and fibrosis (18). However, there is a special device combining ultrasound with computational technique: Transient Elastography (TE). TE has gained a lot of academic interest over the last years, since it has proven to be a reliable method to detect NAFLD (see below). Furthermore, it is painless, rapid, cost-efficient, non-ionizing and observer independent.

Transient Elastography - current state of research

Reliability and Applicability of Transient Elastography

Transient Elastography (TE) is an ultrasound-based examination tool, providing two different methods to examine the liver: Liver Stiffness Measurement (LSM) and Controlled Attenuation Parameter (CAP). LSM is a parameter to estimate liver fibrosis and CAP calculates the percentage of fat accumulation in the liver.

In short, TE technically functions as follows: Low frequency and low amplitude vibrations are sent via a transducer probe which is placed on the vibrator into the liver tissue. In this way a shear wave is induced and propagates through the tissue. These shear waves are measured through pulse-echo ultrasound acquisitions in the probe. The velocity of the shear waves is directly related to the liver stiffness: the stiffer the liver tissue, the faster the shear wave is propagated (19). The results of LSM are displayed in kilopascal (kPa). CAP measures the attenuation of the above-mentioned shear wave propagation, producing results in decibels per meter (dB/m). Higher fat accumulation in the liver results in higher CAP values.

The FibroScan ® device operates with three probes: S, M and XL probe. For our study, we used either the M probe (25-65mm measurement depth) or the XL probe (35-75mm measurement depth). The FibroScan® device includes the Automatic Probe Selection (APS) tool, which indicates which of the two probes should be used for measurement. The selection of probes depends on the distance between probe and liver capsule. As soon as the probe is in contact with the skin of the participant it uses the ultrasound signals to estimate the distance between skin and liver capsule and recommends the right probe on this basis. The measurement was successful when 10 valid data points could be measured.

The necessity of 10 valid measurements shows that one single measurement is not necessarily fully reliable. Taking that into account, we checked for the reliability and reproducibility of our

results while conducting the tests. Therefore, we performed double measurements on 249 participants. With the aim of assessing intra-observer reliability, we calculated the overall concordance correlation coefficient (OCCC), the overall precision (OPREC) and overall accuracy (OACCU) (20,21).

Transient Elastography results in pediatric context

Transient Elastography has become an established examination method in adult hepatology (22,23). Yet, by now, several recent studies established that TE is a reliable method to detect NAFLD respectively liver fibrosis in children as well (24–31). About a decade ago, Nobili et al. already investigated a small cohort of children with regards to the reproducibility and accuracy of TE in detecting NAFLD in children and presented good results for both (25). Nobili et al. also conducted a review about the usefulness of TE as a diagnostic tool for the assessment of liver fibrosis in pediatrics and predicted the great potential of it (28). Likewise, Alkhouri et al. stated that TE is an accurate method to detect significant fibrosis in children (32).

In a different approach, presenting a very valuable contribution to the field, Fitzpatrick et al. performed TE measurements in 104 children who underwent liver biopsy for different reasons, compared the results of biopsy and TE and came to the conclusion that TE is a reliable method detecting liver fibrosis in children (24).

In their review about different non-invasive diagnostic tools for NAFLD in children, Draijer et al. stated that most of them still need to be validated, but, amongst two others, TE (by FibroScan ®) showed the best performance (33).

Yet to date, reliable reference values for LSM and CAP have been only available for adults (22,34–36). A couple of studies also examined TE test results of children, but most of them investigated children with pre-existing diseases like obesity, NAFLD or other liver diseases like hepatitis (24,34,37–40).

Tackling that research gap, a few recent studies investigated healthy children and adolescents (41–45). However, these studies mostly examined rather small or partially age-homogenous cohorts, therefore lacking generalizability. Zeng et al. (41), for instance, presented reference values based on a very large cohort, yet the cohort only covered five-year-olds, thus lacking evidence for other age groups. Tokuhara et al. investigated a more heterogenous age range, yet their cohort consisted only of 123 children (43). A similar limitation applies to the study of Mjelle et al., who investigated a rather small cohort of 230 children (42). Moreover, studies

often research solely into either LSM or CAP values. Li et al., for instance, investigated a large cohort of children (652 participants), yet only conducted LSM measurements (45).

Further on, the study results are partially contradictory with regards to TE test results and corresponding potential influencing factors. To take the age-dependency of LSM test results as an example: While some studies reported an increasement of test results over age (42,43,45–47), Ramirez et al., who investigated a cohort of 462 healthy children and provided reference values, found no effect of age on LSM (44). Potential reasons for this inconsistency include a different age range (12 – 20 years) as well as a different ethnic and geographic background of the cohort. A recent meta-analysis with 1702 participants, on the other hand, ascertained that values increase with age (45). Overall, Mjelle et al. (42) state that there is about the same number of studies indicating an age-dependency of LSM values as there is for age-independency.

The same accounts for the sex-dependency of LSM test results. Several studies could show that LSM test results are higher for boys (42,46,47). Tokuhara et al., on the other hand, could not find any influence of sex on LSM results (43). Likewise, the above-mentioned study by Ramirez et al. (44) did not find sex-dependent alterations of LSM.

This clearly highlights the necessity of further investigation into the age- and sex-dependency of LSM values in the pediatric context.

With regard to pediatric CAP measurements, there are only a few published studies providing reference values. Ramirez et al. (44) presented stable, age- and sex-independent CAP values from the ages 12 to 20. This result was also endorsed by a recent study by Ferraioli et al. (48). Yet, the data basis researched into is still too thin to arrive at reliable, generalizable conclusions.

Therefore, definitely, more studies researching into the TE measurements LSM and CAP of, ideally, a large cohort of healthy children and adolescents are needed. Furthermore, to make use of the potential of TE as a reliable tool to detect NAFLD at an early stage, one would, first of all, need respective reference values of healthy pediatric populations.

Exactly this is the underlying endeavor of our research which is addressed in this dissertation: We present pediatric percentiles with respective reference values for TE measurements derived from a large cohort of healthy children and adolescents.

Influencing factors

In medicine, and especially in pediatrics, diagnostics and the assessment of test results must always be seen in context with potential influencing factors, like, amongst many others, the age of a patient.

Take, e. g., bodyweight, to name the most simple and obvious example for that requirement: Whether the bodyweight of 10 kg of a hypothetical pediatric patient is physiological or pathological can only be assessed when we know about influencing factors, especially age. Whether the patient is 2 months, 2 years or twelve years makes a major difference for the classification of that weight.

Capturing this necessity, in our study we examined the potential influence of age and sex on TE values to attain an informational background against which practitioners can better evaluate test results. Furthermore, to increase, both, breadth and accuracy of our study results and the consequent reference values, we also examined the influence of weight status and pubertal stage on TE test results. The factors of age and sex are more or less self-explanatory, being derived from the study participants' birth dates (age: number in years; sex: male or female), only the influencing factors of weight status and pubertal stage will be explained more precisely in the following.

Weight status

We classified the participants´ weight status according to the guidelines of the German Obesity Association (49,50) as follows:

Normal weight	$\geq 10^{th}$ and $\leq 90^{th}$ age- and sex-specific percentile or
	BMI-SDS < 1.28 BMI-SDS
Overweight	$>90^{th}$ and $<97^{th}$ age- and sex-specific percentile or
	BMI-SDS >1,28 and < 1.88
Obesity	$>97^{th}$ and $\leq 99.5^{th}$ age- and sex-specific percentile or
	BMI-SDS ≥1.88

BMI was calculated and transformed into standard deviation scores (SDS) according to the same guidelines. In adult medicine, the BMI thresholds are the same for every age: BMI <18.5 = underweight, BMI 18.5 – 24.9 = normal weight, BMI 25 – 29.9 = overweight, BMI >30 obesity (51). Yet, in pediatrics BMI reference/standard values do not account equally for every age but differ dependent on the age of a patient. In order to be able to classify BMI for every age, one could either compare the percentiles or, more simply, one can translate them to SDS values and in this way categorize them (49).

Pubertal status

Pubertal stage was assessed according to Tanner (52,53).

To define the Tanner stage for girls, the stages of development of two secondary sex characters are assessed: first, the breast development (B1-5) and, second, the development of pubic hair (P 1-6) (53).

Accordingly, the Tanner stage for boys is assessed by the stages of development of secondary sex characters as well. Instead of breast development, the changes in the genitalia (penis, testes and scrotum) (G1-5) are classified combined with the development of the pubic hair (P1-6) analogous to girls (52).

These changes are then being classified in five, respectively six different stages of development: B1-5/G1-5, P1-6).

For our study, we used 5 pubertal stages, based on the Tanner stages, reaching from 1 (= pre-adolescent) to 5 (= adult stage) (54).

LIFE Child Study

Study design

The data for our study originated from the LIFE Child study which is a part of `Leipziger Forschungszentrums für Zivilisationserkrankungen´ (LIFE) of Leipzig University.

LIFE Child is a prospective longitudinal population-based cohort study with a life course approach to health and disease (55). As a part of LIFE, a research project conducted at the Leipzig Research Center for Civilization Diseases, LIFE Child aims to monitor healthy child

development from birth to adulthood as well as to understand the development of non-communicable diseases such as obesity (54). The study was designed in accordance with the Declaration of Helsinki (56). The Ethics Committee of the University of Leipzig approved the study (Reg. No. 26410-19042010), which is registered with ClinicalTrials.gov under the clinical trial number NCT02550236.

Setting

Fully informed and written consent to participate in this study was obtained from all participants' parents, respectively from the legal guardians of participants and from the age of twelve on also from the participants themselves.

Participants

Children from Leipzig or neighboring municipalities in Germany were recruited via advertisement at different institutions, by media or by word of mouth. The children were primarily healthy, without severe disorders like malignancies, syndromal diseases or diabetes. Accordingly, the acquired test results are qualified for generating reference values.

Data Acquisition

Each study visit contained age-customized interviews, medical examinations, standardized tests, questionnaires and the collection of biological samples, as well as the implementation of FibroScan® measurements (54,55).

For our study the following measurement results were analyzed:

Age: Number in years according to the birth dates/personal documents of the participants.

Sex: Categories female or male according to the birth certificates/personal documents of the participants.

Height: Height was measured using a stadiometer ("Prof. Keller"; Längenmesstechnik GmbH Limbach, Limbach-Oberfrohna, Germany, measurement accuracy 0.10 cm).

Weight: Participants were weighed with the "Seca701" scale (seca GmbH & Co.KG, Hamburg, Germany, accurate to 50 g).

Pubertal status: Pubertal stage was assessed by specially trained and regularly instructed investigators and classified to 5 pubertal stages, based on the Tanner stages (see above).

TE: The examination was carried out after an overnight fast by specially trained and regularly re-certified examiners. The participants were asked to lie on the back, the right arm maximally abducted, and to stay immobile during the examination. Those participants who were designated for dual measurements were asked to stay in the same position after the first measurement, and the second measurement was performed by the same examiner immediately afterwards.

Laboratory parameters: Blood samples were taken from the participants after an overnight fast. Serum glucose concentrations were measured by the photometric method (Roche, Basel, Switzerland). Serum insulin concentrations were measured using a quantitative electrochemiluminescence method (Roche) (57). Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as described in Matthews et al. (58).

Hypothesis

Given the above-mentioned relevance of Transient Elastography and the apparent lack of reference values, respectively percentiles in a pediatric context, we used the large study population of the LIFE Child study to derive reference values and to investigate the possible influencing factors age, sex, weight status and pubertal stage.

We did so due to the following assumptions:

Backed by latest research in our field, we started from the assumption that TE is a reliable tool to detect liver pathologies by identifying alterations in liver stiffness and/or percentage of fat in the liver tissue, especially NAFLD, in children at an early stage.

Furthermore, we presumed that to detect liver diseases, especially NAFLD, in children early by alterations of test results, one needs reference values of healthy children to be able to identify deviations from the norm (that is, from the reference values).

Following that approach, we premised that to generate genuine reference values, one needs to research into a large study cohort of healthy children.

Further on, we assumed that to evaluate test results and their potential ‘healthy’ development over time, one needs to consider potential influencing factors on these results to check for alterations that are merely occurring due to ‘normal’/’healthy’ physiological development.

We postulated that, presumably, the most relevant potential influencing factors on TE test results are age, sex, weight status and pubertal stage.

Thus, we formed our research question as follows:

Q: Do age, sex, weight status and pubertal stage have an impact on TE test results of children and adolescents?

Combining the above-mentioned premises and research question we obtained our hypothesis:

H: By investigating into the TE test results of a large study group of healthy children and checking for the potential influence of age, sex, weight status and pubertal stage on that test results, one can generate reliable reference values for TE measurements in children and, thus, establish generalizable percentiles for TE test results in the pediatric context.

Attempting to validate our hypothesis, we

- performed double measurements to assess accuracy and precision of our TE measurements;
- defined percentiles using data of children with normal-weight and children with overweight;
- assessed the influence of age, sex, weight status and pubertal stage on TE results.

Publication

Pediatric Percentiles for Transient Elastography Measurements - Effects of Age, Sex, Weight Status and Pubertal Stage

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Pediatric percentiles for transient elastography measurements - effects of age, sex, weight status and pubertal stage

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Background and aims: Transient Elastography is a non-invasive, cost-efficient, non-ionizing, observer-independent and reliable method to detect liver fibrosis using Liver Stiffness Measurement (LSM) and the degree of fat accumulation in the liver using Controlled Attenuation Parameter (CAP). This study aims to derive reference values for both measures from healthy children and adolescents. Further, we aim to assess the potential influence of age, sex, puberty, and BMI-SDS on CAP and LSM.

Methods: Within the LIFE Child study, amongst others, anthropometric data and pubertal status were assessed. Transient Elastography (TE) was performed using the FibroScan® device in a population-based cohort at 982 study visits of 482 healthy children aged between 10 and 18 years. Percentiles for LSM and CAP were estimated, and the effects of age, sex, puberty and weight status were assessed through hierarchical regression models.

Results: There was a strong age dependency for LSM with higher values for older children, most pronounced in the upper percentiles in boys. Contrarily, CAP was relatively stable across the age span without considerable difference between boys and girls. We found a significant positive correlation between BMI-SDS and both CAP and LSM for BMI-SDS >1.28. For BMI-SDS < 1.28, the association was also positive but reached statistical significance only for CAP. Further, the association between BMI-SDS and CAP was significantly stronger in younger than in older children. There was no association between pubertal status and CAP. For LSM, we found that children with a high BMI-SDS but not children with normal weight had significantly higher LSM values in Tanner stage 4.

Conclusions: Age, sex, pubertal status and weight status should be considered when interpreting LSM and CAP in pediatric patients to facilitate and improve

early detection of abnormal liver function, which is associated with common pathologies, such as NAFLD.

KEYWORDS

non-alcoholic fatty liver disease – NAFLD, fibroscan, liver stiffness, reference values, obesity, pediatrics

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in children and adolescents. A recent systematic review and meta-analysis estimated a global NAFLD prevalence of 7.6% in children. Moreover, in studies focusing on children with obesity, the prevalence was as high as 34.2% (1). Due to its association with obesity, NAFLD has already become a health issue of pandemic dimensions (2). Considering the growing number of children and adolescents with obesity worldwide (3), the impact of NAFLD on public health will likely increase even further.

NAFLD can lead to liver fibrosis and cirrhosis and increases the risk of developing hepatocellular carcinoma (HCC) (4, 5). Moreover, NAFLD is associated with an increased risk of cardiovascular disease, type 2 diabetes and increased mortality at adult age (2). Detected at early stages, before the liver is irreversibly damaged, NAFLD is treatable with lifestyle modifications, e. g., improved diet, increased physical activity and weight loss (6). There is a high probability of successful development of future pharmacological treatment options, since several drugs for children with NAFLD have been tested in phase 2 trials recently (2). To facilitate successful treatment, detecting NAFLD in pediatric patients accurately at an early stage is imperative.

Until today, the gold standard for diagnosing NAFLD is the histopathological examination of a liver biopsy. However, liver biopsy in children raises several ethical issues and is therefore reluctantly performed. Children often need general anesthesia, which entails a risk for the patient. Additionally, there is the risk of bleeding or mispuncture. Furthermore, since only a tiny part of the liver is examined, there is a risk of misdiagnosis due to sampling bias (7). Hence, reliable non-invasive diagnostic tools are urgently needed.

Various serum parameters and imaging procedures have been evaluated in several studies over the last years, but mostly with rather disappointing results (2, 6, 8–10). Measurement of alanine transaminase (ALT), for instance, is the most common serum parameter for screening, but physiological levels are no reliable predictor for the absence of NAFLD. Moreover, most imaging procedures bring their own disadvantages. CT detects fibrosis and steatosis reliably; however, it must not be used regularly in pediatric patients because of radiation burden. MRI, which works without radiation and is also very sensitive, is expensive and not widely available, rendering it unsuitable to be the standard procedure for detecting NAFLD. Regular ultrasound, on the other hand, is non-ionizing, inexpensive and widely available, but not reliable in detecting NAFLD (2).

Transient Elastography (TE, by FibroScan ® (Echosens, Paris, France)) has drawn a high amount of academic interest since it is a cost-efficient, observer-independent and non-ionizing method to detect fibrosis and steatosis reliably (11–18). FibroScan ® provides two different methods to examine the liver: liver stiffness measurement (LSM) and controlled attenuation parameter (CAP). While LSM is a parameter to estimate liver fibrosis, CAP quantifies the percentage of liver fat.

However, to use TE in pediatric practice, reliable reference values of healthy children - including the potential influence of age, sex, weight and pubertal status - are needed. By drawing from a large, longitudinal, deeply characterized cohort of healthy children, this study aims to provide percentiles for both LSM and CAP measurement. Moreover, we will examine the potential influence of sex, age, BMI and pubertal status on these two parameters. Hereby, we hope to facilitate a better interpretation of test results and, thus, to make a beneficial contribution to pediatric practice with regard to detecting and, ideally, treating NAFLD.

Materials and methods

This article is structured according to the STROBE (Strengthening the Reporting of Observational studies in Epidemiology) Statement checklist for cohort studies (19).

Study design

The LIFE Child study is a prospective longitudinal population-based cohort study with a life course approach to health and disease (20). As a part of LIFE, a research project conducted at the Leipzig Research Center for Civilization Diseases, LIFE Child aims to monitor healthy child development from birth to adulthood and to understand the

development of non-communicable diseases such as obesity (21). The study was designed in accordance with the Declaration of Helsinki (22). The Ethics Committee of the Medical Faculty of the University of Leipzig approved the study (Reg. No. 26410-19042010), which is registered with ClinicalTrials.gov under the clinical trial number NCT02550236.

Setting

Fully informed and written consent was obtained from all participants (from the age of twelve) and their parents. Each study visit contained age-customized interviews, medical examinations, standardized tests, questionnaires and the collection of biological samples, as well as the implementation of FibroScan® measurements (20, 21).

Participants

Children from Leipzig or neighboring municipalities in Germany were recruited *via* advertisement at different institutions, by media or by word of mouth. The children were primarily healthy, without severe disorders like malignancies, syndromal diseases or diabetes. Accordingly, the acquired test results are qualified for generating reference values. Height was measured using a stadiometer ("Prof. Keller"; Längenmesstechnik GmbH Limbach, Limbach-Oberfrohna, Germany, measurement accuracy 0.10 cm). Participants were weighed with the "Seca701" scale (seca GmbH & Co.KG, Hamburg, Germany, accurate to 50 g). BMI was calculated and transformed into standard deviation scores (SDS) according to the guidelines of the German Obesity Association (23, 24). Overweight and obesity were defined according to the same guidelines (23, 24) as 1.28 < BMI-SDS < 1.88 and BMI-SDS ≥ 1.88, respectively. Pubertal stage was assessed according to Tanner (25, 26) by specially trained and regularly instructed investigators.

Study size

Data from 1491 visits provided by 698 individuals from the LIFE Child cohort with a complete data set (CAP, LSM, sex, age, pubertal stage, and BMI) were available. In N=249 cases, we performed double measurements.

Our exclusion criteria were:

1. Measurements from participants younger than 10 years and older than 18 years of age were excluded (N=71 visits and 41 children), due to the small number of measurements below and above that age.
2. Participants with the intake of at least 1 of 92 potentially hepatotoxic drugs (listed in Supplementary Table 1) at the time of measurement were excluded, N= 62 visits and 10 children.

The remaining visits N=1358 from 647 children were used for the assessment of influence factors (sex, age, BMI-SDS, pubertal status).

For the calculation of LSM and CAP percentiles, we excluded 165 participants (231 visits) with a BMI-SDS < 3rd and >97th percentile (BMI-SDS < -1.88 and BMI-SDS>1.88), resulting in data from 982 visits from 482 individuals.

Glucose and insulin measurements were available from 625 visits from 196 individuals.

Transient elastography measurement

The examination was carried out after an overnight fast by specially trained and regularly re-certified examiners. The participants were asked to lie on the back, the right arm maximally abducted, and to stay immobile during the examination. Those participants who were designated for dual measurements were asked to stay in the same position after the first measurement, and the second measurement was performed by the same examiner immediately afterwards.

LSM and CAP values were measured using the FibroScan® device with the M probe (25 - 65 mm measurement depth) or XL probe (35 - 75 mm measurement depth). The FibroScan® device includes the Automatic Probe Selection (APS) tool, which indicates which of the two probes should be used for measurement. LSM measures the propagation of produced shear waves, and the results are displayed in kilopascals (kPa). CAP measures the attenuation of the above-mentioned shear wave propagation, producing results in decibels per meter (dB/m). The measurement was successful when 10 valid data points could be measured.

Laboratory parameters

Blood samples were taken from the participants after an overnight fast. Serum glucose concentrations were measured by the photometric method (Roche, Basel, Switzerland). Serum insulin concentrations were measured using a quantitative electrochemiluminescence method (Roche) (27). Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as described in Matthews et al. (28).

Statistical analyses

Descriptive statistics are given as mean and standard deviations for continuous and counts and percentages for categorical variables.

References for LSM and CAP were estimated as a continuous function of age, stratified by sex using the LMS method as implemented in the package gamlss (29). We corrected for multiple measurements per person by setting weights on the observations accordingly. Subsequently, CAP and LSM measurements were transformed to standard deviation scores applying the new references.

Associations between LSM and CAP as outcome and the assumed predictors (sex, age, BMI-SDS, and pubertal stage) were assessed using hierarchical regression analysis. To assess the effect of puberty, raw measurements of LSM and CAP were used because of the strong dependency between age and puberty; in all other models, the age- and sex-adjusted SDS were used as outcome. All models were adjusted for multiple measurements per subject by adding the subject as random effect. The nature of associations was investigated using non-parametrical generalized additive models. The association between LSM and BMI-SDS required polynomial modeling (3^{rd} degree). Otherwise, linear approximation yielded a sufficient fit. We tested for relevant interactions between predictors. Model terms were only kept if they were necessary. In addition, models were tested for variance inflation. Results were reported as (non-standardized) coefficients and the respective 95%-confidence interval.

To assess intraobserver reliability, we calculated the overall concordance correlation coefficient (OCCC) (30, 31). In addition, we report the components overall precision (OPREC) and overall accuracy (OACCU) and present the respective Bland-Altman plots. The chosen strength-of-agreement categories are orientated to those of the Pearson product-moment correlation: CCC ≥ 0.9 ("excellent"); < 0.9 and ≥ 0.7 ("good"); < 0.7 and ≥ 0.5 ("moderate"); and < 0.5 ("low").

The mediating effect of hepatic insulin resistance was assessed by mediation analyses using HOMA-IR implemented *via* a structural equation model.

Analyses and visualization were performed using the packages gamlss (29), lme4 (32) (version 1.1.30) and ggplot2 (3.3.6) in R (version 4.2.1; R Foundation for Statistical Computing, Vienna, Austria) (33).

Results

Participants

We used the data of 482 (252 male, 231 female) healthy individuals, aged between 10 and 18 years with a BMI-SDS between 3^{rd} and 97^{th} percentile, who were examined between December 2013 and June 2022 in the context of the Leipzig Research Centre for Civilization Diseases (LIFE). Since LIFE Child is a longitudinal study, some participants were measured more than once over the period of 8 years, resulting in a total of 982 (624 male, 587 female) visits for the calculation of the percentiles. Dual measurements for the evaluation of FibroScan® validity were performed in 249 individuals. The population characteristics for the entire study population (N=1358) are listed in Table 1.

Reproducibility/FibroScan ® validity

For both LSM and CAP, we could show an "excellent" OACCU. OCCC and OPREC were "good" for LSM and "moderate" for CAP. The results are shown in Table 2 and Figures 1A, B.

Percentiles for LSM and CAP are influenced by sex and age

The 3^{rd}, 10^{th}, 50^{th}, 90^{th} and 97^{th} percentile curves for LSM and CAP are shown for boys and girls in Figures 2A, B. The respective parameter values are shown in Supplementary Tables 2 (A)-(D).

LSM percentiles show increasing values for both sexes with, in general, higher values for boys, which becomes more pronounced in the upper percentiles (e.g., 16.5 years p50: girls=4.6kPa boys=5.1kPa; p97: girls=7.2kPa boys=8.5kPa). Also, the curve shapes differ from each other with regard to sex: The curves for girls ascend for the first 1.5 years, then slightly flatten for about 1.5 years, after which they ascend again until they reach their peak at about 16.5 years (P50 5.95kPa) which is followed by another slight drop in the 3^{rd}, 10^{th} and 50^{th} percentile. The curves P50, P90 and P97 for boys, on the other hand, show continuous slopes until reaching their peaks, followed by a slight flattening. The age at which boys reach the highest values is comparable with that of girls (about 16.5 years) in P50, 90 and 97. In the lower percentiles, however, the highest values were measured at 18 years.

CAP percentiles show similarly shaped curves for boys and girls. The reference values are comparable as well. Comparing the reference values at the age of ten and 18 years, the lower percentiles show a tendency to descend slightly while the higher percentiles tend to ascend slightly, reaching their peaks at about 14 years. P50 depicts rather stable values during the eight years (boys: 200dB/m at age 11 and 15 years and 198db/m at age 18 years; girls: 188dB/m at age 11 years and 197dB/m from age 14.5 – 18 years).

The parameter tables are provided as part of the R package childs (version 0.8.0). The package also contains functions to transform measurement values into SDS and to create percentile curves. It is available from CRAN (https://cran.r-project.org/package=childsds).

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TABLE 1 Baseline characteristics of the study population.

	[ALL] N = 1358	male N = 692	female N = 666	p.overall
Sex:				
male	692 (51.0%)			
female	666 (49.0%)			
Age (years)	14.0 (2.81)	13.9 (2.87)	14.1 (2.74)	0.432
Pubertal Stage:				<0.001
1	154 (15.3%)	91 (20.5%)	63 (11.2%)	
2	146 (14.5%)	77 (17.3%)	69 (12.2%)	
3	111 (11.0%)	42 (9.46%)	69 (12.2%)	
4	170 (16.8%)	73 (16.4%)	97 (17.2%)	
5	428 (42.4%)	161 (36.3%)	267 (47.3%)	
Weight status:				0.421
underweight/normal weight	885 (65.3%)	455 (65.8%)	430 (64.8%)	
overweight	129 (9.51%)	71 (10.3%)	58 (8.73%)	
obese	342 (25.2%)	166 (24.0%)	176 (26.5%)	
BMI-SDS	0.71 (1.39)	0.64 (1.33)	0.78 (1.45)	0.055

Values are given as mean and standard deviations for continuous and counts and percentages for categorical variables.

TABLE 2 Results of the calculation of the OCCC, the OPREC and the OACCU for LSM and CAP of N=249 dual measurements.

	OCCC	OPREC	OACCU
LSM	0.74	0.76	0.97
CAP	0.66	0.66	1.0

OCCC, overall concordance correlation coefficient; OPREC, overall precision; OACCU, overall accuracy; LSM, Liver Stiffness Measurement; CAP, Controlled Attenuation Parameter. Results were classified as ≥ 0.9 "excellent"; < 0.9 and ≥ 0.7 "good"; < 0.7 and ≥ 0.5 "moderate"; and < 0.5 "low".

Influence of BMI-SDS on LSM and CAP

After establishing percentiles, we assessed the association between weight status and both LSM and CAP values. In children with a BMI-SDS <1.28, there was a slightly positive association between LSM-SDS and BMI-SDS. However, it did not reach statistical significance (beta=0.07, p=0.48). In children with overweight and obesity, the respective effect size was three times as high, and the association became significantly positive (beta=0.26, p=0.025) (see Figure 3A). The two slopes were not significantly different from each other ($beta_{Interaction}$=0.19, p=0.289). The effects of weight status on LSM were not different, regardless of age and sex.

In children with a BMI-SDS <1.28, we found a significant positive association between CAP-SDS and BMI-SDS (beta=0.15, p<0.001). In children with a BMI-SDS >1.28, the effect size was six times as high (beta=0.95, p<0.001) (see Figure 3B). The two slopes were significantly different from each other ($beta_{interaction}$=0.85, p<0.001). In addition, the effect varied significantly with age, having the strongest effect for younger children ($beta_{10years}$=1.6, p<0.001) and the weakest effect for older adolescents ($beta_{18years}$=0.6, p<0.001). The effect of weight status on CAP did not differ between sexes.

Influence of pubertal status on LSM

LSM increased significantly with advancing puberty in boys. The values were significantly higher in Tanner stage (TS) 3 (beta=1.1, p=0.029), TS 4 (beta=1.2, p=0.004), and TS 5 (beta=1.5, p<0.001) than in TS 1. In girls, there was no such distinct pattern. Considering weight status, there was a significant interaction between Tanner stage and BMI-SDS: While we found no effect of puberty in children with a BMI-SDS around or below 0, we found significantly higher LSM values for children with BMI-SDS of 1.88 or higher in TS 4 and 5. The effects were remarkably stronger in TS 4 ($beta_{3BMI-SDS}$=4.3, p<0.001; $beta_{2.6BMI-SDS}$=2.6, p<0.001; $beta_{2BMI-SDS}$=1.5, p=0.005) than in TS 5 ($beta_{3BMI-SDS}$=1.3, p=0.14; $beta_{2.5BMI-SDS}$=1.5, p=0.004; $beta_{2BMI-SDS}$=1.5, p=0.001) (see Figure 4). The association did not differ between sexes. The association of LSM with Tanner stage 4-5 was partly (approximately 1/3, p = 0.047)

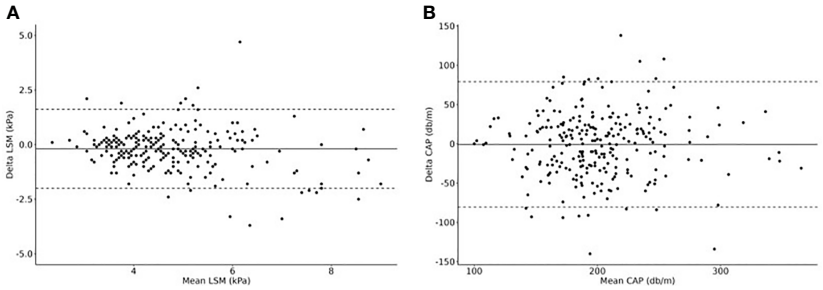

FIGURE 1
Dual measurements of **(A)** LSM and **(B)** CAP to calculate the overall concordance correlation coefficient (OCCC), the overall precision (OPREC) and the overall accuracy (OACCU). Delta values are plotted in relationship to mean values (Bland-Altman Plots). N=249 cases.

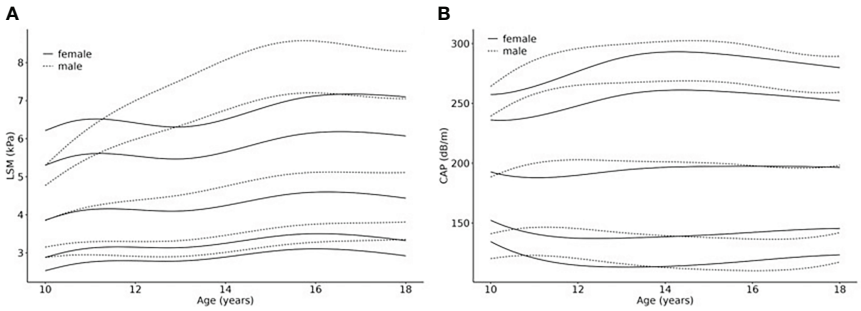

FIGURE 2
Age- and sex-related percentiles of **(A)** LSM (kPa) and **(B)** CAP (dB/m) values. Smoothed percentile curves are shown separately for females and males in relationship to age (10 – 18 years), based on a normal weight reference population from a LIFE Child study sample (N =982 cases (624 male, 587 female) of 482 (252 male, 231 female) healthy individuals). The 3rd (P3), 10th (P10), 50th (P50, median), 90th (P90) and 97th (P97) percentile are shown.

explained by hepatic insulin resistance, which we measured as Homeostatic Model Assessment of Insulin Resistance (HOMA-IR).

Influence of pubertal status on CAP

There was no significant association between CAP results and puberty. Moreover, the association between BMI-SDS and CAP did not differ between Tanner stages. Values were, on average, 10 kPa higher for males than for females (p=0.012). There was no interaction between sex and Tanner stage or BMI-SDS.

Discussion

Considering the rising prevalence of obesity and concomitant liver diseases, especially NAFLD, in children and adolescents, non-invasive diagnostic tools to accurately detect liver pathologies in pediatric patients are urgently needed. Transient Elastography has been used extensively to aid the diagnosis of fatty liver disease and fibrosis in the adult population for which TE reference values are available (34–36). Several studies have already postulated the need for reliable TE reference values for children, respectively the necessity of further detailed research on TE measurement in the pediatric context (6, 37).

With the aim to close this knowledge gap, this study provides pediatric reference values and presents the respective percentiles for the Transient Elastography measurements LSM and CAP, based on our investigation of a large and well-characterized cohort of healthy children and adolescents. We decided to include children with overweight when estimating percentiles because our analyses revealed that the influence of BMI-SDS was similar as in children with normal weight. In contrast, increasing BMI-SDS had considerably stronger effects on LSM and CAP for children with obesity (Figure 3).

Furthermore, we analyzed the influence of age, sex, weight and pubertal status on LSM and CAP. Thereby, we enable

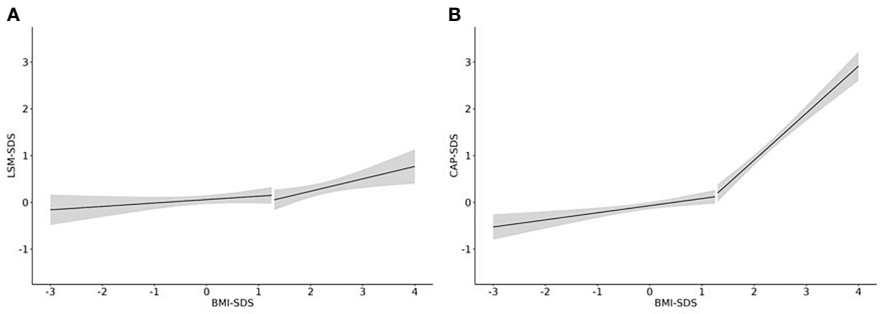

FIGURE 3
Effect of weight status on LSM and CAP. Linear regression curves including a 95%-confidence band are shown for the association of **(A)** LSM-SDS and BMI-SDS and **(B)** CAP-SDS and BMI-SDS. N = 1358 from 647 children.

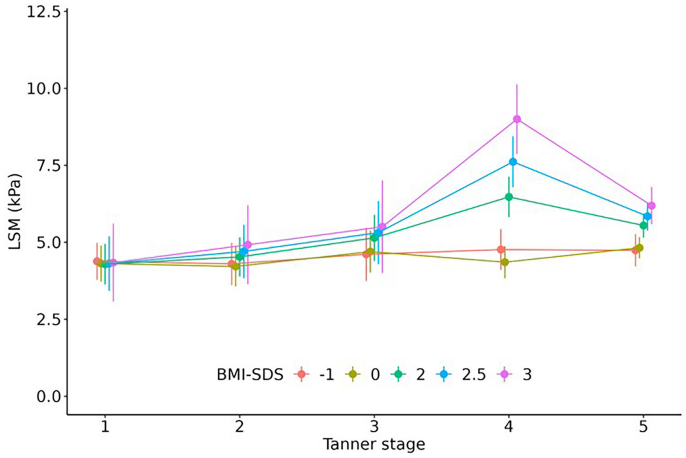

FIGURE 4
Effect of pubertal status on LSM. Children with overweight/obesity (BMI-SDS ≥ 2) but not with normal weight had significantly higher LSM values in Tanner stage 4 and 5. Regression estimates including 95% confidence interval are shown.

examiners and practitioners to interpret LSM and CAP test results in children more accurately, having appropriate reference values at hand.

Our research has shown that LSM is age-dependent, and LSM test results tend to increase with age in the pediatric context. Our reference values for LSM are generally in line with findings of other recent studies examining healthy children (38–43). Likewise, the increase over age was also observed in other studies (38–42). Contrary to this, Ramirez et al., who investigated a cohort of 462 healthy children and provided reference values as well, found no effect of age on LSM (43). Potential reasons for this inconsistency include a different age range (12 – 20 years) as well as a different ethnic and geographic background of the cohort. A recent meta-analysis with 1702 participants, on the other hand, also found that values increase with age (40). Zeng et al. (44) provided reference values for five-year-olds based on a very large cohort. The reference values they established were remarkably lower than ours. Since we included participants starting at age 10 who, from the start, showed higher values compared to those of five-year-olds (Zeng 2019: LSM_{5years} median 3.2 kPa *vs*. Brunnert 2022: $LSM_{10years}$ median 3.9 kPa), we regard their study results, taken together with ours, as strongly supporting the validity of the assumption that pediatric LSM values increase with age. However, Mjelle et al. (39) state that there is about the same number of studies indicating an age-dependency of LSM values as there is for age-independency. This clearly highlights the need to further investigate the age-dependency of LSM values in the pediatric context. In our study, LSM values peak at 14.5 years and stay more or less stable afterwards. This leads to the assumption that after age 18 no further increase in LSM values will occur. This assumption is in line with the so far published studies of the

adult population stating that LSM results show no age-dependency (45, 46).

Moreover, our research has shown that LSM test results are higher for boys. This is also confirmed by results from other relevant recent studies (38, 39, 42). Tokuhara et al., on the other hand, could not find any influence of sex on LSM results (41). Likewise, the above-mentioned study by Ramirez et al. (43) did not find sex-dependent alterations of LSM. Since our study clearly shows the sex-dependency of LSM test results, we expect that future research will further validate this outcome.

We could not identify any correlation between age, sex and CAP. With regard to pediatric CAP measurements, there are only a few published studies providing reference values. Ramirez et al. (43) presented stable, age- and sex-independent CAP values from the ages 12 to 20. Their findings are in line with our observation that CAP values are neither age- nor sex-dependent. This was also shown by a recent study by Ferraioli et al. (47). However, Zeng et al. (44) identified a median CAP value of 171db/m for five-year-olds, so there might be a tendency for lower CAP values at younger ages, if we take into account that our values for older children and adolescents are remarkably higher (median LSM at age 15: 197dB/m for girls, 200dB/m for boys). Since we only analyzed results of children aged 10 years and older, our study could not add further insights on the question of whether CAP values increase below age 10.

We found a positive correlation between weight status and LSM as well as CAP test results, also found by Zeng et al. (44). In addition, Ferraioli et al. (47) examined CAP values of children categorized as 'normal weight', 'overweight' and 'obese'. They, too, found a significant positive association between CAP and weight status (30). Lee et al. evaluated LSM in children with obesity. Values were remarkably higher (16) than in our reference population, which further supports our finding of a considerable impact of weight status on LSM values.

Furthermore, we found that LSM but not CAP values differ across puberty. To our knowledge, we present the first examination of the impact of pubertal status on TE measurements. Partly, the effect might be explained by the increasing hepatic insulin resistance during puberty (48) as our results suggest. Another reason for increased hepatic insulin resistance is obesity (49). Accordingly, we found that adolescents with obesity had significantly higher LSM, especially in Tanner stage 4 and 5. The underlying mechanisms of this phenomenon are unclear and should be subject to future research.

Evaluating dual measurements, we could show that TE is a method with medium reproducibility. Our findings are in line with results of other studies investigating the reproducibility of TE measurements: Ferraioli et al. reported a concordance correlation coefficient (CCC) for CAP of 0.82 for children with normal weight and 0.6 for children with obesity (17). Rowland et al. reported a CCC for LSM of 0.85 (50). We would, therefore, suggest the implementation of a second measurement in case of borderline TE results, to improve the reliability of the results.

There are some limitations to our study. We only used data from a single study center with limited access to subjects with diverse ethnic background. Thus, our results are not necessarily representative of pediatric patients worldwide. Furthermore, families participating in the LIFE Child research project generally have a socio-economic status above average (51) which could also render our findings less representative with regard to both the global pediatric population as well as the general pediatric population of a particular state or region. In addition, the HOMA-IR was only available for a subpopulation (n = 196) which led to less power in the related analyses. Moreover, for evident ethical reasons, we did not perform liver biopsies to validate our test results.

Nevertheless, our study has several strengths. To our knowledge, this paper is the first to provide reference values for both LSM and CAP based on a large pediatric cohort from 10 to 18 years. Additionally, we established that age, sex, BMI-SDS and pubertal status have an impact on TE test results and, thus, should be considered when evaluating LSM and CAP values. Accordingly, we suggest our sex- and age-adapted reference values to interpret TE results in pediatric practice. There are numerous studies evaluating the usefulness and feasibility of TE for pediatric subjects, but most of them only examine patients with NAFLD or obesity. However, in pediatric practice, we need reference values guiding us in our endeavor to identify potential risks or existing diseases in patients. Thus, the reference values and percentiles we present in this paper can help us to red-flag conspicuous test results.

Given the already high and, most likely, further increasing prevalence of liver diseases such as NAFLD, it is paramount to detect potential diseases at an early stage. Our paper attempts to make a valuable contribution to this endeavor in terms of research as well as practice.

Data availability statement

The datasets presented in this article are not readily available because data cannot be shared publicly because there exist ethical restrictions. The LIFE Child study is a study collecting potentially sensitive information. Publishing data sets is not covered by the informed consent provided by the study participants. Furthermore, the data protection concept of LIFE requests that all (external as well as internal) researchers interested in accessing data sign a project agreement. Researchers that are interested in accessing and analyzing data collected in the LIFE Child study may contact the data use and access committee (dm@life.uni-leipzig.de). Requests to access the datasets should be directed to dm@life.uni-leipzig.de.

Ethics statement

This study was reviewed and approved by The Ethics Committee of the Medical Faculty of the University of Leipzig. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

Conceptualization, SG, MP, WK, AG and GF. Methodology, TK, NG, GF and MV. Software MV. Validation, IP, NG and MV. Formal analysis IP, LB and MV. Investigation, IP, NG, GF, MV. Resources, TK, GF, WK and MV. Data curation, IP, LB and MV. Writing—original draft preparation LB, IP and MV. Writing—review and editing, LB, AG, GF and MV. Visualization, IP, LB and MV. Supervision, GF, MV, AG and WK. Project administration, AG. Funding acquisition, SG, MP and WK. All authors contributed to the article and approved the submitted version.

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Conflict of interest

TK received unrestricted research grants from Echosens SA, France, not related to this project. TK took part in a clinical advisory board meeting of Echosens SA.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1030809/full#supplementary-material

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Summary

Dissertation zur Erlangung des akademischen Grades

Dr. med.

Pediatric Percentiles for Transient Elastography Measurements - Effects of Age, Sex, Weight Status and Pubertal Stage

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NAFLD has become the most common liver disease in children and adolescents due to its correlation with obesity which is a pandemic disease with a still rising prevalence. Since there are therapeutic options when NAFLD is detected at an early stage, a reliable and early diagnose is paramount. In recent years, Transient Elastography (TE) has emerged as an appropriate tool in that regard. It is non-invasive, painless, observer-independent, cost-efficient, non-ionizing as well as reliable.

Until today, TE reference values are only available for adults and it is well known that reference values for adult populations are rarely applicable to pediatric populations. Thus, our aim was to generate pediatric percentiles and reference values for the TE measurements LSM and CAP.

Therefore, we analyzed the test results of the TE measurements LSM and CAP of a large and healthy cohort from the LIFE child study and derived reference values from these data. To calculate these reference values, we used the data of 982 visits from 482 individuals aged 10 to

18 years. Participants with a BMI-SDS < 3rd and >97th percentile (BMI-SDS < -1.88 and BMI-SDS>1.88) and/or with intake of potentially hepatotoxic drugs were excluded. TE measurements were implemented on fasting participants by trained examiners. From the resulting CAP and LSM values, we derived our reference values with corresponding percentiles.

We could show that LSM values increase with age and are higher for boys than for girls. This age- and sex-dependency is in line with other recent studies, yet there are also studies which state the opposite.

Our CAP results, on the other hand, are not influenced by age and sex. This result is also mirrored by other recent studies.

Beside the establishment of age- and sex-adjusted percentiles, our aim was to analyze the potential influence of weight status and pubertal stage on TE results. In this regard, we used the data of 1358 visits (of 647 children); children with under- or overweight were included.

We found a positive association between weight status and, both, LSM and CAP values.

The inquiry into the interaction between LSM values and weight status yielded the following: In children with a BMI-SDS <1.28, respectively participants with normal weight, there was a slightly positive association between LSM-SDS and BMI-SDS. Yet, the results did not reach statistical significance. In children with overweight and obesity, the respective effect size was three times higher, and the association became significantly positive. The effects of weight status on LSM were the same, independent of age and sex.

The same inquiry into CAP values showed the following results: In children with a BMI-SDS <1.28, we found a significantly positive association between CAP-SDS and BMI-SDS. In children with a BMI-SDS >1.28, the effect size was six times higher. Furthermore, the effect varied significantly depending on the age of the patient, having the strongest effect for younger children and the weakest effect for older adolescents. There was no effect of weight status on CAP dependent on sex.

This result is backed up by other recently published studies.

To the best of our knowledge, to date, there is no published study investigating the potential influence of puberty on TE results. Thus, we attempted to close this research gap. Whereas we

could not find a significant association between CAP results and puberty, LSM test results showed a significant increasement with advancing puberty in boys. The values were significantly higher in Tanner stage (TS) 3, TS 4, and TS 5 than in TS 1. We could not find a similar pattern in girls.

Focusing upon weight status, we recognized a significant interaction between Tanner stage and BMI-SDS: While we could not identify an effect of puberty in children with a BMI-SDS about or below 0, we detected significantly higher LSM values for children with BMI-SDS of 1.88 or higher in TS 4 and 5. Further on, we found remarkably stronger effects in TS 4 than in TS 5. Sex had no effect on that association.

We could partially shed light on the association of LSM with Tanner stage 4-5 by looking into hepatic insulin resistance, which we measured as Homeostatic Model Assessment of Insulin Resistance (HOMA-IR). Therefore, we analyzed 625 glucose and insulin measurements (625 visits; 196 individuals). The increasement of the hepatic insulin resistance during puberty could present an adequate explanation, since it leads to higher liver stiffness. Also, obesity is leading to an increase in hepatic insulin resistance. Accordingly, we found significantly higher LSM values in adolescents with obesity, especially in Tanner stage 4 and 5. The underlying logic of this association is yet to be found. Thus, future research should engage with this phenomenon.

To evaluate the validity of FibroScan®, we performed dual measurements in 249 individuals. With the aim of assessing intraobserver reliability, we calculated the overall concordance correlation coefficient (OCCC). Furthermore, we accounted for the components overall precision (OPREC) and overall accuracy (OACCU) and present the respective Bland-Altman plots. The chosen strength-of-agreement categories are orientated to those of the Pearson product-moment correlation: CCC ≥ 0.9 = excellent; < 0.9 and ≥ 0.7 = good; < 0.7 and ≥ 0.5 = moderate; and < 0.5 = low. For both LSM and CAP, we could show an “excellent” OACCU. OCCC and OPREC were “good” for LSM and “moderate” for CAP.

In evaluation of the results, TE presented itself as a method with medium reproducibility. This is in line with results of other studies investigating the reproducibility of TE measurements. Thus, the implementation of a second measurement in case of borderline TE results seems advisable and does improve the reliability of the results.

There are also limitations regarding the generalizability of our test results: Albeit our research cohort is rather large, it is not particularly heterogenous in terms of socio-economic status as

well as geographically limited. Hence, further investigation into cohorts of a more diverse descent is needed.

Overall, in our study, we present age- and sex-adjusted percentiles and reference values for LSM and CAP measurements, which should be used to classify results in clinical practice. Furthermore, by providing results sensitive to the influencing factors weight status and pubertal stage, we enable the practitioner to see the results in context of the individual situation of the patient.

Therefore, we are confident that our study makes a valuable contribution to, both, research and practice, for we present reliable reference values and percentiles for TE measurement in children and adolescents that are sensitive for potential influencing factors and, thus, enable practitioners to an accurate assessment of individual TE test results, leading to an earlier and more precise identification as well as treatment of liver diseases in children, especially NAFLD.

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Appendix

Supplementary Table 1. List of potentially hepatotoxic drugs. Individuals with intake of ≥ 1 of these drugs were excluded from the study population.

Abacavir Sulfate	Flutamide	Phenazopyridine Hydrochloride
Acetaminophen	Fluvastatin Sodium	Phenytoin Sodium
Allopurinol	Glimepiride	Pilocarpine Hydrochloride
Amiodarone Hydrochloride	Glipizide	Pravastatin Sodium
Amoxicillin	Glyburide	Probenecid
Amoxicillin Trihydrate; Clavulanate	Indinavir Sulfate	Progesterone
Potassium	Isoniazid	Propylthiouracil
Atorvastatin Calcium	Itraconazole	Pyrazinamide
Azathioprine	Ketoconazole	Quinine Sulfate
Bicalutamide	Labetalol Hydrochloride	Repaglinide
Bromocriptine Mesylate	Lamivudine	Rifabutin
Carbamazepine	Leflunomide	Ritonavir
Carbidopa; Levodopa	Leucovorin Calcium	Rivastigmine Tartrate
Cerivastatin Sodium	Lovastatin	Saquinavir
Chlorpropamide	Medroxyprogesterone Acetate	Simvastatin
Chlorzoxazone	Megestrol Acetate	Stavudine
Cholesterol Lowering Drug – Unspec.	Nomegestrol and Estradiol	Sulfamethoxazole
Clotrimazole	Mesalamine	Sulfasalazine
Colchicine	Mestranol; Norethindrone	Tamoxifen Citrate
Desogestrel; Ethinyl Estradiol	Methimazole	Terbinafine Hydrochloride
Diclofenac	Methotrexate Sodium	Testosterone
Didanosine	Methyldopa	Tetracycline Hydrochloride
Divalproex Sodium	Minocycline Hydrochloride	Tizanidine Hydrochloride
Efavirenz	Montelukast Sodium	Tolazamide
Erythromycin	Nabumetone	Tretinoin
Estrogen	Niacin	Trimethoprim
Estrogen and Progesteron	Nitrofurantoin	Troglitazone
Ethambutol Hydrochloride	Norethindrone	Valproic Acid
Fenofibrate	Olanzapine	Zafirlukast
Fluconazole	Pemoline	Zidovudine
Fluorouracil	Permethrin	

Supplementary Table 2. Reference values of the study reference population (N=982) for Liver Stiffness Measurement (LSM) for boys (A) and girls (B) and Controlled Attenuation Parameter (CAP) for boys (C) and girls (D) including mu, coefficient of variation (sigma) and skewness (nu), tabulated in half-year steps.

(A)

sex	age	3rd percentile	10th percentile	50th percentile	90th percentile	97th percentile	mu	sigma	nu
male	10	120.28	141.10	188.49	239.37	264.26	188.4861	0.2037	0.7283
male	10.5	122.45	144.69	195.44	250.07	276.83	195.4381	0.2107	0.7283
male	11	122.98	146.39	199.95	257.76	286.10	199.9537	0.2176	0.7283
male	11.5	122.11	146.43	202.21	262.56	292.18	202.2145	0.2244	0.7283
male	12	120.36	145.37	202.87	265.22	295.87	202.8683	0.2309	0.7283
male	12.5	118.18	143.72	202.56	266.50	297.97	202.5624	0.237	0.7283
male	13	116.04	142.00	201.94	267.21	299.36	201.9444	0.2424	0.7283
male	13.5	114.27	140.59	201.50	267.93	300.68	201.5018	0.2471	0.7283
male	14	112.87	139.50	201.18	268.55	301.78	201.1845	0.2508	0.7283
male	14.5	111.82	138.63	200.83	268.81	302.37	200.8296	0.2535	0.7283
male	15	111.06	137.92	200.27	268.46	302.13	200.2739	0.2549	0.7283
male	15.5	110.54	137.28	199.35	267.24	300.76	199.3544	0.2549	0.7283
male	16	110.26	136.69	198.02	265.04	298.13	198.0154	0.2534	0.7283
male	16.5	110.48	136.47	196.68	262.42	294.86	196.6814	0.2504	0.7283
male	17	111.53	137.00	195.91	260.12	291.78	195.9117	0.2457	0.7283
male	17.5	113.74	138.70	196.27	258.87	289.70	196.2654	0.2394	0.7283
male	18	117.50	141.99	198.30	259.37	289.40	198.3018	0.2314	0.7283

LSM reference values in kPa for boys, age in years.

(B)

sex	age	3rd percentile	10th percentile	50th percentile	90th percentile	97th percentile	mu	sigma	nu
female	10	134.54	152.34	192.77	236.18	257.44	192.7662	0.1698	0.6733
female	10.5	126.36	145.45	189.10	236.33	259.57	189.0982	0.1877	0.6733
female	11	120.70	141.06	187.94	239.01	264.23	187.9407	0.2036	0.6733
female	11.5	116.94	138.51	188.45	243.17	270.27	188.4518	0.217	0.6733
female	12	114.68	137.34	190.05	248.06	276.87	190.0519	0.2277	0.6733
female	12.5	113.56	137.13	192.16	252.93	283.15	192.1616	0.2355	0.6733
female	13	113.21	137.46	194.20	256.99	288.26	194.2015	0.2406	0.6733
female	13.5	113.31	137.96	195.69	259.64	291.51	195.6892	0.2431	0.6733
female	14	113.78	138.56	196.62	260.94	292.99	196.6177	0.2433	0.6733
female	14.5	114.59	139.29	197.13	261.15	293.05	197.1252	0.2416	0.6733
female	15	115.69	140.15	197.35	260.59	292.07	197.3501	0.2385	0.6733

female	15.5	117.03	141.15	197.43	259.55	290.44	197.4305	0.2344	0.6733
female	16	118.54	142.25	197.48	258.30	288.52	197.4754	0.2297	0.6733
female	16.5	120.09	143.37	197.46	256.92	286.43	197.4635	0.2247	0.6733
female	17	121.52	144.37	197.34	255.44	284.25	197.3378	0.2199	0.6733
female	17.5	122.69	145.12	197.04	253.89	282.04	197.0413	0.2157	0.6733
female	18	123.45	145.52	196.52	252.27	279.86	196.5169	0.2122	0.6733

LSM reference values in kPa for girls, age in years.

(C)

sex	age	3rd percentile	10th percentile	50th percentile	90th percentile	97th percentile	mu	sigma	nu
male	10	2.88	3.15	3.85	4.77	5.30	3.8512	0.1617	-0.3079
male	10.5	2.94	3.25	4.07	5.18	5.84	4.066	0.1822	-0.3079
male	11	2.95	3.29	4.22	5.52	6.30	4.217	0.2011	-0.3079
male	11.5	2.93	3.30	4.31	5.78	6.69	4.3147	0.2179	-0.3079
male	12	2.91	3.30	4.38	5.99	7.00	4.3821	0.232	-0.3079
male	12.5	2.90	3.30	4.44	6.17	7.27	4.4424	0.2432	-0.3079
male	13	2.91	3.32	4.52	6.35	7.53	4.5185	0.2516	-0.3079
male	13.5	2.95	3.38	4.63	6.55	7.81	4.6256	0.2571	-0.3079
male	14	3.01	3.46	4.75	6.75	8.07	4.7522	0.2599	-0.3079
male	14.5	3.09	3.55	4.88	6.94	8.30	4.8812	0.2606	-0.3079
male	15	3.17	3.64	5.00	7.09	8.48	4.9956	0.2594	-0.3079
male	15.5	3.24	3.71	5.08	7.19	8.57	5.0783	0.2568	-0.3079
male	16	3.28	3.76	5.12	7.20	8.57	5.1173	0.2534	-0.3079
male	16.5	3.31	3.78	5.12	7.17	8.50	5.1231	0.2495	-0.3079
male	17	3.32	3.79	5.11	7.12	8.41	5.1129	0.2456	-0.3079
male	17.5	3.33	3.79	5.10	7.07	8.33	5.1036	0.2421	-0.3079
male	18	3.35	3.81	5.11	7.05	8.30	5.112	0.2394	-0.3079

CAP reference values in dB/m for boys, age in years.

(D)

sex	age	3rd percentile	10th percentile	50th percentile	90th percentile	97th percentile	mu	sigma	nu
female	10	2.53	2.88	3.86	5.30	6.21	3.8611	0.2375	-0.2687
female	10.5	2.68	3.04	4.04	5.51	6.43	4.0413	0.232	-0.2687
female	11	2.76	3.13	4.14	5.61	6.52	4.139	0.2273	-0.2687
female	11.5	2.79	3.16	4.16	5.60	6.50	4.1585	0.2235	-0.2687
female	12	2.79	3.15	4.13	5.547	6.42	4.135	0.2204	-0.2687
female	12.5	2.78	3.13	4.10	5.49	6.34	4.1036	0.218	-0.2687
female	13	2.79	3.14	4.10	5.47	6.31	4.0994	0.2164	-0.2687

female	13.5	2.83	3.18	4.15	5.53	6.37	4.1499	0.2153	-0.2687
female	14	2.89	3.25	4.24	5.65	6.51	4.2437	0.215	-0.2687
female	14.5	2.97	3.34	4.36	5.80	6.69	4.358	0.2152	-0.2687
female	15	3.04	3.42	4.47	5.96	6.87	4.4696	0.2161	-0.2687
female	15.5	3.09	3.48	4.56	6.09	7.03	4.5556	0.2177	-0.2687
female	16	3.11	3.50	4.60	6.16	7.13	4.5977	0.2198	-0.2687
female	16.5	3.09	3.49	4.60	6.18	7.17	4.5986	0.2226	-0.2687
female	17	3.05	3.45	4.57	6.17	7.17	4.5665	0.226	-0.2687
female	17.5	2.99	3.40	4.51	6.13	7.14	4.5097	0.2301	-0.2687
female	18	2.92	3.32	4.44	6.07	7.10	4.4368	0.235	-0.2687

CAP reference values in dB/m for girls, age in years.

Description of the own contribution

Description of the own contribution

Lina Brunnert´s contribution:

- formal analysis
- data curation
- writing: original draft preparation, review and editing
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