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STUDY PROTOCOL

Protocol for a Longitudinal Cohort Study to Understand Characteristics and Risk Factors Underlying Vibration-Controlled Transient Elastography-Diagnosed Metabolic Dysfunction-Associated Fatty Liver Disease Children

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Background: Metabolic-associated steatotic liver disease (MASLD) is a novel term proposed in 2023 to replace non-alcoholic fatty liver disease (NAFLD) with the aim of better reflecting its pathogenesis and clinical manifestations. Vibration-controlled transient elastography (VCTE) is an evidence-based, non-invasive imaging device used to evaluate liver fat deposition and fibrosis. It can effectively detect liver fat infiltration greater than 5%, which is much higher than the previous ultrasound detection rate (it is difficult to detect liver fat deposition below 30%). Nevertheless, the prevalence and characteristics of MASLD children diagnosed based on these updated criteria are currently not well established.

Methods: Currently, a prospective multi-center population-based cohort study is being conducted in Wuxi, China, spanning from 2023 to 2035, involving 5600 children from four primary schools. Throughout the study's baseline and follow-up periods, yearly physical examinations, laboratory tests, VCTE assessments, and bioelectrical impedance analysis are being conducted to measure MASLD-related biomarkers. Additionally, a questionnaire is being administered to inquire about dietary habits. MASLD is being diagnosed based on clinical and laboratory criteria, and the corresponding prevalence is being assessed.

Results: Recruitment began in March 13, 2023. To date, 1475 participants have completed the physical examination and questionnaire survey.

Discussion: Our study investigated the prevalence of MASLD and its influencing factors in Chinese school-age children and adolescents. By collecting and analyzing data from physical examinations and survey questionnaires, it may propose new avenues for guiding the treatment and early-stage prevention of MASLD in children.

Trial Registration: Chinese Clinical Trials Registry (NO. ChiCTR2400080508).

Keywords: MASLD, student, incidence, VCTE, cohort study

Introduction

Nonalcoholic fatty liver disease (NAFLD) has become the most prevalent cause of chronic liver disease worldwide, accounting for 39.89% of the prevalence in the general Chinese population.[1] However, due to differences in population characteristics, lifestyle habits, and genetic susceptibility, NAFLD shows significant heterogeneity in clinical

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presentation, pathological features, and clinical outcomes. To better reflect the underlying disease pathology and its close correlation with metabolic comorbidities, a multisociety Delphi consensus statement proposes a change of name to "metabolic-associated steatotic liver disease" (MASLD).[2] The definition of MASLD is hepatic steatosis with at least one cardiometabolic risk factor, which can more accurately screen out the population with MASLD, differentiate more high-risk patients, and better identify late-stage patients.[2,3] Approximately 3% to 10% of the general pediatric population suffers from MASLD, with this percentage significantly increasing to 80% among overweight and obese children.[1] MASLD can not only progress to hepatocellular carcinoma but also result in extrahepatic complications such as hypothyroidism, osteoporosis, and cognitive dysfunction.[4,5]

When it comes to diagnostic methods for MASLD, liver biopsy, and pathology remain the gold standard for evaluating steatosis and fibrosis.[6] However, this technique is invasive, expensive, and prone to sampling errors and interobserver variability.[7,8] Other alternatives for assessing fatty degeneration and fibrosis include B-mode ultrasound imaging and magnetic resonance elastography. Nevertheless, these methods have limitations such as subjectivity, semi-quantitative nature, and high cost, respectively.[9–11] In addition to the aforementioned detection methods, the most commonly used screening criterion in pediatrics is to observe the elevation of alanine aminotransferase (ALT) levels (cut-point for males ≥25.8 IU/L, cut-point for females ≥22.1 IU/L). However, this blood test has low sensitivity in children, and even when ALT results are within the normal range, children may still have MASLD. This situation has led to many children not receiving further screening and clinical monitoring.[12,13] The European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD) recommend using Vibration-controlled transient elastography (VCTE), a non-invasive and user-friendly modality with high accuracy in assessing hepatic fat deposition and liver stiffness, for evaluating liver fibrosis in MASLD patients.

Existing studies have explored human measurements, laboratory tests, and pharmacology parameters to quickly analyze the most discriminative risk factors for adult MASLD using methods like machine learning.[14,15] However, these studies are often conducted in single centers and focus primarily on MASLD risk factors in specific disease populations (such as patients with diabetes or cardiovascular diseases), limiting the broad applicability of the research results.[16] Given the significant differences in growth characteristics and metabolic needs between children and adults, the risk factors identified in adult studies may not be entirely applicable to children.

Currently, there are studies on pediatric MASLD, focusing primarily on its definition, prevalence, etiology, pathogenesis, diagnostic methods and criteria, treatment approaches, as well as its associations with related metabolic syndromes.[17,18] In comparison, there is relatively limited research on risk factors for pediatric MASLD. These studies often concentrate on overweight or obese children, making it challenging to generalize the research results to the entire population or to lean MASLD individuals.[19] Research parameters typically revolve around exploring simple indicators like blood biochemistry and anthropometric measurements.[20,21] In contrast, there is a lack of research on various biomarkers (ie, body composition, blood, and urine routine tests), which can provide a more comprehensive evaluation of MAFLD and offer more comprehensive and valuable information.

Overall, our understanding of the incidence, influencing factors, screening systems, and early biomarkers of pediatric MASLD is limited. Therefore, Wuxi Children's Hospital, an affiliated hospital of Jiangnan University, has conducted a cohort study on the occurrence of MASLD in healthy children who undergo physical examinations, aiming to use VCTE technology to explore the characteristics and early biomarkers of MASLD in children, identify modifiable risk and protective factors, and provide a scientific basis for optimizing the management system and intervention measures for children at high risk of MASLD.

Objectives

The primary objective of this study is to develop early markers for pediatric MASLD. Secondarily, we will also address the following objectives:

- To investigate the incidence rate of MASLD among generally healthy children.
- To determine the extent to which gender and age influence the developmental trajectories of MASLD.
- To examine the characteristics of MASLD in pediatric patients, including demographic information, environmental factors, dietary and exercise habits, and their association with MASLD.

- To identify modifiable risks and protective factors.
- To identify exercise and dietary patterns associated with MASLD and non-MASLD.

Materials and Methods

Study Design

This is a prospective cohort study that spans from March 13, 2023, to March 31, 2035. Data collection occurs each spring and fall semester, and each semester will achieve two schools' physical examinations. Baseline data collection for students in grades 1–6 from four schools will be completed in the first year. Each participant will undergo a comprehensive physical examination and questionnaire survey annually until they reach the age of 18 or voluntarily withdraw from the study. The contents of a physical examination include a general physical examination, a laboratory examination, VCTE assessments, and a bioelectrical impedance analysis (BIA). The study will be conducted in accordance with the Declaration of Helsinki, with written informed consent obtained from children's parents or legal guardians. The Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) schedule of enrolment is reported in Figure 1 and the flow diagram in Figure 2.

Setting

As shown in Figure 3, this study will be conducted at four campuses under an educational group in Wuxi City, China. Each of these campuses has approximately 2100 students, who diversely represent various ethnic backgrounds, providing a robust platform for comprehensive research.

Participants

The objectives of this study encompass the recruitment of 5600 participants. Students from grades 1 to 6 in each school district are invited to participate in this cohort study, which will span until they reach the age of 18 or voluntarily withdraw from the study. Guardians are required to sign a consent form on behalf of their wards, thereby ensuring their participation is informed and consensual.

The exclusion criteria for this study include: coexistence of liver cirrhosis (liver stiffness measurement (LSM) ≥13 kPa during elastography) or chronic liver diseases caused by hepatitis B/C or other reasons (such as autoimmune hepatitis, Wilson's disease, hemochromatosis, primary biliary cholangitis, right-sided heart failure, HIV co-infection, alcoholic liver disease, etc).; male alcohol consumption ≥21 drinks/week (30 g alcohol/day), female alcohol consumption ≥14 drinks/week (20 g alcohol/day); impaired kidney function (estimated glomerular filtration rate <30 mL/min/1.73m^2); and individuals taking orlistat and statins.

Test Items

Medical Examination

The routine physical examination includes measurements of height, weight, systolic and diastolic blood pressure, all of which are conducted by professional physicians. Among these, BMI >1 SD above the WHO growth reference median (for children aged 6 to 15 years), and BMI ≥23 kg/m^2 (adolescents older than 16 years), systolic blood pressure or diastolic blood pressure >90th percentile (children aged 6 to 9 years), systolic blood pressure >130 mmHg or diastolic blood pressure >85 mmHg (children aged 10 to 15 years), and blood pressure ≥130/85 mmHg or specific antihypertensive drug treatment (adolescents older than 16 years), can all serve as biomarkers suggesting the presence of cardiometabolic risk factors.[2] The specialist tests are carried out by trained and experienced specialists:

Oral and dental hygiene are examined by a dentist using the relevant checklist and the WHO guide.[22]

Ophthalmic examination: visual acuity is determined by an LED chart, optometry (refraction and automatic refraction), and a lesion of anterior segment tissue is examined by slit microscopy.

Ear, nose, and throat examination: anterior rhinoscopy was used to examine bilateral nasal passages, and otoscopy was used to examine bilateral external auditory canals and tympanic membranes.

	STUDY PERIOD							
	Enrolment	Allocation	Post-allocation					Close-out
TIMEPOINT	$-t_1$	0	t_1	t_2	t_3	*etc.*	t_{12}	t_{12}
ENROLMENT:								
Eligibility screen	X							
Informed consent	X							
Demographics	X							
Allocation		X						
INTERVENTIONS:								
Intervention group			X	X	X	etc.	X	X
Control group			X	X	X	etc.	X	X
ASSESSMENTS:								
Pregnancy and Birth History	X							
Diet and Exercise	X							
Living Environment	X							
Anthropometric Measures (weight, height, blood pressure, ophthalmic, ear, nose, and throat, oral and dental hygiene, medical and surgical, and body composition)			X	X	X	etc.	X	X
Physiopathology			X	X	X	etc.	X	X
Liver Steatosis			X	X	X	etc.	X	X

Figure 1 SPIRIT schedule of enrolment, interventions, and assessments.

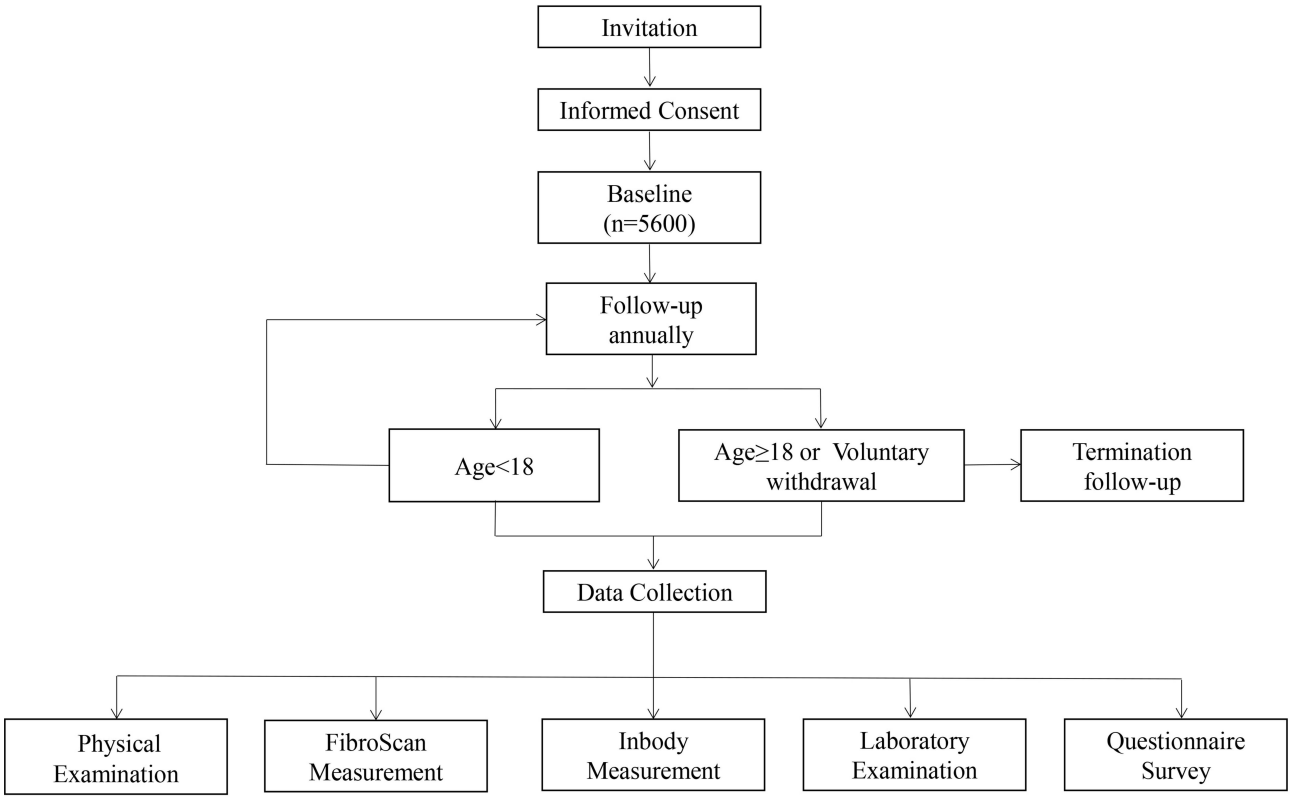

Figure 2 Flow chart of the prospective cohort study.

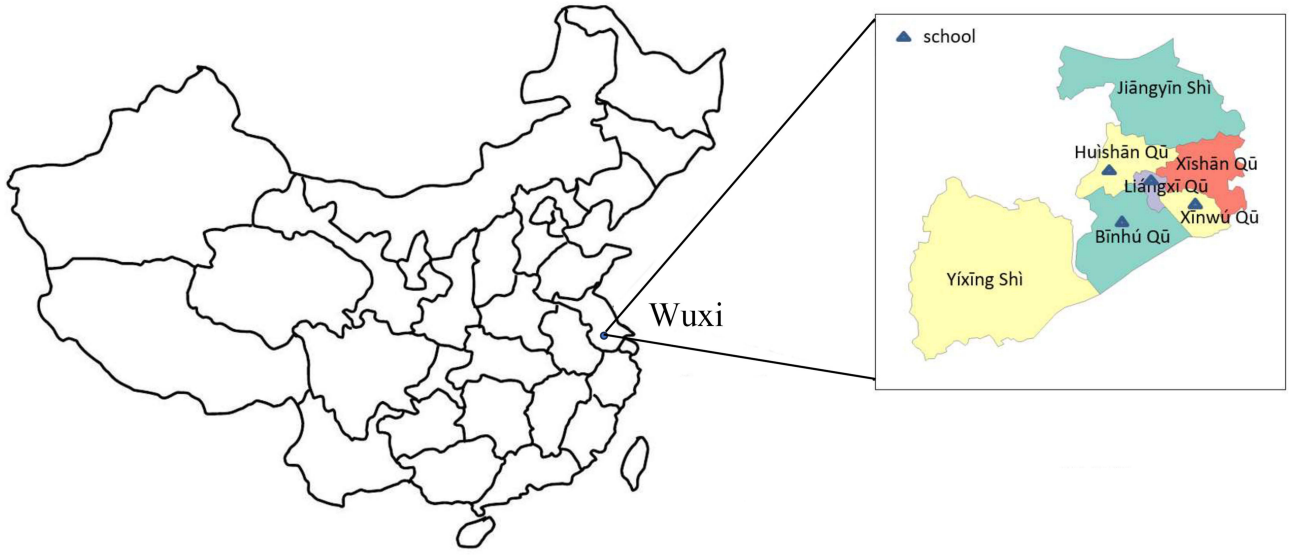

Figure 3 Locations of four campuses under an educational group in Wuxi, China.

Medical and surgical examination: including auscultation of the heart and lungs, visual examination, palpation, and percussion examination of the skin, lymph nodes, head, neck, chest, spine, limbs, liver, and spleen.

Laboratory Tests

On the day before blood collection, the teacher instructed the students to fast the following day and avoid consuming any food or drinks before the test, and distributed urine tubes and sampling vessels with numbered information to each

subject. Considering the young age of the students, the teacher distributed two urine tubes to each of them and instructed them to take them home. The purpose was for the parents to assist the children in collecting urine samples (20 mL) on the morning of the physical examination. After collecting the samples, the students were required to bring the urine tubes back to the examination venue so that the samples could be centrally stored and analyzed.

On the day of blood collection, a specialist is assigned to check whether the collection equipment is sterile and dry. The blood collection process is carried out by experienced laboratory doctors who have received professional training. Before blood collection, the doctor will confirm the subject's name, number, and whether they are fasting and calm. During blood collection, medical staff will select an appropriate vein based on the subject's condition and strictly follow the order of collecting blood in the yellow tube before the purple tube, ensuring that each tube collects at least 3 mL of blood. After collection, the blood is stored at 4°C, and the daily blood collection is completed before 8:30 a.m..

When all the samples have been collected, a specialized sample transport vehicle will transport the samples at low temperatures to the laboratory of the Wuxi Children's Hospital for testing. To prevent sample contamination during transportation, the samples will be checked again to ensure their accuracy (eg, checking for empty tubes), confirm the sample temperature, check whether the tube caps are tightened, and ensure that there is no liquid leakage. Trained professionals at the hospital will separate the blood and urine samples collected on the day of the examination, with 1 mL and 300 μL units of blood and 4.5 mL units of urine, respectively, and perform corresponding biological tests, including blood routine, urine routine, blood lipids, blood biochemistry, and insulin tests (as shown in Table S1). After completing the tests, trained personnel will collect and package the remaining biological samples uniformly. Blood samples will be frozen at −80°C, while urine samples will be kept at −30°C. These samples will be stored carefully for the next step of testing.

If the testing results show that plasma triglyceride concentration >90th percentile, plasma HDL cholesterol concentration ≤10th percentile; systolic blood pressure or diastolic blood pressure >90th percentile (children aged 6 to 9 years); systolic blood pressure >130 mmHg or diastolic blood pressure >85 mmHg, plasma triglycerides >150 mg/dL, plasma HDL cholesterol <40 mg/dL (children aged 10 to 15 years); for adolescents older than 16 years plasma triglycerides ≥1.70 mmol/L (150 mg/dL) or lipid lowering treatment, plasma HDL-cholesterol ≤1.0 mmol/L (40 mg/dL) (male) and ≤1.3 mmol/L (50 mg/dL) (female) or lipid lowering treatment; fasting serum glucose ≥5.6 mmol/L (100 mg/dL) or 2-hour post-load glucose levels ≥7.8 mmol/L (140 mg/dL) or HbA1c ≥39 mmol/mol (5.7%) or type 2 diabetes, indicates the presence of cardiometabolic risk.[2,23]

Vibration-Controlled Transient Elastography Test

The VCTE test utilizes the FibroScan HANDY® (Echosens, Paris, France), which is an evidence-based, transient elastography instrument for non-invasive evaluation of liver steatosis and fibrosis. It is fast, reliable, and reproducible, with good interobserver levels of agreement. The ability to effectively detect >5% of steatosis is much higher than that of previous ultrasound tests.[24–26]

All the operators in this study have received professional training. Before the measurement, the subjects maintained an empty stomach and a calm state. During the measurement, the subjects are supine, lying flat on the test bed, with their right hand behind their head and their body slightly bent to the left, fully exposing the costal space in the right lobe of the chest and liver. The operator set the stool on the right side of the subject, facing the subject's chest and the instrument screen, and attached the M+ probe coated with conductive gel to the subject's costal space (intercostal space No. 7–9 was selected).[27] The low-frequency vibration wave emitted by the probe is measured, and at least ten valid personal measurements are eventually.[28]

As shown in Figure S1A, the median value is used to evaluate liver fibrosis. The measurement of liver stiffness is based on Hook's law, which states that the velocity of shear waves that travel through an elastic object is proportional to the object's stiffness (ie, inversely proportional to the object's elasticity). Young's modulus clinically corresponds to the LSM and is typically referred to as E or LSM, whose values ranging from 1.5 to 75 kPa; lower values indicate a more elastic liver.[9] The normal liver hardness range is about 4 ~ 6 kPa, cirrhosis is more than 12 kPa. A controlled attenuation parameter (CAP) with a fixed and known frequency (3.5 MHz), is directly affected and proportional to the level of steatosis. CAP values range from 100 to 400 dB/m, and higher numbers indicate more pronounced steatosis.[24,29]

Referring to the user manual, the subjects were divided into non-MASLD (CAP value < 248 dB /m) and MASLD (CAP value ≥ 248 dB /m) according to the CAP value.[30] To assess significant liver fibrosis (stages F2-F4), LSM ≥ 7.4 kPa was used, while LSM ≥ 5.1 kPa is accurate for the diagnosis of any liver fibrosis (stage ≥ F1).[31]

Clinical and Biomarker Scores

HOAM-IR Score

The equation was HOMA-IR = fasting serum insulin × fasting glucose (mmol/L) / 22.5, where glucose was expressed in mmol/L and insulin in μU/mL.[32]

Evaluation of Liver Fibrosis

The formulas used to calculate noninvasive measures of liver fibrosis are as follows:

APRI: (AST [U/L]/upper limit of normal AST range [U/L])/platelet count [10^9/L] × 100.[33]

FIB-4: (age [years] × AST [U/L]) / (platelet count [10^9/L] × ALT [U/L] 1/2).[34]

NFS: −1.675 + 0.037 × age [years] + 0.094 × BMI [kg/m^2] + 1.13 × impaired fasting glucose/diabetes (yes = 1, no = 0) + 0.99 × AST [U/L]/ALT [U/L] − 0.013 × platelet count [10^9/L] − 0.66 × albumin [g/dL].[35]

Bioelectrical Impedance Analysis

BIA is a fast, accurate, and comprehensive non-invasive body composition analysis method that calculates body composition by passing a small amount of alternating current through the body, utilizing the varying electrical conductivity of different body components.[36–38] This project is performed by a professionally trained and experienced endocrinologist. The instrument is calibrated before the measurement, and the subjects are confirmed to be in a calm state on an empty stomach. The subjects' number and height (measured during a medical examination) are entered, and the subjects are asked to take off their shoes and socks, with their feet flat on the electrodes on the bottom of their feet and their hands holding the handle. The measurement consists mainly of the following:

1. Body composition analysis: measurement of total body moisture, protein, inorganic salts, and body fat.
2. Muscle fat analysis: weight, skeletal muscle and body fat are measured and classified by percentage as low standard, standard and over standard.
3. Obesity analysis: body mass index (kg/m²) and body fat percentage (%) are measured and classified as low standard, standard and over standard according to the different levels.
4. Muscle balance and segmental fat analysis: the content and percentage of muscle and segmental fat were measured for the right (left) upper limb, right (left) lower limb and trunk respectively, and showed whether the muscle or segmental fat content and percentage are standard for that area. Of note is that the segmental fat analysis is a presumptive value.

As shown in Figure S1B, the results report the BIA score (n/100 points, reflecting the evaluation value of body composition, where individuals with well-developed muscles may score over 100 points), body shape (divided according to BMI (kg/m²) and body fat percentage (%)), basal metabolic rate, waist-to-hip ratio, visceral fat grade, bone mineral content, and bioelectrical impedance values at 5 kHz, 50 kHz, and 250 kHz for the right (left) upper (lower) limbs and trunk. Furthermore, it categorizes individuals as normal, underweight, overweight, or severely overweight based on BMI and as normal, mildly obese, or obese based on body fat percentage. If the test results reveal that the waist circumference of children under 16 years old is above the 90th percentile, or the waist circumference of adolescents, who are aged 16 and above, is greater than 94 cm for males and greater than 80 cm for females, it indicates the presence of cardiometabolic risk factors.[2]

Questionnaire Survey

In addition to the above tests, a paper version of the child nutrition questionnaire is distributed to collect the basic lifestyle of participants (Figure S2). The survey questionnaire was designed based on the content of the PhenX Toolkit

Report combined with local circumstances. The questionnaire has been validated for reliability and validity, with a Cronbach's α of 0.870, an item-level content validity index of 0.83 to 1.00, and a scale-level content validity index of 0.995. It mainly includes the following parts:

General Information

It mainly includes the child's name and date of birth; parents' height, weight, date of birth, highest level of education and contact information, etc.

Maternal Pregnancy and Lactation Status of Children

The mother's pregnancy information mainly includes the mother's age when she became pregnant, the gestational week at delivery, the weight gain during pregnancy, whether she failed any physical examination during pregnancy, whether she had any pregnancy-related illnesses, and whether the living environment has been renovated during pregnancy until now. The breastfeeding period includes information about the child's birth weight and feeding after birth.

Children's Exercise and Diet Status

The evaluation of physical activity mainly includes children's daily screen time, weekly exercise frequency, and duration of each exercise. The evaluation of dietary habits mainly includes children's habits of washing vegetables and fruits, drinking water, daily food consumption, and family eating habits. The frequency of various types of food intake among children is divided into the following categories: rarely (<1 time/week), occasionally (1–3 times/week), frequently (4–7 times/week), and almost every time (≥8 times/week). For liquid foods (eg, milk and fruit juices), each time refers to 250 mL, except for eggs and fruits, which refer to 1 each, while for other foods, each time refers to 100g.

Family Living Environment and Daily Living Conditions

This questionnaire involves information about household members, including the total number of family members (seniors, adults, and children), the average monthly income per family member, and whether they work in occupations with higher occupational health risks (such as sanitation workers, traffic police, chemical industry, metal smelting, etc).

In addition, the questionnaire also covers information about the home environment, including the living years of the current house, the floor, whether there has been renovation in the past five years, how long after the last renovation, the materials used in the renovation, whether new furniture has been added in the past year, whether there has been water leakage or mold on the top, whether the carpet has been used, whether flowers have been planted, and whether lampblack equipment has been installed. Furthermore, the questionnaire also involves the fuel used, the electronic equipment used, and the amount of cooking in the home. Finally, the questionnaire will also ask whether there are traffic pollution sources near the house, such as gas stations, traffic trunks, high-voltage transformers, high-voltage towers, or high-voltage power lines.

Follow-Ups and Assessments

This study will achieve long-term follow-up of fixed study participants for 12 years until 2035, or until they reach the age of 18 or voluntarily withdraw from the study, by collecting data from annual school physical examinations. According to preliminary research, the dropout rate at this school has been below 0.5% over the past decade, and as the school encompasses three stages (ie, primary, middle, and high school), we can easily achieve continuous tracking and follow-up.

The follow-up contents are consistent with the first-year physical examination, including a full-body examination, laboratory tests, VCTE, BIA, and a questionnaire survey. Questionnaires and physical examinations will be performed with the same standard as the baseline investigation. The diagnostic criteria for pediatric MASLD also adhere to international standards, which involve the presence of steatosis (evaluated by CAP) in addition to at least one cardiometabolic risk factor, as previously mentioned.

By analyzing the overall prevalence and annual incidence rate, as well as relevant physical examination and questionnaire data, a more comprehensive and accurate assessment of the incidence and risk factors of MASLD among Chinese children can be obtained. In addition, by comparing the differences between the diseased and non-

diseased groups, potential risk factors and preventive measures can be identified more effectively, providing a reliable scientific basis for the treatment and prevention of this disease.

Quality Control and Data Management

Quality Control of Testing Equipment and Personnel

Before carrying out daily physical examination operations, the testing equipment is checked and calibrated one by one to ensure that the equipment can function properly. After completing the daily testing tasks, each piece of equipment undergoes routine maintenance and calibration, including keeping the equipment dry, cleaning the probe, and timely replacement of the probe cover, to ensure the stability and accuracy of the equipment. In addition, all measurement personnel involved in this physical examination project have undergone professional training and have more than two years of work experience.

Quality Control of Questionnaire Survey

After the questionnaires are collected, they will be evaluated by two reviewers who will check the completeness and reliability of each section. For questionnaires with lower quality, the reviewers will contact parents or guardians within one week to conduct phone interviews, and complete and modify the questionnaire. To ensure the accuracy of data entry, two people will be responsible for data entry, and logic check functions will be pre-written in the data input system to prevent input errors.

Quality Control of Data

In this study, different approaches are used to ensure the traceability of data, including registering the contact information of students and guardians (student name, date of birth, grade class, ID card or passport number, and parents' phone number), coding each student with an ID card or passport number, building a database to aggregate and integrate data information, etc. In addition, throughout the data collection, the data are controlled daily and in case any issues are detected, the quality control manager refers the issue to the director by completing a form. Then the director finds the corresponding school official according to the information provided in the form and arranges for the student to be re-examined. Finally, we will print out the physical examination results and send them back to the school, and parents and medical professionals will be arranged to give lifestyle health guidance to the school, to improve the participation of students and parents and ensure the integrity of follow-up data.

Handling of the Missing Data

A multiple imputation method based on the mice package in R 4.2.2 will be employed to handle missing data. By setting to generate 5 complete datasets for imputation (m=5), specifying a maximum of 50 iterations (maxit=50), and opting for predictive mean matching for imputation (method= pmm), data analysis will be conducted. This approach not only prevents automatic exclusion of participants due to missing data but also allows them to continue participating in the study by providing data in subsequent years, thus ensuring data integrity and reliability.

Statistical Analysis

Statistical analysis will be performed using R 4.2.2. Initially, patients with MASLD will be screened according to MASLD diagnostic criteria combined with measurements. Descriptive statistics will be used to describe the population characteristics of the participants. Also, the Chi-square test will be used to analyze the relationship between demographic characteristics (ie, gender and age) and the incidence of MASLD. Linear (or logistics) regression will be used to assess whether there are significant differences between the incidence of MASLD and outcomes. The effect size was represented with BETA±SE (for linear regression) or odds ratios [OR] (95% confidence interval [CI]), respectively. Statistical significance will be considered when $P < 0.05$.

Adverse Event Reporting and Harms

During our MASLD investigation, any adverse events experienced by research participants, encompassing unfavorable changes in physical or mental health regardless of study-relatedness, will be meticulously documented in the Adverse

Event Log. Types and frequencies of these events will be promptly reported to the China National Adverse Drug Reaction Monitoring Center. Upon confirming abnormal findings, we swiftly notify the child's physician with a comprehensive report outlining the details and recommended actions. Additionally, we maintain open communication with families, explaining the situation thoroughly and addressing their concerns, thereby ensuring timely medical intervention and fostering collaboration between professionals and families for the best possible care of the child.

Confidentiality

Any information and data obtained about the subjects during the course of the study will be kept strictly confidential. Blood and urine specimens from the subjects will be identified by a code rather than by name, and information that could reveal the identity of the subjects will not be disclosed to members outside the research team. Any public reports regarding the results of this study will not include the personal identities of the subjects.

Dissemination Plans

The findings of this study will be showcased at academic conferences and published in open-access journals. Authorship will be determined by the steering group, and the order of authors will reflect the individual contributions of each member.

Discussion

MASLD has become a global health burden with an increasing prevalence in the pediatric population: a 15-year meta-analysis showed that the average prevalence of fatty liver in children and adolescents aged 1–19 years was 7.6%.[39] Different races have different prevalence rates; the prevalence among children and adolescents in Asia was 10.2%;[40] the prevalence rate in Europe was 2.5%;[41] and the prevalence rate among Hispanics was 11.8%, which is the highest prevalence rate. Previous studies have shown that: besides liver-related morbidity, MASLD is also associated with an increased risk of cardiovascular disease, type 2 diabetes, and mortality at adult age.[42,43]

According to current guidelines,[12] there is no approved pharmacotherapy for it, lifestyle intervention remains the first line of intervention for MASLD in children. A recent prospective study on 204 severely obese adolescents showed that an intensive, multidisciplinary lifestyle management program can not only significantly reduce weight (median weight loss of 16.0%), but also improve liver fat and fibrosis, with a fibrosis improvement rate of up to 75.0%. Therefore, early detection and management of children with MASLD is the most important step to prevent the progression of the disease.

In this protocol, we describe the study design, data collection methods, and analysis of a prospective cohort study in four primary schools. By collecting information on factors such as children's family environment, lifestyle, and population characteristics, this study will analyze the clinical data and laboratory test results collected in children with MASLD from the perspectives of society and the environment. The objectives are to develop early markers for pediatric MASLD, determine how gender and age influence the developmental trajectories of MASLD, examine the characteristics of MASLD in pediatric patients (ie, demographic information, environmental factors, dietary and exercise habits, and their association with MASLD), identify modifiable risks and protective factors, and pinpoint exercise and dietary patterns associated with MASLD and non-MASLD.

This study conducts a cohort investigation on pediatric MASLD, exploring the incidence, influencing factors, screening systems, and early biomarkers of MASLD in children. If effective, the tools and methodologies employed in this study could assist policymakers and healthcare providers in promptly identifying and diagnosing children with MASLD, enhancing the prognosis and treatment outcomes of pediatric MASLD, and facilitating the formulation of personalized treatment and intervention strategies. Moreover, the study will contribute to identifying unhealthy lifestyles, offering insights for crafting healthy dietary and physical activity guidelines, ultimately reducing the occurrence of MASLD and lowering healthcare expenses.

The major strengths of our study lie in the robust and complete data from a population-based cohort with a representative sample of school children aged 6 to 18 in Wuxi, China. Secondly, both the prevalence of MASLD and the aggregation of metabolic components change with development, so the results of this study based on the cohort study are more accurate.

The study also faces a few challenges and limitations. First, a high participation rate of schools and children is required for sufficient power to analyze different ages and grades within classes and schools, which could otherwise be rather limited in a study collecting venous blood samples in children. Second, this is a single-center study. In addition, the diagnosis of MASLD was based on ultrasonography rather than liver biopsy, which may lead to misclassification and underestimate the prevalence. However, due to shortcomings such as sampling errors, severe complications and the high cost involved in liver biopsy, non-invasive diagnostic methods such as VCTE or ultrasound are the main detection methods for clinical applications, particularly in children.[44]

Patient and Public Involvement

Patients or patient advisors have not been directly involved in the planning or conduct of the study. Results will be disseminated via public media channels to inform patients and parents about results.

Abbreviations

MASLD, Metabolic associated steatotic liver disease; NAFLD, Nonalcoholic fatty liver disease; VCTE, Vibration-controlled transient elastography; BIA, bioelectrical impedance analysis; ALT, alanine aminotransferase; TG, triglycerides; TCHOL, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

Data Sharing Statement

Data is available from the corresponding author (Le Zhang) on reasonable request.

Ethics Approval and Consent to Participate

This study was approved by the Affiliated Children's Hospital of Jiangnan University (WXCH2022-09-044). Written informed consent obtained from all the participant's parents or legal guardians.

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Disclosure

The authors declare that they have no competing interest.

References

1. En Chan K, Jia Ling Koh T, Shao Pin Tang A, et al. Global prevalence and clinical characteristics of metabolic-associated fatty liver disease: a meta-analysis and systematic review of 10 739 607 individuals. *J Clin Endocrinol Metab*. 2022;107(9):2691–2700. doi:10.1210/clinem/dgac321
2. 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
3. Lim GEH, Tang A, Ng CH, et al. An observational data meta-analysis on the differences in prevalence and risk. *Clin Gastroenterol Hepatol*. 2023;21(3):619–629. doi:10.1016/j.cgh.2021.11.038
4. Pipitone RM, Ciccioli C, Infantino G, et al. MAFLD: a multisystem disease. *Ther Adv Endocrinol Metab*. 2023;14:20420188221145549. PMID: 36726391; PMCID: PMC9885036. doi:10.1177/20420188221145549

5. Guan L, Zhang X, Tian H, et al. Prevalence and risk factors of metabolic-associated fatty liver disease during 2014-2018 from three cities of Liaoning Province: an epidemiological survey. *BMJ Open*. 2022;12(2):e047588. doi:10.1136/bmjopen-2020-047588 PMID: 35177440; PMCID: PMC8860048.
6. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. *Hepatology*. 2012;55(6):2005–2023. doi:10.1002/hep.25762 PMID: 22488764.
7. Ratziu V, Charlotte F, Heurtier A, et al. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. *Gastroenterology*. 2005;128 (7):1898–1906. doi:10.1053/j.gastro.2005.03.084 PMID: 15940625.
8. Kuwashiro T, Takahashi H, Hyogo H, et al. Discordant pathological diagnosis of non-alcoholic fatty liver disease: a prospective multicenter study. *JGH Open*. 2019;4(3):497–502. doi:10.1002/jgh3.12289 PMID: 32514460; PMCID: PMC7273711.
9. Oeda S, Tanaka K, Oshima A, Matsumoto Y, Sueoka E, Takahashi H. Diagnostic accuracy of FibroScan and factors affecting measurements. *Diagnostics*. 2020;10:11. doi:10.3390/diagnostics10110940 PMID: 33198092; PMCID: PMC7696616.
10. Marshall RH, Eissa M, Bluth EI, Gulotta PM, Davis NK. Hepatorenal index as an accurate, simple, and effective tool in screening for steatosis. *AJR Am J Roentgenol*. 2012;199(5):997–1002. doi:10.2214/AJR.11.6677 PMID: 23096171.
11. Shen L, Li JQ, Zeng MD, Lu LG, Fan ST, Bao H. Correlation between ultrasonographic and pathologic diagnosis of liver fibrosis due to chronic virus hepatitis. *World J Gastroenterol*. 2006;12(8):1292–1295. doi:10.3748/wjg.v12.i8.1292 PMID: 16534888; PMCID: PMC4124446.
12. Vos MB, Abrams SH, Barlow SE, et al. NASPGHAN clinical practice guideline for the diagnosis and treatment of nonalcoholic fatty liver disease in children: recommendations from the expert committee on NAFLD (ECON) and the North American society of pediatric gastroenterology, hepatology and nutrition (NASPGHAN). *J Pediatric Gastroenterol Nutr*. 2017;64(2):319–334. doi:10.1097/mpg.0000000000001482 PMID: 28107283; PMCID: PMC5413933.
13. Koot BGP, Nobili V. Screening for non-alcoholic fatty liver disease in children: do guidelines provide enough guidance? *Obes Rev*. 2017;18 (9):1050–1060. doi:10.1111/obr.12556 PMID: 28544608.
14. Deng J, Ji W, Liu H, et al. Development and validation of a machine learning-based framework for assessing metabolic-associated fatty liver disease risk. *BMC Public Health*. 2024;24(1):2545. doi:10.1186/s12889-024-19882-z PMID: 39294603; PMCID: PMC11412026.
15. Nabrdalik K, Kwiendacz H, Irlik K, et al. Machine learning identification of risk factors for heart failure in patients with diabetes mellitus with metabolic dysfunction associated steatotic liver disease (MASLD): the Silesia diabetes-heart project. *Cardiovasc Diabetol*. 2023;22(1):318. doi:10.1186/s12933-023-02014-z PMID: 37985994; PMCID: PMC10661663.
16. Nabrdalik K, Kwiendacz H, Irlik K, et al. Machine learning identifies metabolic dysfunction–associated steatotic liver disease in patients with diabetes mellitus. *J Clin Endocrinol Metab*. 2024;109(8):2029–2038. doi:10.1210/clinem/dgae060 PMID: 37985994; PMCID: PMC10661663.
17. Maxwell SL, Price JC, Perito ER, Rosenthal P, Wojcicki JM. Food insecurity is a risk factor for metabolic dysfunction-associated steatotic liver disease in Latinx children. *Pediatric Obes*. 2024;19(6):e13109. doi:10.1111/ijpo.13109 PMID: 38453472; PMCID: PMC11146202.
18. Zhang L, El-Shabrawi M, Baur LA, et al. An international multidisciplinary consensus on pediatric metabolic dysfunction-associated fatty liver disease. *Med*. 2024;5(7):797–815.e792. doi:10.1016/j.medj.2024.03.017 PMID: 38677287.
19. Xing Y, Zhang P, Li X, et al. New predictive models and indices for screening MAFLD in school-aged overweight/obese children. *Eur J Pediatrics*. 2023;182(11):5025–5036. doi:10.1007/s00431-023-05175-x PMID: 37648793.
20. Liang C, Yu Z, Bai L, et al. Association of serum bilirubin with metabolic syndrome and non-alcoholic fatty liver disease: a systematic review and meta-analysis. *Front Endocrinol*. 2022;13:869579. PMID: 35937795; PMCID: PMC9346511. doi:10.3389/fendo.2022.869579
21. Yodoshi T, Orkin S, Romantic E, et al. Impedance-based measures of muscle mass can be used to predict severity of hepatic steatosis in pediatric nonalcoholic fatty liver disease. *Nutrition*. 2021;91-92:111447. PMID: 34583137; PMCID: PMC8595713. doi:10.1016/j.nut.2021.111447
22. World Health Organization. *Oral Health Surveys*. 2013. Google Scholar.
23. Eslam M, Alkhouri N, Vajro P, et al. Defining paediatric metabolic (dysfunction)-associated fatty liver disease: an international expert consensus statement. *Lancet Gastroenterol Hepatol*. 2021;6(10):864–873. doi:10.1016/S2468-1253(21)00183-7 PMID: 34364544.
24. Ferraioli G, Soares Monteiro LB. Ultrasound-based techniques for the diagnosis of liver steatosis. *World J Gastroenterol*. 2019;25(40):6053–6062. doi:10.3748/wjg.v25.i40.6053 PMID: 31686762; PMCID: PMC6824276.
25. Dasarathy S, Dasarathy J, Khiyami A, Joseph R, Lopez R, McCullough AJ. Validity of real time ultrasound in the diagnosis of hepatic steatosis: a prospective study. *J Hepatol*. 2009;51(6):1061–1067. doi:10.1016/j.jhep.2009.09.001 PMID: 19846234; PMCID: PMC6136148.
26. van Werven JR, Marsman HA, Nederveen AJ, et al. Assessment of hepatic steatosis in patients undergoing liver resection: comparison of US, CT, T1-weighted dual-echo MR imaging, and point-resolved 1H MR spectroscopy. *Radiology*. 2010;256(1):159–168. doi:10.1148/radiol.10091790 PMID: 20574093.
27. Mikolasevic I, Orlic L, Franjic N, Hauser G, Stimac D, Milic S. Transient elastography (FibroScan(®)) with controlled attenuation parameter in the assessment of liver steatosis and fibrosis in patients with nonalcoholic fatty liver disease - Where do we stand? *World J Gastroenterol*. 2016;22 (32):7236–7251. doi:10.3748/wjg.v22.i32.7236 PMID: 27621571; PMCID: PMC4997649.
28. Wai-Sun Wong V, Petta S, Hiriart J-B, et al. Validity criteria for the diagnosis of fatty liver by M probe-based controlled attenuation parameter. *J Hepatol*. 2017;67(3):577–584. doi:10.1016/j.jhep.2017.05.005 PMID: 28506907.
29. Sasso M, Beaugrand M, de Ledinghen V, et al. Controlled attenuation parameter (CAP): a novel VCTE™ guided ultrasonic attenuation measurement for the evaluation of hepatic steatosis: preliminary study and validation in a cohort of patients with chronic liver disease from various causes. *Ultrasound Med Biol*. 2010;36(11):1825–1835. doi:10.1016/j.ultrasmedbio.2010.07.005 PMID: 20870345.
30. Karlas T, Petroff D, Sasso M, et al. Individual patient data meta-analysis of controlled attenuation parameter (CAP) technology for assessing steatosis. *J Hepatol*. 2017;66(5):1022–1030. doi:10.1016/j.jhep.2016.12.022 PMID: 28039099.
31. Alkhouri 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. doi:10.1111/liv.12024 PMID: 23146095.
32. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28(7):412–419. doi:10.1007/bf00280883 PMID: 3899825.
33. Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology*. 2003;38(2):518–526. doi:10.1053/jhep.2003.50346 PMID: 12883497.

34. Shah AG, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol*. 2009;7(10):1104–1112. doi:10.1016/j.cgh.2009.05.033 PMID: 19523535; PMCID: PMC3079239.
35. Angulo P, Hui JM, Marchesini G, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology*. 2007;45(4):846–854. doi:10.1002/hep.21496 PMID: 17393509.
36. Larsen MN, Orcid I, Krustrup P, Araújo Póvoas SC, Castagna C. Accuracy and reliability of the InBody 270 multi-frequency body composition analyser in 10-12-year-old children. *PLoS One*. 2021;16(3):0247362. doi:10.1371/journal.pone.0247362 PMID: 33770106; PMCID: PMC7996997.
37. Kabiri LS, Hernandez DC, Reliability MK. Validity, and diagnostic value of a pediatric bioelectrical impedance analysis scale. *Child Obes*. 2015;11(5):650–655. doi:10.1089/chi.2014.0156 PMID: 26332367.
38. McLester CN, Nickerson BS, Kliszczewicz BM, McLester JR. Reliability and agreement of various inbody body composition analyzers as compared to dual-energy X-Ray absorptiometry in healthy men and women. *J Clin Densitom*. 2020;23(3):443–450. doi:10.1016/j.jocd.2018.10.008 PMID: 30472111.
39. Yu EL, Golshan S, Harlow KE, et al. Prevalence of nonalcoholic fatty liver disease in children with obesity. *J Pediatrics*. 2019;207:64–70. PMID: 30559024; PMCID: PMC6440815. doi:10.1016/j.jpeds.2018.11.021
40. Fang Y-L, Chen H, Wang C-L, Liang L. Pathogenesis of non-alcoholic fatty liver disease in children and adolescence: from "two hit theory" to "multiple hit model". *World J Gastroenterol*. 2018;24(27):2974–2983. doi:10.3748/wjg.v24.i27.2974 PMID: 30038464; PMCID: PMC6054950.
41. Lawlor DA, Callaway M, Macdonald-Wallis C, et al. Nonalcoholic fatty liver disease, liver fibrosis, and cardiometabolic risk factors in adolescence: a cross-sectional study of 1874 general population adolescents. *J Clin Endocrinol Metab*. 2014;99(3):E410–E417. doi:10.1210/jc.2013-3612 PMID: 24471572; PMCID: PMC3955251.
42. Draijer L, Benninga M, Koot B. Pediatric NAFLD: an overview and recent developments in diagnostics and treatment. *Expert Rev Gastroenterol Hepatol*. 2019;13(5):447–461. doi:10.1080/17474124.2019.1595589 PMID: 30875479.
43. Sandireddy R, Sakthivel S, Gupta P, Behari J, Tripathi M, Singh BK. Systemic impacts of metabolic dysfunction-associated steatotic liver disease (MASLD) and metabolic dysfunction-associated steatohepatitis (MASH) on heart, muscle, and kidney related diseases. *Front Cell Develop Biol*. 2024;12:1433857. doi:10.3389/fcell.2024.1433857 PMID: 39086662; PMCID: PMC11289778.
44. Draijer LG, van Oosterhout JPM, Vali Y, et al. Diagnostic accuracy of fibrosis tests in children with non-alcoholic fatty liver disease: a systematic review. *Liver Int*. 2021;41(9):2087–2100. doi:10.1111/liv.14908 PMID: 33894100; PMCID: PMC8453517.