

An international multicenter study of MASLD prevalence among children and adolescents assessed by VCTE

Corresponding Author: Professor Ming-Hua Zheng

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The study represents an impressive effort to investigate the prevalence of MASLD across both hospital settings and community populations by using VCTE. Population based data from primary care have been particularly limited, making this contribution especially valuable. Manuscript is well written and statistical methods are clear and appropriate. I have only minor recommendations to further improve the manuscript.

Main manuscript:

On the line 267 it is mentioned that BMI was used to define overweight and obesity. Why not use BMI Z-score, which is more accurate in pediatric populations?

Starting from line 382, "When discussing the prevalence of MASLD across different studies, could you expand the interpretation by considering whether the observed variation might be related to differences in the severity of pediatric obesity and the age distributions of the study populations?"

Figures/tables:

Could you add information on the prevalence of overweight and obesity in the different populations to the figure legend of Figure 3, or clarify whether the children from the various continents were comparable in terms of age, BMI z score, and sex distribution?

Supplementary files:

To the supplementary file methods, could you consider adding the prevalence of MASLD in the children under 6 years of age, since this would be very interesting data and information is limited.

Were the exclusion of other liver affecting conditions systematic within study population, or based on clinical decision-making? If not systematic, this should be mentioned as a limitation.

Reviewer #2

(Remarks to the Author)

Thank you for the opportunity to review this manuscript. Zhang and colleagues have undertaken an impressive task of delineating paediatric MASLD to a greater extent, with greater concordance to adult literature.

This multicentre study (17 countries) estimates the global paediatric MASLD prevalence using VCTE, which positions this uniquely as prior studies have relied on either USG imaging or ALT levels. The authors report a prevalence of 18% in community practices vs 58% in hospital practices. They also demonstrate a marked regional variation in prevalence. Adolescents and males appeared at greater risk. Also, the prevalences appear to be more in the more recent years.

The key strengths are as follows:

- 1) Use of VCTE measurements, which is being used as prognostication to MALO / LRE in adult populations. This is the largest VCTE based study in paediatric populations, and provides valuable insight.
- 2) Clear definition of MASLD in concordance to existing literature.
- 3) Clearly identified at risk individuals from multivariate models

4) Alignment to NASPGHAN guidance.

My criticisms (and suggestions) are as follows:

- 1) Given the wide geographical variance, were the patients recruited differently? Details on how the patients were recruited from each site / country should be provided in a supplementary table. Also, referral thresholds from community / primary care should be delineated.
- 2) Is the higher prevalence in more recent years suggestive of greater awareness and case finding rather than true prevalence? This should be discussed.
- 3) I would suggest including a paragraph in the discussion mapping operational definitions to adult nomenclature as well as paediatric consensus recommendations.
- 4) Tone down the language on estimating risk - this is based on fibrosis score, and not on hard outcomes
- 5) Please discuss the reproducibility and repeatability of VCTE in the limitations of the modality (Pierce et al, Radiology 2024)

Reviewer #3

(Remarks to the Author)

This manuscript presents a cross-sectional study evaluating the global prevalence of MASLD in children and adolescents using vibration-controlled transient elastography (VCTE). The topic is timely and clinically relevant, given the increasing burden of pediatric obesity and metabolic syndrome worldwide.

High-quality epidemiological data on pediatric MASLD is still scarce. The study includes a large, multinational sample with participation from multiple centers and countries and use vibration-controlled transient elastography, a very reliable test. Moreover, inclusion of two target populations (community-based and hospital-based cohorts) adds important clinical context to the estimated prevalence.

However, geographic representation appears uneven, with a relatively low proportion of centers from Europe and South America, which may restrict global extrapolation. No data are provided on family history of cardiometabolic or cardiovascular risk factors, which are particularly relevant in pediatric MASLD and could help define screening strategies and risk stratification.

Overall, the study addresses an important knowledge gap and has several notable strengths, but there are also methodological and reporting issues that should be clarified or expanded before the work can be considered for publication.

Major comments

Study design and patient identification

1. Please, revise whether the term "Registry" is accurate to describe the cohort in which the results are based upon. This seems to be a retrospective cohort study composed of patients that were not planned to be included in a preconfigured database or registry but happened to have available VCTE.
2. It is also unclear whether anthropometric measurements, laboratory tests, and VCTE assessments were performed at the same visit. If assessments were performed at different time points, please report the median and interquartile range of the time interval between measurements.
3. Please specify whether participants were previously diagnosed with obesity, diabetes, hypertension, or other cardiometabolic risk factors before inclusion, and whether the study evaluation was part of routine care or dedicated screening.
4. Please, justify and clarify in the limitations' section the following (selection criteria in Supplementary Material): "Children under 6 years of age were excluded due to the low prevalence of MASLD and the limited number of participants in this age group (n = 268)". Introducing such selection bias in a study that is designed to investigate the prevalence of MASLD as its primary outcome should be justified.

Definition of cardiometabolic risk factors

5. The definitions used for cardiometabolic risk factors should be described in greater detail. For pediatric populations, several variables (especially blood pressure) depend on age-, sex-, and height-specific percentiles. Please specify which pediatric guidelines or percentile cutoffs were used.
6. Definition of MASLD as CAP threshold attained + single metabolic factor (already mentioned in the introduction). In order for a single metabolic factor to be enough, it should be one of the following list (note that thresholds vary according to sex): 1) BMI ≥ 25 kg/m² or waist circumference ≥ 94 cm for males and ≥ 80 cm for females; 2) fasting glucose ≥ 100 mg/dl or 2-hour post-load glucose levels ≥ 140 mg/dl or HbA1c ≥ 5.7 % or T2DM; 3) blood pressure $\geq 130/85$ mmHg; 4) plasma triglycerides ≥ 150 mg/dl; 5) plasma HDL-cholesterol < 40 mg/dl for males and < 50 mg/dl for females. Therefore, for instance, total cholesterol of 210 mg/dl with normal HDL would not account for itself as a MASLD-defining criterion.
7. For overweight/obesity, specify whether the definitions based on BMI changed across geographic settings (e.g. Asia vs. America). For dyslipidemia, clarify how abnormal values were defined across different age groups

Clinical and familiar history

8. Please clarify whether the following baseline data was collected:

Family history of cardiovascular disease (especially at young age), diabetes, obesity, or MASLD.

Pre-existing cardiometabolic conditions prior to enrollment.

Current medications or treatments at the time of evaluation (e.g., antidiabetic drugs, lipid-lowering agents, antihypertensives, weight management therapies).

Current or prior personal and familiar diagnosis as this may affect differences observed between community and hospital population.

9. If such data are available, they should be reported and, if sample size allows, explored in subgroup analyses. It would be particularly valuable from a clinical perspective to assess whether children with a positive family history represent a higher-risk group for MASLD screening strategies.

Outcomes

10. Results regarding F4 should also be shown, both in the main text and supplementary materials. See comment on F4 VCTE thresholds in minor comments.

11. It is undeniable that evaluating the prevalence of MASLD in the children and adolescent population is a worthy endeavor as it remains largely unknown. Therefore, studying the potential risk factors for MASLD through a multivariate model is correct, as these might differ from those of the adult population. However, it would be likely more clinically relevant to investigate the risk factors for either significant or advanced fibrosis, as the use of VCTE is primarily intended for this rather than diagnosing MASLD (which is clinically more relevant, as fibrosis primarily drives the risk of complications and events). Moreover, given the large number of individuals with significant/advanced fibrosis in this study (see comments regarding limitations in the discussion section), a multivariate model can be calculated a priori without problem.

12. In the regression analysis of MASLD predictors, variables such as obesity, overweight, and elevated glucose appear to be included as independent predictors despite being part of the MASLD definition. Including definitional components of the dependent variable may introduce circularity and overfitting. Please justify this modeling approach and consider revising the model to avoid redundancy.

13. Thus, the suggestion would be to move the model of MASLD to the supplementary material and shortly mention the results in the main text and focus on a model of fibrosis to identify those individuals that should be prioritized in future screening programs.

Minor comments

Title

14. "Global"- 38 countries, but not country-wide representation but rather single-center cohorts. All continents? Scarce representation in several of them, with highly populated or reference countries (in terms of MASLD epidemiology) missing or slightly present.

Abstract

15. Number of continents/regions not mentioned

16. IQR for median age not shown

17. If the prevalence in South America includes data from Mexico and appears to be the highest, this should be revisited (Mexico is North America, or perhaps Central America).

18. The statement that the prevalence of MASLD appears to have increased in the 2021-2024 compared to 2017-2024 is not supported by any figures nor statistical indicators.

19. As mentioned in the comments for Methods, "Increased glucose levels" as a factor associated with MASLD in the multivariable analysis should be revised and/or clarified.

20. Last sentence: "supports" should be in plural as it refers to "these findings". Moreover, the last assertion "supports disease management in high-risk populations" is not supported by other information in the abstract, as the variables identified in the multivariable analysis do not lead to a clear identification of such high-risk clusters for early intervention (older age, overweight/obesity, increased glucose levels, and elevated transaminase), in any case for case-finding screening.

Introduction

21. In the section discussing liver biopsy (around lines 208–209), please add a brief comment on interobserver variability and sampling variability, which are recognized limitations of liver biopsy and help justify the use of noninvasive techniques such as VCTE.

Methods

22. Although general aspects of patients' eligibility are provided in the Supplementary material, it is not clearly described how participants were identified and recruited at each site and population. Please clarify the recruitment strategy (consecutive sampling, voluntary participation, screening programs, clinic-based recruitment, etc.) as this may explain differences between subgroups (community-based and hospital-based cohorts).

23. Supplementary material. Definition of VCTE kPa thresholds for fibrosis stages. There is no threshold for F4-cirrhosis. Please, clarify whether the VCTE definition for fibrosis in children and adolescents defined in the article by Nobili et al (2008) stands current as of today for a global examination given the sharp changes the global epidemiology of metabolic syndrome occurred in the last three decades.

24. Please clarify whether patients were fasting prior to VCTE assessment, and if so, for how long. Current recommendations generally suggest at least 3–4 hours of fasting to avoid variability in liver stiffness and steatosis measurements. If fasting status was not standardized, this should be acknowledged as a limitation.

Results

25. Ethnicity representation. In the subgroup analysis section (around line 266), please clarify whether all major ethnic groups are adequately represented and provide more detail on how ethnicity was categorized and recorded across sites.

26. It is noteworthy that approximately 26% of patients without MASLD exhibited some degree of fibrosis (F1 or higher). Please clarify whether these cases were further investigated to identify alternative explanations for fibrosis or other causes of chronic liver disease were systematically excluded. Similarly, a substantial number of patients (n = 154) presented with elevated CAP values in the absence of a MASLD diagnosis. The authors should clarify whether this finding is attributable to the absence of cardiometabolic risk factors and whether other causes of steatotic liver disease were evaluated and ruled out, or rather due to a selection bias because these are hospital-based cohorts built in reference centers with monographic MASLD clinics.

27. Although the generalizability of the results given the involvement of tertiary institutions is the enrollment is already discussed in the limitations' paragraph, the finding of 9.5% suspected advanced fibrosis amongst individuals with MASLD should be further nuanced.

Discussion

28. The discussion would benefit from a clearer explanation of the observed differences between hospital-based and

community-based populations, including further details on hospital cohort recruitment criteria and potential selection bias. 29. The estimation of global population prevalence appears somewhat overreaching given the unequal geographic distribution of participating centers and the limited representation of some regions. This limitation should be more explicitly acknowledged and the conclusions regarding global prevalence should be tempered accordingly.

Reviewer #4

(Remarks to the Author)

I read this paper on a topic of great importance with great interest. Although it is interesting, I believe that some issues need to be resolved before its publication can be considered.

- Due to the significant imbalance in recruitment across regions (with the majority of participants hailing from Asia, primarily China, and minimal representation from Europe and Oceania), the term 'global prevalence' could be misleading. As a few very large datasets make up most of the sample, pooled estimates are likely to be dominated by these centres. To assess the robustness of the results, please consider using more cautious wording (e.g. 'international registry-based estimates') and performing simple sensitivity analyses (e.g. centre-level averaging or excluding the largest datasets).
- In the introduction, 'procedure risks' appears redundant alongside 'invasiveness'. It would also be appropriate to mention inter-observer variability. Conceptually, a biopsy should be described as the reference standard for MASH rather than MASLD.
- Neither the abstract nor the aims stated in the introduction mention the stratification by disease severity based on LSM. It refers exclusively to MASLD prevalence. I would ask the authors to clarify this point.
- Please clarify the rationale for selecting 6 years as the minimum age for inclusion.
- Specify what is meant by 'community-based' populations. Where were these participants recruited (general practice, community medicine, referral to hepatologists?). The same observation applies to hospital populations (are these participants already being monitored for MASLD? Are they being followed up for metabolic comorbidities?). This is an important point that needs to be clarified because it significantly influences the epidemiological data derived from this study.
- Specify who performed the Fibroscan (clinicians? nurses? technicians?) and whether different probes were used (and, if so, based on what criteria).
- For participants with elevated transaminases, please clarify whether other causes of liver injury were excluded, particularly as these variables are included in the regression models.
- Please double-check the blood pressure definition for participants ≥ 13 years, as only DBP is reported and SBP appears to be missing.
- Since MASLD is defined by the presence of steatosis plus cardiometabolic risk factors, and these same variables are later included as predictors in the regression models, the authors should discuss the potential for incorporation bias or circularity, which may artificially inflate associations. The very large odds ratios observed for obesity (e.g., OR >90) suggest potential issues such as collinearity or incorporation bias. Please provide variance inflation factors or sensitivity analyses to assess model stability.
- Please clarify the rationale for selecting CAP ≥ 248 dB/m as the diagnostic threshold in children, and whether this cut-off has been validated specifically in pediatric populations.
- Given the heterogeneous sampling across countries and settings, did the authors consider weighted or age-standardized prevalence estimates to improve generalizability?

Reviewer #5

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

All my previous comments have been appropriately addressed, and the manuscript has improved markedly. I have no further recommendations to improve the manuscript.

Reviewer #2

(Remarks to the Author)

Thank you for addressing my comments, I have no further issues.

Reviewer #3

(Remarks to the Author)

The authors have carefully addressed all reviewers' comments. In general, they have been able to implement a series of relevant changes that have improved the methods and the way results shown, hence enhancing the interpretability of

findings. Regarding my comments in particular, although in some cases the authors have not conducted changes aligned with my recommendations, they have either provided detailed responses with reasonable grounds for not doing so or they have acknowledge so in the limitations' paragraph.

Overall, I believe the current version of the manuscript is much improved and of interest for a broad audience.

Reviewer #4

(Remarks to the Author)

The authors have addressed my concerns.

Reviewer #5

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

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Response Letter

May 11, 2026

Dear Editor,

On behalf of my coauthors, I am pleased to resubmit our revised manuscript entitled “**Prevalence of MASLD among children and adolescents assessed by vibration-controlled transient elastography: An international multicenter study**” (NCOMMS-25-97144A-R1) for consideration for publication in *Nature Communications*.

We sincerely thank you and the Reviewers for allowing us to submit our revised manuscript. All suggestions from the 5 Reviewers have been carefully considered, resulting in further improvements to our manuscript. We believe that all points raised by the Reviewers have been adequately addressed. Revised portions are marked with highlighted changes in this revised manuscript.

Prof. Ming-Hua Zheng

Our responses to the Reviewers' comments are as follows:

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

The study represents an impressive effort to investigate the prevalence of MASLD across both hospital settings and community populations by using VCTE. Population based data from primary care have been particularly limited, making this contribution especially valuable. Manuscript is well written and statistical methods are clear and appropriate. I have only minor recommendations to further improve the manuscript.

Response: Thank you for these supportive comments.

Main manuscript:

On the line 267 it is mentioned that BMI was used to define overweight and obesity. Why not use BMI Z-score, which is more accurate in pediatric populations?

Response: Thank you for the comment. We apologize for the confusion caused by the unclear wording in the original manuscript. In our study, weight categories were based on BMI z-scores. To avoid misunderstanding, we have revised the relevant text in the revised manuscript and, where appropriate, replaced “BMI” with “weight status” to clarify the description.

Starting from line 382, “When discussing the prevalence of MASLD across different studies, could you expand the interpretation by considering whether the observed variation might be related to differences in the severity of pediatric obesity and the age distributions of the study populations?”

Response: Thank you for the comment. In the revised manuscript, we have added the relevant discussion as follows:

“As a new method with broad accessibility potential, VCTE-measured CAP values can detect >5% hepatic steatosis and accurately distinguish mild hepatic steatosis from moderate and severe steatosis, demonstrating good diagnostic performance.”²⁴ However, studies on CAP-based assessment of MASLD prevalence in children and adolescents are scarce. Using the 2017-2018 National Health and Nutrition Examination Survey (NHANES) database, Ciardullo et al.²⁵ reported that the prevalence of MASLD (CAP ≥ 248 dB/m) in US adolescents aged 12-18 years was 24.16%. A study from China that followed 848 children aged 8 years estimated the prevalence of MASLD at 3.42%.²⁶ However, there are substantial differences between studies published in the literature. A study from China reported that MASLD (CAP ≥ 238 dB/m) was present in 23.83% of 1,309 school-aged children and adolescents aged 7-18, with a MASLD prevalence of 71.68% among those with obesity.²⁷ Similarly, Lefere et al.²⁸ conducted a study of 204 children with severe obesity (BMI at least 25% above than 95th weight percentile) in Belgium, and they found that 68.6% had hepatic steatosis (CAP ≥ 248 dB/m). These differences in prevalence across studies could be attributed to factors such as limited sample sizes, variations in CAP cut-off values, and differences in population characteristics, including age and weight status. Therefore, using a large sample and international multicenter data, our study provides robust VCTE-based prevalence estimates of MASLD in children and adolescents.” (Page 11, Lines 294–308)

Figures/tables:

Could you add information on the prevalence of overweight and obesity in the different populations to the figure legend of Figure 3, or clarify whether the children from the various continents were comparable in terms of age, BMI z score, and sex distribution?

Response: Thank you for this insightful comment. In the revised supplementary file, we have added “Supplementary Table 2” to present the distribution of age, BMI z-score, and sex among participants across continents. In addition, we have added a discussion of this issue to the revised manuscript, as follows: “Additionally, children and adolescents across continents exhibited differences in population characteristics, such as age and body weight, which may also partially contribute to the observed differences in MASLD prevalence.” (Page 13, Lines 354–355)

Supplementary files:

To the supplementary file methods, could you consider adding the prevalence of MASLD in the children under 6 years of age, since this would be very interesting data and information is limited.

Response: Thank you for the comment. In the revised supplementary file, we have further clarified the rationale for excluding children under 6 years of age and provided prevalence estimates for this population, as follows: “Children under 6 years of age were excluded because participants in this age group were recruited only from community-based populations in China, and the sample size was limited (n = 268; median age, 4.75 years). In this population, the estimated prevalence of MASLD; S1, S2, and S3 steatosis; and F1, F2, and $\geq$ F3 fibrosis were 4.48% (95% CI: 2.33–7.69), 7.46% (95% CI:

4.62–11.29), 3.36% (95% CI: 1.55–6.28), 1.49% (95% CI: 0.41–3.78), 19.78% (95% CI: 15.18–25.06), 2.61% (95% CI: 1.06–5.31), and 0.00%, respectively.” (Supplementary Methods)

Were the exclusion of other liver affecting conditions systematic within study population, or based on clinical decision-making? If not systematic, this should be mentioned as a limitation.

Response: Thank you for the important comment. Other conditions that could affect the liver were assessed using available clinical information, including medical history and routine clinical evaluation. However, this was not performed using a fully standardized, systematic screening protocol for all participants. We agree that this may have introduced potential residual confounding and should be acknowledged as a limitation. This point has been added to the limitation section of the revised manuscript as follows: “Furthermore, the exclusion of other chronic liver conditions was based on available clinical information rather than a standardized screening protocol for all participants, which may have introduced residual confounding.” (Page 15, Lines 408–410)

Reviewer #2 (Remarks to the Author):

Thank you for the opportunity to review this manuscript. Zhang and colleagues have undertaken an impressive task of delineating paediatric MASLD to a greater extent, with greater concordance to adult literature.

This multicentre study (17 countries) estimates the global paediatric MASLD prevalence using VCTE, which positions this uniquely as prior studies have relied on either USG imaging or ALT levels. The authors report a prevalence of 18% in community practices vs 58% in hospital practices. They also demonstrate a marked regional variation in prevalence. Adolescents and males appeared at greater risk. Also, the prevalences appear to be more in the more recent years.

The key strengths are as follows:

- 1) Use of VCTE measurements, which is being used as prognostication to MALO / LRE in adult populations. This is the largest VCTE based study in paediatric populations, and provides valuable insight.
- 2) Clear definition of MASLD in concordance to existing literature.
- 3) Clearly identified at risk individuals from multivariate models
- 4) Alignment to NASPGHAN guidance.

Response: Thank you for these supportive comments.

My criticisms (and suggestions) are as follows:

- 1) Given the wide geographical variance, were the patients recruited differently? Details on how the patients were recruited from each site / country should be provided in a supplementary table. Also, referral thresholds from community / primary care should be delineated.

Response: Thank you for this important comment. As noted in our response to another reviewer's comment, the present study was not based on a preconfigured registry or a prospectively designed uniform recruitment framework. Therefore, we have removed the term "registry-based" from the title and manuscript and now describe the study as an international multicenter study based on retrospectively collected VCTE data. Participating centers were invited to contribute data from their existing local cohorts of children and adolescents with available VCTE measurements, in accordance with predefined inclusion and exclusion criteria and using a standardized data collection form with uniform study variables. Community-based populations were defined as those recruited from community-based, school-based, population screening, or general health examination settings, whereas hospital-based populations were defined as those recruited from hospital clinics or departments. However, detailed site-specific recruitment procedures and referral thresholds from community or primary care settings were not systematically collected across all participating centers. Therefore, we are unable to provide a complete supplementary table of recruitment strategies or referral thresholds for each site/country without risking incomplete or inaccurate reporting. We have now clarified the study design and data collection process in the Methods section and acknowledged this issue as a limitation in the Discussion section of the revised manuscript.

2) Is the higher prevalence in more recent years suggestive of greater awareness and case finding rather than true prevalence? This should be discussed.

Response: Thank you for the insightful comment. In the revised manuscript, we have added a discussion of this issue as follows:

"The possible drivers behind this increase may include obesity and type 2 diabetes, both of which have risen significantly among adolescents.⁴⁰ In addition, greater clinical awareness, wider use of VCTE, and more active case finding in recent years may also have increased the detection of MASLD. That said, the rising prevalence of pediatric MASLD raises concerns about its future clinical burden in adolescents." (Page 13, Lines 363–366)

3) I would suggest including a paragraph in the discussion mapping operational definitions to adult nomenclature as well as paediatric consensus recommendations.

Response: Thank you for the comment. In the revised manuscript, we have added the relevant description in the Discussion section as follows:

"The prevalence of MASLD varies by detection method, which may include screening with serum ALT levels, imaging for hepatic steatosis, or confirmation by liver biopsy. Fernandes et al.⁴⁴ analyzed liver specimens obtained from autopsies of 583 children aged 2 to 19 years in the United States and reported a MASLD prevalence of 4.5%. Using serum ALT elevation to estimate MASLD, a study based on the NHANES database found that the prevalence of MASLD among adolescents increased from 3.9% in 1988-1994 to 10.7% in 2007-2010.⁴⁵ Some studies have shown that CAP as measured by FibroScan is feasible and accurate for quantifying hepatic steatosis in the pediatric population.^{24,33} However, the CAP cutoff values used to grade hepatic steatosis vary across studies, as there is currently no consensus on the optimal cutoff values for hepatic steatosis in children. In addition, although VCTE is a widely

used noninvasive tool for assessing hepatic steatosis and fibrosis, CAP and LSM values may be influenced by measurement variability, operator-related factors, probe selection, fasting status, body posture, and examination quality. A reproducibility study suggested that changes in VCTE-based liver stiffness measurements below a certain threshold might also reflect measurement variability rather than true biological changes in hepatic fibrosis.⁴⁶ Therefore, future studies and expert consensus are needed to establish standardized pediatric VCTE protocols, including appropriate probe selection, right-lobe intercostal measurement positioning, repeated valid measurements, and technical quality criteria, as well as validated VCTE thresholds for assessing hepatic steatosis and fibrosis in the pediatric population.” (Page 14, Lines 379–394)

4) Tone down the language on estimating risk - this is based on fibrosis score, and not on hard outcomes.

Response: Thank you for the comment. In the revised manuscript, we have re-performed regression analyses across grades of hepatic steatosis and stages of fibrosis and revised the wording to clarify that these analyses assessed factors associated with VCTE-measured grades of hepatic steatosis and fibrosis stages, rather than estimating the risk of hard clinical outcomes.

5) Please discuss the reproducibility and repeatability of VCTE in the limitations of the modality (Pierce et al, Radiology 2024).

Response: Thank you for the insightful comment. In the revised manuscript, we have added a discussion of VCTE reproducibility and repeatability in the Discussion section and cited the following reference: “ In addition, although VCTE is a widely used noninvasive tool for assessing hepatic steatosis and fibrosis, CAP and LSM values may be influenced by measurement variability, operator-related factors, probe selection, fasting status, body posture, and examination quality. A reproducibility study suggested that changes in VCTE-based liver stiffness measurements below a certain threshold might also reflect measurement variability rather than true biological changes in hepatic fibrosis.⁴⁶ ” (Page 14, Lines 387–391)

Reviewer #3 (Remarks to the Author):

This manuscript presents a cross-sectional study evaluating the global prevalence of MASLD in children and adolescents using vibration-controlled transient elastography (VCTE). The topic is timely and clinically relevant, given the increasing burden of pediatric obesity and metabolic syndrome worldwide.

High-quality epidemiological data on pediatric MASLD is still scarce. The study includes a large, multinational sample with participation from multiple centers and countries and use vibration-controlled transient elastography, a very reliable test. Moreover, inclusion of two target populations (community-based and hospital-based cohorts) adds important clinical context to the estimated prevalence.

However, geographic representation appears uneven, with a relatively low proportion of centers from Europe and South America, which may restrict global extrapolation. No data are provided on family history of cardiometabolic or cardiovascular risk factors, which are particularly relevant in pediatric MASLD and could help define screening strategies and risk stratification.

Overall, the study addresses an important knowledge gap and has several notable strengths, but there are also methodological and reporting issues that should be clarified or expanded before the work can be considered for publication.

Major comments

Study design and patient identification

1. Please, revise whether the term “Registry” is accurate to describe the cohort in which the results are based upon. This seems to be a retrospective cohort study composed of patients that were not planned to be included in a preconfigured database or registry but happened to have available VCTE.

Response: Thank you for this comment. We agree that the term “registry-based” may not accurately describe all participating cohorts. In this study, participating centers were contacted and asked to contribute data in accordance with predefined inclusion and exclusion criteria. A standardized data collection form with uniform variables was provided to all centers to ensure consistent data collection. Some centers may have contributed data from established local registries, whereas others provided data from existing clinical or research cohorts with available VCTE measurements. Therefore, we have removed the term “registry-based” from the title and throughout the manuscript and now describe the study as an international multicenter study.

Accordingly, the manuscript title has been revised to “Prevalence of MASLD among children and adolescents assessed by vibration-controlled transient elastography: an international multicenter study.”

We have also clarified in the Methods section that data were collected using a standardized data collection form completed by each participating center. (Supplementary Methods)

2. It is also unclear whether anthropometric measurements, laboratory tests, and VCTE assessments were performed at the same visit. If assessments were performed at different time points, please report the median and interquartile range of the time interval between measurements.

Response: Thank you for the comment. In our study, all data were collected during the same visit or health survey for each participant. We have clarified this point in the Methods section of the revised manuscript as follows: “All data were collected during the same visit or health survey for each participant.” (Page 16, Lines 440–441)

3. Please specify whether participants were previously diagnosed with obesity, diabetes, hypertension, or other cardiometabolic risk factors before inclusion, and whether the study evaluation was part of routine care or dedicated screening.

Response: Thank you for the comment. Because our study included participants from multiple centers

in different countries, the recruitment settings were heterogeneous. Participants from community-based cohorts were more likely to be screened during general population health assessments, whereas those from hospital-based cohorts may have presented to MASLD specialty clinics for existing health conditions. Regrettably, to allow broader inclusion of available populations, information on prior diagnoses of obesity, diabetes, hypertension, other cardiometabolic risk factors, and the exact context of evaluation (routine care vs. dedicated screening) was not uniformly predefined in the inclusion/exclusion criteria across all participating centers. As a result, these characteristics were not consistent across the study population. In the revised manuscript, we have added further clarification regarding this issue.

4. Please, justify and clarify in the limitations' section the following (selection criteria in Supplementary Material): "Children under 6 years of age were excluded due to the low prevalence of MASLD and the limited number of participants in this age group (n = 268)". Introducing such selection bias in a study that is designed to investigate the prevalence of MASLD as its primary outcome should be justified.

Response: Thank you for the comment. We apologize for the unclear explanation of the exclusion of children under 6 years of age in the original manuscript. Our study was designed to estimate the prevalence of MASLD across community- and hospital-based populations and their relevant subgroups. However, children under 6 years of age were recruited exclusively from community-based populations in China, resulting in an uneven distribution across population sources and regions. Therefore, given the limited representativeness of the sample and potential imbalance in subgroup comparisons, these participants were not included in the main analyses. In the revised supplementary file, we have further clarified this rationale and added the prevalence estimates for this population, as follows:

"Children under 6 years of age were excluded because participants in this age group were recruited only from community-based populations in China, and the sample size was limited (n = 268; median age, 4.75 years). In this population, the estimated prevalence of MASLD; S1, S2, and S3 steatosis; and F1, F2, and $\geq$ F3 fibrosis were 4.48% (95% CI: 2.33–7.69), 7.46% (95% CI: 4.62–11.29), 3.36% (95% CI: 1.55–6.28), 1.49% (95% CI: 0.41–3.78), 19.78% (95% CI: 15.18–25.06), 2.61% (95% CI: 1.06–5.31), and 0.00%, respectively." (Supplementary Methods)

Definition of cardiometabolic risk factors

5. The definitions used for cardiometabolic risk factors should be described in greater detail. For pediatric populations, several variables (especially blood pressure) depend on age-, sex-, and height-specific percentiles. Please specify which pediatric guidelines or percentile cutoffs were used.

Response: Thank you for the valuable comment. In the revised manuscript, we have added the blood pressure criteria based on age-, sex-, and height-specific percentiles as follows "3) blood pressure $\geq$ the 95th percentile²⁵ or $\geq$ 130/80 mmHg for children aged <13 years and $\geq$ 130/85 mmHg for children aged $\geq$ 13 years". Regrettably, to the best of our knowledge, universally recognized international standards based on age- and sex-specific criteria are currently unavailable for several other indicators in children. Therefore, the criteria used in our study were derived from the expert consensus definition

of MASLD proposed by Rinella et al. (Ref: Rinella ME, Lazarus JV, Ratziu V, et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. *J Hepatol.* 2023;79(6):1542-1556. doi:10.1016/j.jhep.2023.06.003). This consensus definition has also been adopted by many researchers in previous studies on pediatric MASLD.

6. Definition of MASLD as CAP threshold attained + single metabolic factor (already mentioned in the introduction). In order for a single metabolic factor to be enough, it should be one of the following list (note that thresholds vary according to sex): 1) BMI ≥ 25 kg/m² or waist circumference ≥ 94 cm for males and ≥ 80 cm for females; 2) fasting glucose ≥ 100 mg/dl or 2-hour post-load glucose levels ≥ 140 mg/dl or HbA1c ≥ 5.7 % or T2DM; 3) blood pressure $\geq 130/85$ mmHg; 4) plasma triglycerides ≥ 150 mg/dl; 5) plasma HDL-cholesterol < 40 mg/dl for males and < 50 mg/dl for females. Therefore, for instance, total cholesterol of 210 mg/dl with normal HDL would not account for itself as a MASLD-defining criterion.

Response: Thank you for the comment. We agree with your view that all relevant indicators, such as waist circumference and HbA1c, should ideally be included in the diagnosis of MASLD, as these variables are also part of its diagnostic criteria. However, because our study was based on data from several centers, the availability and completeness of certain variables varied across cohorts, making it difficult to include all recommended indicators. Accordingly, we adapted the definition of MASLD based on the available data and the existing diagnostic criteria. In the revised manuscript, we have clarified this point in the limitations section as follows: “Third, due to incomplete data availability, some cardiometabolic criteria relevant to MASLD diagnosis were not fully incorporated into the definition used in this study, which may have influenced MASLD classification to some extent.” (Page 15, Lines 410–412).

7. For overweight/obesity, specify whether the definitions based on BMI changed across geographic settings (e.g. Asia vs. America). For dyslipidemia, clarify how abnormal values were defined across different age groups

Response: Thank you for the comments. First, for overweight/obesity, we used the WHO criteria in the present study and did not apply region-specific BMI definitions, such as those for Asia or the Americas. We have further clarified this point in the revised manuscript as follows: “BMI z-scores were calculated using age- and sex-specific criteria as recommended by the WHO.⁴⁸ Weight status was classified as normal weight or below (BMI z-score ≤ 1), overweight ($1 < \text{BMI z-score} \leq 2$), or obesity (BMI z-score > 2)”. In addition, for lipid indicators, due to the lack of a recognized, unified standard, we did not evaluate or classify them separately. Only in the definition of MASLD did we adopt the cutoff values provided in the expert consensus proposed by Rinella et al. (Ref: Rinella ME, Lazarus JV, Ratziu V, et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. *J Hepatol.* 2023;79(6):1542-1556. doi:10.1016/j.jhep.2023.06.003).

Clinical and familiar history

8. Please clarify whether the following baseline data was collected:

- ☐ Family history of cardiovascular disease (especially at young age), diabetes, obesity, or MASLD.
- ☐ Pre-existing cardiometabolic conditions prior to enrollment.
- ☐ Current medications or treatments at the time of evaluation (e.g., antidiabetic drugs, lipid-lowering agents, antihypertensives, weight management therapies).
- ☐ Current or prior personal and familial diagnosis as this may affect differences observed between community and hospital population.

Response: Thank you for the comment. Your suggestions prompted us to reconsider this issue carefully. Regrettably, these data were not collected in our study. In the initial study design, we asked collaborators to collect data in accordance with the predefined inclusion and exclusion criteria and the data collection list. However, the final pooled dataset did not include information on participants' family history or clinical diagnoses. This was partly because most participants were recruited from community-based healthy populations. The original aim can be to screen for previously unrecognized or hidden MASLD in these populations using the VCTE. We have added this limitation in the revised manuscript as follows:

“Fourth, because detailed information on family history of related diseases, prior or current clinical diagnoses, physical activity, and other socioeconomic characteristics was unavailable, we were unable to perform further subgroup or regression analyses based on these factors. These factors should be considered in future studies to provide a more comprehensive evaluation of pediatric MASLD.” (Page 15, Lines 412–415)

9. If such data are available, they should be reported and, if sample size allows, explored in subgroup analyses. It would be particularly valuable from a clinical perspective to assess whether children with a positive family history represent a higher-risk group for MASLD screening strategies.

Response: Thank you for your comment. As noted above, these data were not collected in our study. The primary aim of our study was to estimate the prevalence of pediatric MASLD. Considerations regarding the screening and management of high-risk populations emerged mainly during subsequent data analysis and interpretation. We appreciate your valuable suggestion, which has provided important guidance for our future work. In future studies, we will pay greater attention to this issue and consider collecting relevant data to better identify and manage high-risk populations.

Outcomes

10. Results regarding F4 should also be shown, both in the main text and supplementary materials. See comment on F4 VCTE thresholds in minor comments.

Response: Thank you for the comment. Although VCTE has been widely validated for identifying F3–F4 fibrosis in adults with MASLD, few studies have evaluated its performance in children or adolescents. After an extensive search of the available literature, we did not find a validated VCTE-based cutoff for defining F4 fibrosis in the pediatric population. Notably, the study by Alkhouri et al (Ref: Alkhouri N, Sedki E, Alisi A, et al. Combined pediatric NAFLD fibrosis index and transient

elastography to predict clinically significant fibrosis in children with fatty liver disease. Liver Int. 2013;33(1):79-85.) also classified pediatric liver fibrosis, as assessed by VCTE, into F0, F1, F2, and F3 stages, which is consistent with the grouping strategy used in our study. Given the absence of a well-established pediatric F4 (cirrhosis) threshold and the need for caution when defining severe fibrosis in children, we did not further classify participants with values $\geq$ F3 in the present analysis. In the revised manuscript, we have changed “F3 stages” to “ $\geq$ F3 stages” and updated the corresponding reference.

11. It is undeniable that evaluating the prevalence of MASLD in the children and adolescent population is a worthy endeavor as it remains largely unknown. Therefore, studying the potential risk factors for MASLD through a multivariate model is correct, as these might differ from those of the adult population. However, it would be likely more clinically relevant to investigate the risk factors for either significant or advanced fibrosis, as the use of VCTE is primarily intended for this rather than diagnosing MASLD (which is clinically more relevant, as fibrosis primarily drives the risk of complications and events). Moreover, given the large number of individuals with significant/advanced fibrosis in this study (see comments regarding limitations in the discussion section), a multivariate model can be calculated a priori without problem.

Response: Thank you for the comment. We fully agree that it is inappropriate to include variables used in the MASLD definition in a regression model with MASLD as the outcome, as this may introduce circularity and overestimate the associations. Therefore, following your suggestion, we reconstructed the regression models with hepatic steatosis and fibrosis as outcomes to assess the predictive effects of these variables on them. In the revised manuscript, we have updated the regression models and modified the corresponding results accordingly. In the Results section we added the following text:

“Factors associated with hepatic steatosis and fibrosis in the study population

Older age, overweight or obesity, and elevated serum ALT levels increased across hepatic steatosis grades ($P < 0.05$). Compared with non-Hispanic White participants, non-Hispanic Black participants had a lower risk of S1 steatosis ($P = 0.014$). In addition, male sex and elevated serum glucose and AST levels were significantly associated with an increased risk of S3 steatosis ($P < 0.05$) (Table 2). Across hepatic fibrosis stages, older age, non-Hispanic Black ethnicity, and elevated serum AST levels were significantly associated with an increased risk of having F1, F2, and $\geq$ F3 fibrosis ($P < 0.05$). Male sex and Asian ethnicity were associated with higher odds of F2 fibrosis ($P < 0.05$). Obesity was associated with higher odds of F1 and F2 fibrosis, whereas elevated serum ALT levels were associated with higher odds of F2 and $\geq$ F3 fibrosis ($P < 0.05$). Although hepatic steatosis grades were associated with an increased risk of F1 and F2 fibrosis, only S2 and S3 steatosis were significantly associated with an increased risk of $\geq$ F3 fibrosis ($P < 0.05$) (Table 3).” (Pages 10–11, Lines 272–282)

12. In the regression analysis of MASLD predictors, variables such as obesity, overweight, and elevated glucose appear to be included as independent predictors despite being part of the MASLD definition. Including definitional components of the dependent variable may introduce circularity and overfitting. Please justify this modeling approach and consider revising the model to avoid redundancy.

Response: Thank you for your comment. We have revised the corresponding section of the revised manuscript.

13. Thus, the suggestion would be to move the model of MASLD to the supplementary material and shortly mention the results in the main text and focus on a model of fibrosis to identify those individuals that should be prioritized in future screening programs.

Response: Thank you for the comment. In the revised manuscript, we have removed the regression model with MASLD as the outcome. Instead, we have reconstructed the regression models using pediatric liver steatosis and fibrosis as outcomes to assess associations of these variables with hepatic steatosis and fibrosis.

Minor comments

Title

14. “Global”- 38 countries, but not country-wide representation but rather single-center cohorts. All continents? Scarce representation in several of them, with highly populated or reference countries (in terms of MASLD epidemiology) missing or slightly present.

Response: Thank you for the comment. We agree that the term “Global” may imply country-wide or population-level representativeness, which is not fully supported by the design of the present study. Although our study included participants from 38 countries across multiple continents, the participating cohorts were not nationally representative within each country, and some regions were underrepresented. In particular, the geographic distribution of participants was uneven, with limited representation from several continents and from some highly populated or epidemiologically important countries for MASLD. Therefore, we have removed the term “Global” from the manuscript title and revised the wording throughout the manuscript to avoid overstating the generalizability of our findings. The manuscript title has been revised to “Prevalence of MASLD among children and adolescents assessed by vibration-controlled transient elastography: an international multicenter study.”

Abstract

15. Number of continents/regions not mentioned

Response: Thanks for your careful review of our manuscript. We have made the corrections in the revised manuscript.

16. IQR for median age not shown

Response: Thanks. We have added the IQR for median age in the revised manuscript.

17. If the prevalence in South America includes data from Mexico and appears to be the highest, this should be revisited (Mexico is North America, or perhaps Central America).

Response: Thanks again. We carefully rechecked the data and confirm that, in our study, Mexico was classified as part of North America for the subsequent analysis.

18. The statement that the prevalence of MASLD appears to have increased in the 2021-2024 compared to 2017-2020 is not supported by any figures nor statistical indicators.

Response: Thank you for the comment. In the original manuscript, Figure 5 compared MASLD prevalence from 2017–2020 with that from 2021–2024 in the overall population and across subgroups defined by source of population, sex, ethnicity, weight status, and ALT and AST levels. As shown in Figure 5, MASLD prevalence was higher in 2021–2024 than in 2017–2020 across the overall population and all subgroups. To provide statistical support for this observation, we have now added statistical comparisons between the two time periods and have marked the groups with statistically significant differences in Figure 5. We have also revised the corresponding text in the manuscript, as follows:

“The prevalence of MASLD was compared between 2017–2020 and 2021–2024 in the overall population and across subgroups categorized by population source, sex, ethnicity, weight status, and ALT and AST levels. The prevalence was higher in 2021–2024 than in 2017–2020 across the overall population and all subgroups. Statistically significant differences were observed in the overall population and in all subgroups ($P < 0.05$), except among participants with normal weight or below. In general, the prevalence of MASLD among adolescents showed an upward trend over time (Figure 5).” (Page 10, Lines 265–271)

19. As mentioned in the comments for Methods, “Increased glucose levels” as a factor associated with MASLD in the multivariable analysis should be revised and/or clarified.

Response: Thank you for the comment. We have revised the relevant content in the revised manuscript.

20. Last sentence: “supports” should be in plural as it refers to “these findings”. Moreover, the last assertion “supports disease management in high-risk populations” is not supported by other information in the abstract, as the variables identified in the multivariable analysis do not lead to a clear identification of such high-risk clusters for early intervention (older age, overweight/obesity, increased glucose levels, and elevated transaminase), in any case for case-finding screening.

Response: Thank you for the valuable comments. In the revised manuscript, we have corrected the error and revised the sentence as follows: “Our findings provide useful contemporary data for estimating the epidemiology of pediatric MASLD across diverse populations”.

Introduction

21. In the section discussing liver biopsy (around lines 208–209), please add a brief comment on interobserver variability and sampling variability, which are recognized limitations of liver biopsy and help justify the use of noninvasive techniques such as VCTE.

Response: Thank you for the comment. We have added the following description to the revised manuscript: “In addition, liver biopsy results may be affected by intra- and inter-reader variability and sampling variability.”¹¹ (Page 6, Lines 157–158)

Methods

22. Although general aspects of patients' eligibility are provided in the Supplementary material, it is not clearly described how participants were identified and recruited at each site and population. Please clarify the recruitment strategy (consecutive sampling, voluntary participation, screening programs, clinic-based recruitment, etc.) as this may explain differences between subgroups (community-based and hospital-based cohorts).

Response: Thank you for this comment. We would like to clarify that, as also addressed in response to another comment, the present study was not based on a preconfigured registry or a prospectively designed uniform recruitment framework, but on retrospectively collected VCTE data contributed by participating centers.

Participating centers contributed data from their existing local cohorts, applying predefined inclusion and exclusion criteria and using a standardized data collection form with uniform study variables. In the revised Methods section, we have clarified the definitions of community-based and hospital-based populations as follows: "Community populations were defined as those recruited from community-based, school-based, population screening, or general health examination settings, whereas hospital populations were defined as those recruited from hospital clinics or departments" (Page 16, Lines 444–447). However, detailed site-specific recruitment strategies, such as consecutive sampling, voluntary participation, screening programs, or clinic-based recruitment procedures, were not systematically collected across all participating centers. Therefore, we are unable to provide complete center-level recruitment details without risking incomplete or inaccurate reporting. We have acknowledged this limitation in the Discussion section of the revised manuscript as follows: "Detailed site-specific recruitment strategies, referral thresholds, and clinical indications for VCTE assessment were not uniformly collected across all participating centers; therefore, differences in recruitment settings should be considered when interpreting MASLD prevalence estimates and when comparing subgroups between community-based and hospital-based populations." (Pages 15, Lines 404–408)

23. Supplementary material. Definition of VCTE kPa thresholds for fibrosis stages. There is no threshold for F4-cirrhosis. Please, clarify whether the VCTE definition for fibrosis in children and adolescents defined in the article by Nobili et al (2008) stands current as of today for a global examination given the sharp changes the global epidemiology of metabolic syndrome occurred in the last three decades.

Response: Thank you for the comment. Although transient elastography has been widely validated for identifying F3–F4 fibrosis in adult patients, few studies have evaluated its performance in children or young adults. After an extensive search of the literature, we did not find a validated VCTE-based cutoff to define F4 fibrosis in pediatric populations. Notably, the study by Alkhoury et al (Ref: *Alkhoury N, Sedki E, Alisi A, et al. Combined paediatric NAFLD fibrosis index and transient elastography to predict clinically significant fibrosis in children with fatty liver disease. Liver Int. 2013;33(1):79-85.*) also classified pediatric liver fibrosis, as assessed by VCTE, into F0, F1, F2, and F3 stages, which is consistent with the grouping strategy used in our study. Given the absence of a well-established pediatric F4 (cirrhosis) threshold and the need for caution when defining severe fibrosis in children, we did not further classify participants with values $\geq$ F3 in the present analysis. In the revised manuscript, we have revised the "F3

stages” to “≥ F3 stages” and updated the corresponding reference.

We acknowledge that validated VCTE thresholds for evaluating hepatic steatosis and liver fibrosis in children have long been limited. We sincerely hope that more accurate pediatric-specific cutoffs will be established in future studies, and we aim to contribute to this important area in our future work.

24. Please clarify whether patients were fasting prior to VCTE assessment, and if so, for how long. Current recommendations generally suggest at least 3–4 hours of fasting to avoid variability in liver stiffness and steatosis measurements. If fasting status was not standardized, this should be acknowledged as a limitation.

Response: Thank you for this helpful comment. We agree that fasting status prior to VCTE assessment should be clearly documented. In this study, all participants were asked to fast for at least 4 hours or overnight before transient elastography. We have now added this information to the Methods section of the revised supplementary material.

Results

25. Ethnicity representation. In the subgroup analysis section (around line 266), please clarify whether all major ethnic groups are adequately represented and provide more detail on how ethnicity was categorized and recorded across sites.

Response: Thank you for the valuable comment. We have clarified how ethnicity was categorized and recorded across participating centers. In this study, ethnicity was collected using a standardized data collection form with five predefined categories: non-Hispanic White, non-Hispanic Black, Hispanic, Asian, and other. Each participating center assigned participants to one of these predefined categories according to the information available in its local cohort. We have clarified the categorization of ethnicity in the Data collection section in the revised manuscript. (Page 16, Lines 443–444)

We agree that not all ethnic groups were equally represented in the study population. Asian participants accounted for the majority of the cohort, whereas non-Hispanic White, non-Hispanic Black, Hispanic, and other ethnic groups were relatively underrepresented. Therefore, we have added the following clarification to the revised manuscript: “In addition, the ethnic distribution was imbalanced, with Asian participants comprising the majority of the cohort; therefore, ethnicity-specific subgroup estimates should be interpreted with caution.” (Page 15, Lines 402–404)

26. It is noteworthy that approximately 26% of patients without MASLD exhibited some degree of fibrosis (F1 or higher). Please clarify whether these cases were further investigated to identify alternative explanations for fibrosis or other causes of chronic liver disease were systematically excluded. Similarly, a substantial number of patients (n = 154) presented with elevated CAP values in the absence of a MASLD diagnosis. The authors should clarify whether this finding is attributable to the absence of cardiometabolic risk factors and whether other causes of steatotic liver disease were evaluated and ruled out, or rather due to a selection bias because these are hospital-based cohorts built in reference centers with monographic MASLD clinics.

Response: Thank you for the comments. First, participants with other liver diseases had already been excluded before enrollment, per the predefined inclusion and exclusion criteria. Exclusion criteria included other chronic liver diseases, such as viral hepatitis and HIV infection; excessive alcohol consumption (> 30 g/day for males and > 20 g/day for females); and a history of any tumors. Second, following the above suggestion, we performed regression analyses with grades of liver steatosis and fibrosis as outcomes to identify potential predictors of hepatic steatosis and fibrosis. In our revised manuscript, we have updated the regression models and modified the corresponding Results and Discussion sections.

27. Although the generalizability of the results given the involvement of tertiary institutions is the enrollment is already discussed in the limitations' paragraph, the finding of 9.5% suspected advanced fibrosis amongst individuals with MASLD should be further nuanced.

Response: Thank you for the suggestion. In the revised manuscript, we have added a relevant description in the Results section and further discussed this issue in the Discussion section as follows: "Notably, in our study, moderate and severe hepatic steatosis ($\geq$ S2) increased significantly across fibrosis stages, and 9.5% of participants with MASLD had suspected advanced fibrosis, further supporting AGA's recommendations to screen for hepatic fibrosis in all individuals with steatosis.⁴² This finding also underscores the clinical importance of identifying and monitoring MASLD-related hepatic fibrosis to guide targeted interventions and prevent disease progression.⁴³" (Page 14, Lines 372–376)

Discussion

28. The discussion would benefit from a clearer explanation of the observed differences between hospital-based and community-based populations, including further details on hospital cohort recruitment criteria and potential selection bias.

Response: Thanks again for the suggestion. In the revised manuscript, we have added further explanation in the Discussion section on the differences between hospital-based and community-based populations as follows:

"We observed a significantly higher prevalence of MASLD among children and adolescents in the hospital than those in the community. Specifically, 58.03% of our participants had MASLD, with 45.4% showing severe hepatic steatosis and 22.2% showing significant hepatic fibrosis. Possible explanations for these high rates may include poor metabolic health among participants, high heterogeneity in the population's characteristics, and potential selection bias. Santos et al.³⁴ included 89 adolescents aged 8 to 18 years from a pediatric outpatient clinic and found a MASLD prevalence of 24%. Another study of 1156 pediatric inpatients with mental disorders found that the prevalence of MASLD was 7.35%.³⁵ However, for the hospital population, most published studies have focused on the prevalence of MASLD in children who are overweight or obese. A study from Malaysia reported that 78.6% of children with obesity had hepatic steatosis, and 35.7% had significant hepatic fibrosis, as measured by VCTE.³⁶ Using the same method, Lefere et al.²⁸ observed that 68.8% of children with obesity had hepatic steatosis and 32.8% had significant fibrosis. A similar prevalence was observed in

our study, with 79.3% of children and adolescents with obesity showing hepatic steatosis and 30.5% showing significant fibrosis ($\geq$ F2) in the hospital population. Notably, in both the overall population and specific subgroups, the prevalence of MASLD in the hospital-based population was approximately 2-3 times higher than that in the community-based population. In our study, community-based participants were generally recruited from community-based, school-based, population screening, or general health examination settings, whereas hospital-based participants were recruited from hospital clinics or departments. Accordingly, hospital-based participants may have been more likely to undergo VCTE examination due to obesity, metabolic abnormalities, elevated aminotransferase levels, suspected hepatic steatosis, or other related clinical indications. Therefore, the higher prevalence observed in hospital populations likely reflects a higher-risk clinical population rather than the prevalence expected in the general pediatric population, and potential selection bias should be considered when interpreting comparisons between these cohorts.” (Pages 12–13, Lines 323–344)

29. The estimation of global population prevalence appears somewhat overreaching given the unequal geographic distribution of participating centers and the limited representation of some regions. This limitation should be more explicitly acknowledged and the conclusions regarding global prevalence should be tempered accordingly.

Response: Thank you for this comment. We agree that estimating global population prevalence may be overreaching, given the uneven geographic distribution of participating centers and limited representation of some regions. Although our study included participants from 38 countries, the participating cohorts were not nationally representative within each country, and the geographic distribution of participants was uneven. In response, we have revised the manuscript to more explicitly acknowledge this limitation in the Discussion. We have also tempered the wording in the Conclusions and avoided describing our findings as global population-level prevalence estimates. Instead, we now refer to them as prevalence estimates derived from an international multicenter data.

The following text has been added to the Discussion section of the revised manuscript: “Second, selection bias cannot be entirely eliminated. Although this study included children and adolescents from 38 countries, their geographic distribution was uneven, with a large proportion recruited from Asia and relatively limited representation from South America, Europe, and Oceania. Several centers also contributed small sample sizes, and the pooled estimates may have been influenced by large contributing centers. In addition, the ethnic distribution was imbalanced, with Asian participants comprising the majority of the cohort; therefore, ethnicity-specific subgroup estimates should be interpreted with caution. Detailed site-specific recruitment strategies, referral thresholds, and clinical indications for VCTE assessment were not uniformly collected across all participating centers; therefore, differences in recruitment settings should be considered when interpreting MASLD prevalence estimates and when comparing subgroups between community-based and hospital-based populations. Furthermore, the exclusion of other chronic liver conditions was based on available clinical information rather than a standardized screening protocol for all participants, which may have introduced residual confounding.” (Pages 14–15, Lines 398–410)

Reviewer #4 (Remarks to the Author):

I read this paper on a topic of great importance with great interest. Although it is interesting, I believe that some issues need to be resolved before its publication can be considered.

- Due to the significant imbalance in recruitment across regions (with the majority of participants hailing from Asia, primarily China, and minimal representation from Europe and Oceania), the term 'global prevalence' could be misleading. As a few very large datasets make up most of the sample, pooled estimates are likely to be dominated by these centres. To assess the robustness of the results, please consider using more cautious wording (e.g. 'international registry-based estimates') and performing simple sensitivity analyses (e.g. centre-level averaging or excluding the largest datasets).

Response: Thank you for this comment. We agree that the term “global prevalence” may be misleading given the imbalance in recruitment across regions and the limited representation of some continents. Accordingly, we have removed “global” from the title and revised the wording throughout the manuscript to avoid implying representativeness at the global population level. We now describe our findings as prevalence estimates derived from an international multicenter cohort. The manuscript title has been revised to “Prevalence of MASLD among children and adolescents assessed by vibration-controlled transient elastography: an international multicenter cohort study.”

We also acknowledge that the pooled estimates may have been influenced by several large contributing centers, particularly those in Asia. To better characterize variation in MASLD prevalence across participant profiles, we conducted subgroup analyses by age, sex, weight status, aminotransferase levels, and glucose status. These analyses helped assess prevalence patterns across key demographic and metabolic subgroups. We considered additional sensitivity analyses, such as center-level averaging or excluding the largest datasets. However, given the differences in sample sizes and population characteristics across centers, center-level averaging could disproportionately weight small cohorts and yield unstable estimates, particularly in underrepresented regions. Similarly, excluding the largest datasets may be arbitrary and could reduce statistical precision while discarding valid data from major contributing cohorts. Therefore, we have revised the Discussion section to explicitly acknowledge this limitation and have tempered the interpretation of the prevalence estimates.

The following text has been added to the Discussion section of the revised manuscript: “Second, selection bias cannot be entirely eliminated. Although this study included children and adolescents from 38 countries, their geographic distribution was uneven, with a large proportion recruited from Asia and relatively limited representation from South America, Europe, and Oceania. Several centers also contributed small sample sizes, and the pooled estimates may have been influenced by large contributing centers. In addition, the ethnic distribution was imbalanced, with Asian participants comprising the majority of the cohort; therefore, ethnicity-specific subgroup estimates should be interpreted with caution. Detailed site-specific recruitment strategies, referral thresholds, and clinical indications for VCTE assessment were not uniformly collected across all participating centers; therefore, differences in recruitment settings should be considered when interpreting MASLD prevalence estimates and when comparing subgroups between community-based and hospital-based

populations. Furthermore, the exclusion of other chronic liver conditions was based on available clinical information rather than a standardized screening protocol for all participants, which may have introduced residual confounding.” (Pages 14–15, Lines 398–410)

- In the introduction, 'procedure risks' appears redundant alongside 'invasiveness'. It would also be appropriate to mention inter-observer variability. Conceptually, a biopsy should be described as the reference standard for MASH rather than MASLD.

Response: Thank you for the suggestion. In the revised manuscript, we have revised the corresponding section and added an additional description as follows: “Although liver biopsy remains the reference standard for diagnosing MASLD and MASH,¹⁰ its clinical use is limited, especially in the pediatric population, due to its invasiveness, high cost, and potential post-procedural risks. In addition, liver biopsy results may be affected by intra- and inter-reader variability and sampling variability.¹¹” (Page 6, Lines 155–158)

- Neither the abstract nor the aims stated in the introduction mention the stratification by disease severity based on LSM. It refers exclusively to MASLD prevalence. I would ask the authors to clarify this point.

Response: Thank you for the comment. In the original manuscript, the primary objective of our study was to estimate the prevalence of MASLD in children and adolescents. We conducted additional analyses stratified by CAP and LSM values because these variables were available in our dataset, and to provide readers with more comprehensive information. Accordingly, the prevalence estimates for liver fibrosis were reported in the Supplementary Materials, rather than emphasized as primary outcomes in the main text.

In the revised manuscript, we have clarified in both the Abstract and the Introduction that, in addition to estimating VCTE-assessed MASLD prevalence, this study evaluated CAP-measured hepatic steatosis grades and LSM-measured fibrosis stages among children and adolescents. We also conducted new regression analyses to assess risk factors associated with different grades of hepatic steatosis and fibrosis stages.

- Please clarify the rationale for selecting 6 years as the minimum age for inclusion.

Response: Thank you for the comment. We sincerely apologize for the unclear explanation of the exclusion of children under 6 years of age in the original manuscript. Our study was designed to estimate the prevalence of MASLD across community- and hospital-based populations and their relevant subgroups. However, children under 6 years of age were recruited exclusively from community-based populations in China, resulting in an uneven distribution across population sources and regions. Therefore, given the limited representativeness of the sample and potential imbalance in subgroup comparisons, these participants were excluded from the main analyses. In the revised supplementary file, we have further clarified this rationale and added the prevalence estimates for this population, as follows:

“Children under 6 years of age were excluded because participants in this age group were recruited

only from community-based populations in China, and the sample size was limited (n = 268; median age, 4.75 years). In this population, the estimated prevalence of MASLD; S1, S2, and S3 steatosis; and F1, F2, and $\geq$ F3 fibrosis were 4.48% (95% CI: 2.33–7.69), 7.46% (95% CI: 4.62–11.29), 3.36% (95% CI: 1.55–6.28), 1.49% (95% CI: 0.41–3.78), 19.78% (95% CI: 15.18–25.06), 2.61% (95% CI: 1.06–5.31), and 0.00%, respectively.” (Supplementary Methods)

- Specify what is meant by ‘community-based’ populations. Where were these participants recruited (general practice, community medicine, referral to hepatologists?). The same observation applies to hospital populations (are these participants already being monitored for MASLD? Are they being followed up for metabolic comorbidities?). This is an important point that needs to be clarified because it significantly influences the epidemiological data derived from this study.

Response: Thank you for this comment. In the revised Methods section, we have clarified that community-based populations were recruited from community-based, school-based, population screening, or general health examination settings, whereas hospital-based populations were recruited from hospital clinics or departments (Page 16, Lines 444–447). Hospital-based populations may have been evaluated or followed for obesity, metabolic abnormalities, elevated aminotransferase levels, suspected hepatic steatosis, MASLD, or related clinical indications. For this point, we have added further discussion in the revised manuscript. (Pages 12–13, Lines 335–344)

Because this study relied on retrospectively collected VCTE data from existing local cohorts rather than a prospectively designed, uniform recruitment framework, detailed information on whether each hospital-based participant was already being monitored for MASLD or followed for specific metabolic comorbidities was not uniformly collected across all participating centers. We have therefore clarified the general definitions in the Methods section and, in the Discussion, acknowledged that differences in recruitment settings should be considered when interpreting the prevalence estimates and subgroup comparisons, as follows: “Detailed site-specific recruitment strategies, referral thresholds, and clinical indications for VCTE assessment were not uniformly collected across all participating centers; therefore, differences in recruitment settings should be considered when interpreting MASLD prevalence estimates and when comparing subgroups between community-based and hospital-based populations.” (Page 15, Lines 404–408)

- Specify who performed the Fibroscan (clinicians? nurses? technicians?) and whether different probes were used (and, if so, based on what criteria).

Response: Thank you for the comments. Regarding the operators of FibroScan, we have updated the relevant description in the revised manuscript as follows: “The CAP and LSM values were assessed using a VCTE device (FibroScan, Echosens, Paris, France) by trained health professionals at healthcare centers, following a standard protocol”. (Page 16, Lines 455–456)

Moreover, probe selection followed the manufacturer’s instructions. The S probe was used for participants with a thoracic circumference < 75 cm, whereas the M probe was used initially for those with a thoracic circumference $\geq$ 75 cm. When the device indicated the XL probe should be used, the XL probe was selected accordingly. We have clarified this point in the revised manuscript

(Supplementary Methods).

- For participants with elevated transaminases, please clarify whether other causes of liver injury were excluded, particularly as these variables are included in the regression models.

Response: Thank you for the comment. Because our study aimed to assess the prevalence of MASLD using VCTE, we applied relevant exclusion criteria during participant enrollment to minimize confounding from other causes of liver injury. As stated in the Methods section of the Supplementary file: “Exclusion criteria included having other chronic liver diseases, such as viral hepatitis, HIV infection, excessive alcohol consumption (> 30 g/day for males and > 20 g/day for females), or a history of any tumors.” Therefore, participants with elevated serum transaminases were not managed under a unified protocol in the present study. Instead, each study center provided individualized management according to its local clinical practice. In addition, we have re-performed regression analysis to assess risk factors associated with pediatric liver steatosis and fibrosis.

- Please double-check the blood pressure definition for participants ≥ 13 years, as only DBP is reported and SBP appears to be missing.

Response: Thank you. In the revised manuscript, we have made the following correction: “Elevated blood pressure was defined as $\geq$ the 95th percentile based on age-, sex-, and height- specific criteria ⁴⁹, or as blood pressure $\geq 130/80$ mmHg for children aged < 13 years and $\geq 130/85$ mmHg for children aged ≥ 13 years. ³” (Page 16, Lines 447–449)

- Since MASLD is defined by the presence of steatosis plus cardiometabolic risk factors, and these same variables are later included as predictors in the regression models, the authors should discuss the potential for incorporation bias or circularity, which may artificially inflate associations. The very large odds ratios observed for obesity (e.g., OR >90) suggest potential issues such as collinearity or incorporation bias. Please provide variance inflation factors or sensitivity analyses to assess model stability.

Response: Thank you for the comment. We fully agree that it is inappropriate to include variables used in the MASLD definition in a regression model with MASLD as the outcome, as this may lead to collinearity or incorporation bias. Therefore, in combination with the comments from other reviewers, we reconstructed the regression models with pediatric liver steatosis and fibrosis as outcomes to assess the effects of these variables on hepatic steatosis and fibrosis. In the revised manuscript, we have updated the regression models and modified the corresponding Results and Discussion sections. The revised Results are presented as follows:

“Factors associated with hepatic steatosis and fibrosis in the study population

Older age, overweight or obesity, and elevated serum ALT levels increased across hepatic steatosis grades ($P < 0.05$). Compared with non-Hispanic White participants, non-Hispanic Black participants had a lower risk of S1 steatosis ($P = 0.014$). In addition, male sex and elevated serum glucose and AST levels were significantly associated with an increased risk of S3 steatosis ($P < 0.05$) (Table 2). Across hepatic fibrosis stages, older age, non-Hispanic Black ethnicity, and elevated serum AST levels were

significantly associated with an increased risk of having F1, F2, and $\geq$ F3 fibrosis ($P < 0.05$). Male sex and Asian ethnicity were associated with higher odds of F2 fibrosis ($P < 0.05$). Obesity was associated with higher odds of F1 and F2 fibrosis, whereas elevated serum ALT levels were associated with higher odds of F2 and $\geq$ F3 fibrosis ($P < 0.05$). Although hepatic steatosis grades were associated with an increased risk of F1 and F2 fibrosis, only S2 and S3 steatosis were significantly associated with an increased risk of $\geq$ F3 fibrosis ($P < 0.05$) (Table 3).” (Page 10–11, Lines 272–282)

- Please clarify the rationale for selecting CAP ≥ 248 dB/m as the diagnostic threshold in children, and whether this cut-off has been validated specifically in pediatric populations.

Response: Thank you for the comment. We acknowledge that there is currently no universally accepted CAP cutoff for diagnosing hepatic steatosis in pediatric populations, and the thresholds used in previous studies are not entirely consistent. In the present study, we selected CAP ≥ 248 dB/m as the diagnostic threshold. This cutoff is widely accepted and has been validated for diagnosing hepatic steatosis in adults. After reviewing the relevant literature, we referred to several recent high-quality studies on pediatric fatty liver disease, in which CAP ≥ 248 dB/m was also used as the threshold for defining hepatic steatosis (Ref: 1. Lefere S, Dupont E, De Guchteneere A, et al. *Intensive Lifestyle Management Improves Steatosis and Fibrosis in Pediatric Nonalcoholic Fatty Liver Disease. Clin Gastroenterol Hepatol.* 2022;20(10):2317-2326.e4. 2. Liu Y, Wang Y, Xing Y, et al. *Establish a noninvasive model to screen metabolic dysfunction-associated steatotic liver disease in children aged 6-14 years in China and its applications in high-obesity-risk countries and regions. Lancet Reg Health West Pac.* 2024;49:101150. Published 2024 Jul 25. 3. Ciardullo S, Monti T, Perseghin G. *Prevalence of Liver Steatosis and Fibrosis Detected by Transient Elastography in Adolescents in the 2017-2018 National Health and Nutrition Examination Survey. Clin Gastroenterol Hepatol.* 2021;19(2):384-390.e1.). These references have been added to the revised manuscript.

- Given the heterogeneous sampling across countries and settings, did the authors consider weighted or age-standardized prevalence estimates to improve generalizability?

Response: Thank you for this suggestion. We agree that weighted or age-standardized prevalence estimates would improve the comparability and interpretation of prevalence estimates. However, the present study included participants from multiple countries and recruitment settings without a unified population-based sampling design, and valid country-, center-, or setting-specific sampling weights were not available. Therefore, weighted prevalence estimates could not be reliably calculated.

To account for differences in age structure, we also calculated age-standardized prevalence estimates using the direct standardization method. Age-specific prevalence was estimated for each single-year age group from 6 to 18 years and then weighted using the 2020 world population age distribution as the reference population. These age-standardized estimates have been added to the revised manuscript.

Reviewer #5 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of

the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Response: We sincerely appreciate your comments, which have been very helpful in improving the manuscript, and we hope the revisions we have made adequately address your concerns. We are also pleased you were able to help your junior colleague learn more about the peer review process. Thank you for letting us know.

Response Letter

July 2, 2026

Dear Editor,

Thank you for the in-principle acceptance of our manuscript entitled “**An international multicenter study of MASLD prevalence among children and adolescents assessed by VCTE**” (NCOMMS-25-97144A) for publication in *Nature Communications*.

On behalf of all co-authors, we are pleased to resubmit the revised manuscript, “An international multicenter study of MASLD prevalence among children and adolescents assessed by VCTE” (NCOMMS-25-97144A-R2).

We appreciate the Editor’s and Reviewers’ careful assessment of our work throughout the review process. All comments from the five Reviewers have been considered and addressed, and the manuscript has been further revised accordingly.

Our point-by-point responses are provided below:

Reviewer #1 (Remarks to the Author):

All my previous comments have been appropriately addressed, and the manuscript has improved markedly. I have no further recommendations to improve the manuscript.

Response: Thank you for the recognition and support of our work, and for confirming that the revisions have adequately addressed the previous comments.

Reviewer #2 (Remarks to the Author):

Thank you for addressing my comments, I have no further issues.

Response: Thanks for your positive feedback. We are glad that our revisions have addressed your comments.

Reviewer #3 (Remarks to the Author):

The authors have carefully addressed all reviewers' comments. In general, they have been able to implement a series of relevant changes that have improved the methods and the way results shown, hence enhancing the interpretability of findings. Regarding my comments in particular, although in some cases the authors have not conducted changes aligned with my recommendations, they have either provided detailed responses with reasonable grounds for not doing so or they have acknowledge so in the limitations' paragraph.

Overall, I believe the current version of the manuscript is much improved and of interest for a broad audience.

Response: Thank you for your thoughtful assessment of the revised manuscript. We appreciate your

recognition of the improvements made to the methods, presentation of the results, and interpretation of the findings. We are also grateful that our responses and the acknowledgement of relevant limitations were considered appropriate.

Reviewer #4 (Remarks to the Author):

The authors have addressed my concerns.

Response: Thank you for confirming that the concerns have been addressed.

Reviewer #5 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Response: We appreciate your comments, which have been helpful in improving the manuscript, and are glad that the revisions have addressed your concerns.