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Can ultrasound echo intensity assess muscle quality in children aged 10–14 years? Protocol for a cross-sectional validation study in Czech children

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ABSTRACT

Introduction Ultrasonography is a non-invasive and safe method for assessing muscle morphology. Among its parameters, echo intensity (EI), derived from grayscale image analysis, has emerged as a promising indicator of muscle quality and intramuscular fat infiltration. This study aims to validate EI as a marker for evaluating muscle quality in a population of Czech children, through integration with gold-standard assessments of muscle strength and body composition. The primary aim of this study is to assess the reliability and construct validity of quadriceps muscle EI using ultrasound as a proxy measure of morphological muscle quality in children aged 10–14 years.

Methods and analysis Children aged 10–14 years will undergo ultrasound assessment of the quadriceps femoris (QF). EI will be derived from longitudinal scans of each QF head and the cross-sectional area (CSA_{QF}) from panoramic mid-thigh images. Muscle function will be assessed as maximal voluntary contraction (MVC) of isometric knee extension with muscle quality expressed as MVC/CSA_{QF}. A 30 s sit-to-stand test (30STS) will be used as an additional functional measure. EI reliability (intra-rater, inter-rater and test–retest) will be evaluated with intraclass correlation coefficients (ICC), Bland–Altman plots and complementary indices. Exploratory known-groups validity will be tested by comparing EI between weight-status groups. Control variables include dual-energy X-ray absorptiometry (DXA)-derived body composition, skeletal age (as determined by DXA hand scans) and physical activity (assessed using 7-day accelerometry).

This study will include 200 children (100 girls and 100 boys) aged 10–14 years using an a priori power analysis based on the primary objective of assessing construct validity through multiple linear regression, assuming an alpha level of 0.05 and 80% power. Participants will be recruited from paediatric outpatients of the Paediatric Obesity Clinic and individuals reached through a recruitment campaign. Inclusion criteria require general good health, while exclusion criteria include a history or symptoms of cardiovascular, pulmonary, metabolic or neurological disease, as well as the use of over-the-counter or prescribed medications. Informed consent and assent will be obtained from all participants.

Reliability of ultrasound-derived EI will be assessed for intra-rater, inter-rater and test–retest agreement using ICC coefficients, Bland–Altman plots and complementary indices such as SE of measurement, coefficient of variation and minimal detectable change at 95% CI, following Consensus-based Standards for the selection of health Measurement Instruments guidelines. Construct validity will be examined by modelling associations between EI and functional muscle quality (MVC/CSA_{QF}), with 30STS as an additional functional measure. Known-groups validity will be tested by comparing EI across weight groups, using generalised linear regression models adjusted for skeletal age, body composition and physical activity. All validity analyses will be conducted separately for girls and boys. Ultrasound-derived EI of the QF is expected to show high reliability (ICC≥0.80) and acceptable test–retest reproducibility. Construct validity should be supported by moderate associations with functional muscle quality (MVC/CSA_{QF}), while known-groups validity is expected to reveal higher EI values in children with obesity and/or insufficient physical activity.

Ethics and dissemination The study will be conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Faculty of Physical Education and Sport, Charles University (EK 101/2024). Written parental consent and verbal assent from children will be obtained, with all data handled

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study combines ultrasonography with gold-standard methods (dual-energy X-ray absorptiometry and isokinetic dynamometry) to provide a robust assessment of muscle quality in children.
⇒ Standardised ultrasound procedures for probe placement, depth and positioning are applied to enhance reproducibility and minimise measurement variability.
⇒ Inclusion of both normal-weight and obese participants improves population relevance and generalisability.
⇒ A comprehensive construct validity framework, including functional associations and known-groups comparisons, strengthens interpretability.
⇒ The cross-sectional design limits causal inference and prevents evaluation of longitudinal changes or training effects.

confidentially and anonymised. Results will be disseminated transparently to participants and their families in line with ethical principles of respect, beneficence and justice.

Trial registration number NCT06792279.

INTRODUCTION

The 10-fold global rise in childhood and adolescent obesity in the last four decades is a significant health challenge with profound implications for society and the economy. A 2021 Association of General Practitioners for Children and Adolescents study[1] of 4386 Czech children (aged 5–17 years) found that obesity rose sharply to 16% (an increase of one-third compared with 2016), particularly among early adolescents aged 11 and 13 years (a reminder of how severely COVID-19 restrictions affected the health of children). Severe obesity (above the 99th percentile) was then identified in approximately half of the obese children.

Childhood obesity not only undermines immediate well-being but also increases the risk of serious health conditions, including type 2 diabetes, hypertension, dyslipidaemia and early cardiovascular disease.[2] Moreover, children and adolescents who are obese often experience altered muscle structure and function, which may increase the risk of musculoskeletal problems, including weakness, poor posture and limited mobility.[3] Sedentary lifestyles and high-fat diets are key contributors to obesity and can also impair skeletal muscle metabolism, further exacerbating the problem.[4] This combination of factors highlights the need for strategies to assess muscle quality (the functional capacity of skeletal muscle, encompassing its ability to generate force, contract and relax efficiently, and regulate metabolism) in children early on before more serious complications arise.

Muscle quality is often expressed as strength or power relative to muscle mass, providing a measure of how effectively the muscle performs its functions beyond mere size.[5] Evidence suggests that during early adolescence, muscle size and strength increase substantially, yet muscle quality (ie, torque per muscle volume) remains relatively stable across pubertal stages,[6] enabling more precise detection of structural and functional changes. Although no gold-standard imaging method currently exists for assessing muscle quality,[5] ultrasonography has become increasingly popular in recent research due to its low cost, safety and portability. An ultrasound-based method of assessing muscle quality uses echo intensity (EI),[7] which reflects tissue brightness on ultrasound images. Muscles with higher proportions of non-contractile tissue appear brighter, resulting in higher EI values and lower muscle quality. Using grayscale analysis, EI calculates the average pixel intensity within a defined region of interest (ROI).[8 9] Previous studies in children have shown that EI differs significantly between normal-weight and overweight prepubertal children. Herda *et al*[10] demonstrated, based on a study sample of 30 children, poorer muscle tissue composition and greater velocity-related impairments in muscle strength in obese children. However, there were no differences in cross-sectional area (CSA) when accounting for EI.[11] In another cross-sectional study involving 267 children, the authors demonstrated that the EI of the rectus femoris (RF) in children with an age-specific and sex-specific body mass index (BMI) above the 75th percentile was significantly higher than that of the other participants.[12] The overweight children (n=11) had greater EI, although anatomical CSA was similar to that of children with normal weight (n=17).[13] Previous studies have reported that higher EI is associated with poorer cardiometabolic health in overweight children[14] and that EI shows moderate associations with athletic performance measures in boys (n=29), including jump height, peak velocity, sprint speed and agility.[15] On the other hand, numerous studies have highlighted significant variability in EI measurements due to differences in probe pressure, angle, ROI, subcutaneous fat thickness and disparities in image analysis software. and ultrasound device parameters,[16–21] underscoring the need for standardised protocols in muscle quality assessment to enhance reproducibility and validation against compositional and functional standards.

The quadriceps femoris (QF) is a particularly suitable target in early adolescence, as it plays a central role in locomotion, posture and overall functional performance. It underpins key daily activities such as walking, running, jumping and rising from a seated position, making it a sensitive marker of functional health in children. At the same time, this muscle group is highly vulnerable to the adverse effects of sedentary behaviour and excess adiposity. Evidence shows that prolonged inactivity reduces quadriceps strength and endurance, while childhood obesity is linked to greater intramuscular fat infiltration and impaired muscle quality, reflected in decreased specific force and elevated EI.[15 22] Given its dual importance for locomotor function and its susceptibility to early decline, the QF provides a practical and clinically relevant site for assessing muscle quality in paediatric populations.

This study aims to evaluate the reliability and construct validity of QF muscle EI assessed by ultrasound as a proxy measure of morphological muscle quality in children aged 10–14 years. We hypothesise that ultrasound-derived EI of the QF will provide a reliable and valid non-invasive proxy of muscle quality in children aged 10–14 years, demonstrating strong reproducibility, meaningful associations with functional strength and the ability to discriminate between groups differing in weight and/or physical activity status. By integrating ultrasonography with other gold-standard techniques, such as dual-energy X-ray absorptiometry (DXA) for body composition and isokinetic dynamometry for strength, this study aims to provide a robust, multifaceted assessment of muscle quality in children. Ultimately, this work will contribute to a deeper understanding of muscle quality in children and support future research aimed at developing interventions to improve paediatric muscle health.

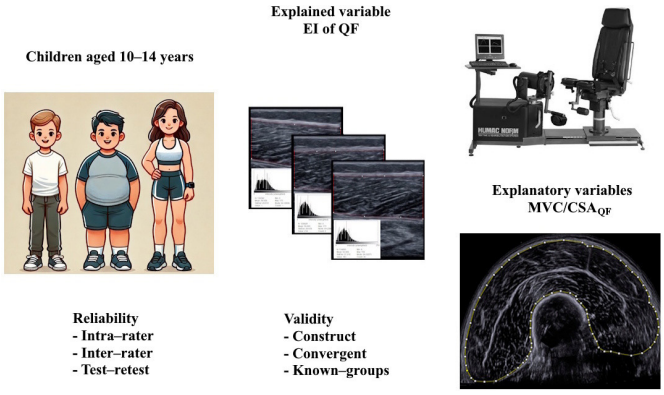

Figure 1 Schematic representation of the study design. CSA, cross-sectional area; EI, echo intensity; MVC, maximal voluntary contraction; QF, quadriceps femoris.

METHODS AND ANALYSIS

Design

This is a cross-sectional study examining the reliability and construct validity of ultrasound-derived EI in children aged 10–14 years. The study design is shown in figure 1.

Study population

We will recruit children aged 10–14 years, targeting a sample of n=200 with approximately equal representation of girls and boys, and a balanced enrolment of normal-weight and obese participants. Weight status will be defined using Czech reference values[23] for BMI according to age and sex (obese: ≥97th percentile; normal weight: 5th–85th percentile).

Sample size

The sample size is determined based on the primary objective of assessing construct validity through multiple linear regression. Using G*Power (V.3.1), a priori power analysis indicated that a minimum of 85 participants per sex group would be required to detect a moderate effect size (Cohen's f-squared=0.15) with four predictors (maximal voluntary contraction (MVC/CSA, skeletal age, body composition and physical activity), assuming an alpha level of 0.05 and 80% power. This effect size reflects the expected contribution of functional muscle quality (MVC/CSA) to the variability in EI, while adjusting for skeletal age, body composition and functional capacity. To account for potential unusable scans, incomplete measurements or participant exclusions, we plan to enrol a total of 200 participants.

Inclusion criteria

Children in general good health, able to understand study instructions in Czech with no known neuromuscular, endocrine or metabolic disorders that affect muscle mass or quality, such as Duchenne muscular dystrophy, Becker muscular dystrophy, limb-girdle muscular dystrophy, facioscapulohumeral muscular dystrophy, congenital muscular dystrophies, Charcot-Marie-Tooth disease, polymyositis, type 1 or 2 diabetes, hypothyroidism or Cushing's syndrome.

Exclusion criteria

Contraindications for participation in the study: any acute diseases; lower limb injury or immobilisation in the previous 3 months; post-traumatic conditions; diseases of the cardiovascular, pulmonary, metabolic or endocrine systems; corticosteroid therapy or other drugs affecting muscle composition; the presence of implanted electronic or metallic devices; and pregnancy.

Recruitment process

We will use a non-probability sampling strategy, specifically purposive sampling. Participants will be recruited from two sources: paediatric outpatients of the Paediatric Obesity Clinic at the Department of Children and Adolescents at University Hospital Královské Vinohrady; and individuals reached through a recruitment campaign leveraging social media posts, flyers, school newsletters, emails and word of mouth in public and private schools, youth sports clubs, youth organisations and community centres in Prague and the surrounding area. To enable assessment of selection bias, we will record the number of families approached, the number declining participation and basic demographics (age and sex) of non-responders where feasible.

Patient and public involvement

Patients and the public will not be involved in the design, conduct, reporting or dissemination plans of this research.

Primary outcome measure

Non-invasive ultrasound measurements will be applied along the longitudinal axis of the four heads of the right QF to estimate EI and determine the CSA of the QF.[24] Participants will lie supine with legs extended and relaxed. The ultrasound probe will be positioned at fixed anatomical landmarks, calculated as a percentage of the distance between the superior iliac spine and the superior edge of the patella: 22% for vastus medialis, 39% for vastus lateralis, and 56% for RF and vastus intermedius.[15 19 25] CSA$_{QF}$ will be assessed at mid-thigh (50% of femur length) from a panoramic axial scan of the QF muscle group. All images will be obtained using a SonoScape E2 portable ultrasound system (SonoScape Medical Corp., China) with a 40 mm linear array probe (7–12 MHz, depth 50 mm and gain 50 dB). If the entire muscle is not visible, the depth may be increased by ≤0.5 cm, a change that is demonstrated not to affect EI values.[15] Still, sagittal images will be captured, with three images obtained per muscle belly. EI will be calculated using ImageJ (V.1.54g; National Institutes of Health, USA) as the mean grayscale value (0=black, 255=white) from a polygonal ROI encompassing the largest visible portion of the muscle. Subcutaneous adipose tissue (SAT) thickness will not be adjusted for, as correction may be unnecessary in children, and existing correction formulas have not been validated in paediatric populations.[26 27] Instead, all scans will be acquired using standardised probe pressure and fixed

gain settings to minimise variability. CSA will be derived from three panoramic scans by manually tracing the QF muscle boundary.

To ensure data integrity, both the sonographer and image analyst will be blinded to the weight status and strength results of the participants. Quality assurance checks will include a repeat analysis of 10% of randomly selected images. Pre-specified exclusion criteria for images include visible motion blur, poor contrast or compression artefacts. Where artefacts occur, a repeat scan will be performed; if this is not possible, an adjacent ROI will be selected.

Explanatory outcome measures

Maximal voluntary contraction (MVC) of isometric knee extension will be measured as the primary explanatory variable using an isokinetic dynamometer (Humac Norm, Cybex CSMI, Stoughton, MA, USA) on the right QF. Participants will be seated with their hips flexed at 85°–90° and right knee at 60° flexion, with their torso and pelvis stabilised, and the dynamometer axis aligned with the lateral femoral condyle. Gravity correction will be applied. Each participant will sustain one MVC for 5 s after a familiarisation measure performed at 30% of the participant's perceived maximal strength, with a 60 s rest between trials. The highest peak torque (Nm) will be used for analysis. Functional muscle quality relating to muscle intrinsic force capacity shall be assessed by normalising the MVC torque of the knee extensor muscles to CSA_{QF} ($Nm \cdot cm^{-2}$).

Exploratory convergent validity will use an additional functional measure, the 30 s sit-to-stand test (30STS), as described by Robinson *et al*,[28] which reflects broader functional capacity but is not adjusted for muscle size.

Control variables

Body composition will be measured using DXA (Hologic Horizon, Marlborough, Massachusetts, USA) in the supine position following standard positioning and scan acquisition guidelines.[29] Standardised positioning includes arms placed at the sides, legs supported to minimise rotation and instructions to remain still during acquisition. Outputs will include total body fat and lean mass (kg), from which we will calculate lean soft tissue mass index and fat mass index, both in (kg/m^2).[30]

Skeletal age, derived from a DXA hand scan, is selected as a proxy for pubertal status in this study because it avoids the limitations of Tanner staging, which include being intrusive, subject to reporting bias and raising ethical concerns in paediatric cohorts.[31] Participants will be seated with their non-dominant forearm pronated and their hand placed flat on the scanning surface, with fingers slightly separated. The acquisition field includes the distal radius and ulna, carpals, metacarpals and phalanges. Each image will be exported for blinded evaluation by two independent raters, who will estimate skeletal age (years) to the nearest 0.1 year based on carpal and phalangeal ossification patterns, following validated DXA-adapted protocols.[32 33] Discrepancies greater than 0.5 years will be resolved by consensus or adjudication by a third rater. The final skeletal age value will be used to derive the difference between bone age and chronological age, which serves as a proxy indicator of pubertal status in subsequent analyses.

All DXA scans and analyses will be performed by trained personnel using the manufacturer's software, with all scans reviewed for movement artefacts and anatomical misclassification. Scans with movement will be repeated if possible. Scans with movement or other artefacts will be excluded from analyses.

Physical activity will be objectively assessed as minutes of moderate-to-vigorous physical activity using accelerometry with an Actigraph wGT3X-BT (ActiGraph, L.L.C., Pensacola, Florida, USA) worn on the non-dominant wrist for 7 consecutive days, 24 hours a day. The measure will be derived from raw accelerometer files using the open access package GGIR in R, according to standard procedures.[34]

All the abovementioned variables will be included as covariates in subsequent analyses. Table 1 summarises outcomes, control variables, measurement details and quality assurance of the study.

Standardised assessment sequence and pre-test procedures

Participants will arrive at the laboratory in a euhydrated state, having followed standardised pre-test instructions to consume their usual breakfast and a prescribed volume of water (≈5 $mL \cdot kg^{-1}$ body mass) at least 2 hours before testing. To minimise variability in muscle hydration and glycogen-related water shifts, participants will be instructed to follow their regular diet and refrain from carbohydrate loading or any unusual dietary practices in the 24 hours preceding the assessment. They will be further instructed to avoid caffeine, to abstain from vigorous exercise for at least 24 hours (preferably 48 hours) prior to testing, and to maintain their normal sleep schedule the night before. On arrival, adherence to these pre-test conditions will be confirmed by a brief interview.

All assessments will be conducted in a standardised order to minimise fatigue and measurement bias. Participants first undergo anthropometric measurements (height, weight and BMI) followed by whole-body and hand DXA scans. Subsequently, ultrasound imaging of the QF will be performed to capture EI and CSA. After imaging, participants will perform the 30STS test, preceded by a 5 min standardised warm-up on a cycle ergometer at a light-to-moderate intensity corresponding to ~50%–60% of age-predicted maximal heart rate (≈100–130 bpm for this age group). The workload will be adjusted individually to maintain a cadence of 60–70 rpm at this target intensity, ensuring adequate preparation without inducing fatigue. This approach aligns with recommendations from the American College of Sports Medicine, which emphasise light-to-moderate aerobic activity as an appropriate warm-up for children and adolescents prior to strength or functional testing.[35]

Table 1 Summary of outcomes, control variables, measurement details and quality assurance of the study

Domain	Measure	Method	Units	Validity
Primary outcome	EI	Ultrasound	Grayscale 0–255	Construct
Explanatory outcome	CSA_{QF}	Panoramic ultrasound	cm^2	Construct
	Functional muscle quality	MVC/CSA_{QF}	$Nm \cdot cm^{-2}$	Construct
	Physical capacity	30-STS	Repetitions	Convergent
Known-groups	Weight status	Anthropometry	BMI percentile	Known-groups
Control variable	Body composition (LSTM and FMI)	DXA	$kg \cdot m^{-2}$	Covariate
	Skeletal age	DXA	Years (BA–CA)	Covariate
	Physical activity	Accelerometry	MVPA	Covariate
Quality Assurance	Blinding	—	—	—
	Reliability	Intra-rater, inter-rater, test–retest	ICC	—
	Phantom calibration	Daily	—	—

BA, bone age; BMI, body mass index; CA, chronological age; CSA, cross-sectional area; DXA, dual-energy X-ray absorptiometry; EI, echo intensity; FMI, fat mass index; ICC, inter-intra class correlation coefficient; LSTM, lean soft tissue mass index; MVC, maximal voluntary contraction; MVPA, minutes of moderate-to-vigorous physical activity; QF, quadriceps femoris; 30STS, 30 s sit-to-stand test.

Maximal voluntary contraction (MVC) testing will be performed at the end, with a sufficient recovery interval to ensure performance is not limited by prior exertion. This sequential and standardised approach, combined with controlled pre-test conditions, is designed to ensure that imaging, strength and functional outcomes reflect true physiological capacity rather than transient influences of diet, hydration or exercise. Figure 2 illustrates the standardised assessment flow.

To maximise attendance and data completeness, participants will receive reminder calls, flexible scheduling options and brief feedback on their body composition results.

Data analysis and statistical considerations

The reliability of ultrasound-derived EI will be assessed in terms of intra-rater, inter-rater and test–retest agreement. Intra-rater reliability will be evaluated using repeated measurements acquired within the same session by the same operator in the whole study sample. A subsample (n≥20) will be independently assessed by a second trained operator to evaluate inter-rater reliability, and a further subsample (n≥20) will be invited for a second scanning session on a separate day to assess test–retest reliability. Intraclass correlation coefficients will be calculated using a two-way mixed-effects model for intra-rater reliability and a two-way random-effects model for inter-rater reliability, both based on absolute agreement. Complementary metrics will include the SE of measurement, the coefficient of variation and the minimal detectable change at the 95% CI. Bland–Altman plots will be used to visualise inter-rater agreement. Missing or unusable scans and incomplete test data will be handled using a complete-case approach. Excluded cases and reasons for exclusion will be documented and reported transparently.

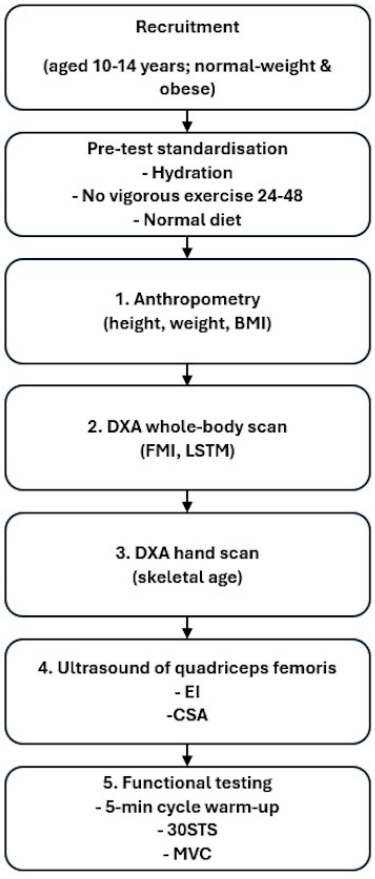

Figure 2 Standardised assessment flow. BMI, body mass index; CSA, cross-sectional area; EI, echo intensity; FMI, fat mass index; LSTM, lean soft tissue mass index; MVC, maximal voluntary contraction; QF, quadriceps femoris; 30STS, 30 s sit-to-stand test.

Reliability results will be presented in accordance with Consensus-based Standards for the selection of health Measurement Instruments guidelines for studies of measurement properties.

Construct validity will be assessed by modelling the association between EI and functional muscle quality, operationalised as maximal voluntary contraction (MVC) of the knee extensors normalised to the CSA of the QF (MVC/CSA). Another functional measure will include the 30STS, which reflects broader functional capacity but is not adjusted for muscle size. EI will be modelled as the dependent variable in a multiple linear regression analysis, with MVC/CSA and the 30STS as predictors, while adjusting for skeletal age, body composition and physical activity. To account for potential non-linearity, polynomial terms or restricted cubic splines will be introduced as needed, based on both visual and statistical evaluations of the model fit. If evidence suggests that the shape or strength of these associations differs across phenotypic subgroups, interaction terms or stratified models will be explored. Model assumptions, including linearity, homoscedasticity and normality of residuals, will be assessed using visual and statistical methods. If assumptions are violated, appropriate transformations or robust regression approaches will be applied. No correction for multiple comparisons will be applied. Secondary analyses will be considered exploratory, and interpretation will focus on effect sizes and CIs, rather than relying solely on p-values.

In addition to assessing associations with functional indicators, the same regression models will allow us to evaluate whether EI is independently associated with demographic and anthropometric variables that are not central to the construct of muscle quality. Specifically, we will examine the contributions of skeletal age, body composition and physical activity in explaining variability in EI, relative to the contributions of MVC/CSA. This approach will help determine whether EI reflects functional muscle quality beyond general developmental characteristics, thereby contributing to both discriminant and incremental validity.

Known-groups validity will be evaluated by testing whether EI differs between groups that are expected to vary in muscle quality. Specifically, we will compare EI values between children classified as obese and those classified as normal weight, adjusting for those who meet and those who do not meet the physical activity recommendations of WHO,[36 37] based on accelerometer-assessed compliance. Group comparisons will be conducted using generalised linear regression models adjusted for skeletal age. These analyses will provide insight into the ability of EI to discriminate between predefined groups with contrasting physiological profiles.

All statistical analyses will be conducted using R (V.4.4.0), using appropriate packages for reliability and regression modelling.

Major confounders

Major confounders in the EI literature stem from inconsistencies in image acquisition and analysis that might complicate comparisons across studies and the establishment of normative data.[9] Key methodological considerations include image depth, participant positioning, probe orientation and EI correction. Standardising image depth has been recommended, as varying depths significantly affect EI values, with recent findings highlighting the heterogeneity of EI at different muscle depths.[25 38] Additionally, participant positioning, such as transitioning from a standing to a supine or lateral recumbent position, significantly affects EI outcomes, underscoring the need for standardised protocols.[39 40] Moreover, subtle methodological factors, such as probe tilt, may influence precision and consistency in data collection. Research indicates that minor probe tilts may alter EI values and reliability, underscoring the importance of engaging a single, skilled assessor to ensure consistency.[41]

To control for these confounders in our study, participants will lie supine for 5 min before scanning to allow for the redistribution and stabilisation of intravascular and interstitial fluids after transitioning from a standing to the testing position. This procedure helps reduce variability in muscle EI caused by acute fluid shifts. To minimise potential variability due to diurnal variation and tissue temperature, testing will be scheduled wherever possible within a consistent 3-hour window and conducted under controlled ambient conditions (~22°C). Probe frequency, image depth, dynamic range, gain and time-gain compensation will be fixed for all scans, and probe pressure will be minimised by applying a thick gel layer and confirming the absence of visible muscle deformation during acquisition.

All devices, including the ultrasound system, DXA and dynamometer, will undergo daily phantom calibration according to the manufacturer's instructions before each session.

Limitations

This study has several limitations that warrant consideration. First, the cross-sectional design, while appropriate for initial validation, limits causal inference and precludes assessment of changes in muscle quality over time or in response to interventions. As such, the sensitivity of EI to developmental trajectories or training-related adaptations remains uncertain. Longitudinal research is necessary to determine whether EI can reliably detect clinically relevant changes in paediatric populations. The study also does not address the long-term clinical relevance or health outcomes associated with variations in EI.

Second, methodological factors may have influenced the reliability of EI measurements. EI is highly sensitive to technical parameters of ultrasonography, including probe tilt, pressure, scanning depth, ROI selection and image gain, as well as the software used for analysis.[9] These findings underscore the importance of consistent operator training, stringent quality control measures

and, ultimately, the development of consensus guidelines to standardise EI acquisition and analysis across studies. In addition, EI remains an indirect marker of muscle quality, reflecting fat and fibrous infiltration, but lacking direct histological confirmation; thus, construct validity relies on functional associations rather than tissue-level evidence. Additionally, it is still plausible that SAT thickness may attenuate ultrasound signals, potentially introducing bias into EI measurements. Future studies should explore paediatric-specific correction procedures to enhance EI precision. Moreover, only the QF will be assessed; although highly relevant to mobility, this single-muscle approach does not capture potential variability across muscle groups. Another methodological limitation concerns skeletal age estimated using a DXA-derived hand scan, which provides an indirect marker of skeletal maturation and, by extension, pubertal status. While DXA-based bone age assessment shows strong agreement with radiographic methods,[33] it reflects skeletal rather than hormonal changes. Hence, residual confounding due to pubertal variability may persist.

Finally, the study sample imposes constraints on generalisability. Recruitment challenges and ethical considerations in paediatric research may limit the sample size and exclude children with certain health conditions, potentially introducing bias. The focus on children aged 10–14 years provides insight into early adolescence; however, the findings may not apply to younger children, older adolescents or clinical populations. Broader age ranges, diverse populations and multiple muscle sites should be considered in future research. Moreover, the findings may not be generalisable to children from different ethnic backgrounds, nutritional environments or healthcare systems, and external validation in multicentre or multinational cohorts is recommended. By acknowledging all these limitations, the present study provides a clear framework for interpreting its findings and highlights priorities for future investigation.

Future directions

If this study demonstrates that EI is a reliable and valid indicator of morphological muscle quality in children, future research will aim to develop age-specific and sex-specific normative reference values using larger, population-representative samples. Such reference values would enable standardised interpretation of individual EI scores, facilitate early identification of low muscle quality and support clinical decision-making. Longitudinal studies will be required to evaluate the predictive validity of EI in relation to future muscle strength, functional capacity and health outcomes. In addition, intervention studies will be needed to assess the responsiveness of EI to changes induced by strength training, physical activity interventions or rehabilitation, thereby determining its utility as a monitoring tool in both healthy and clinical paediatric populations.

Ethics and dissemination

The study will be conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.[42] The protocol was reviewed by the institutional ethics committee of the Faculty of Physical Education and Sport at Charles University (EK 101/2024). Written consent from a parent or legal guardian will be obtained before a child can be included in the study. In addition, verbal consent will be obtained from child participants to ensure they understand the purpose, procedures and potential risks in an age-appropriate and cognitively appropriate manner. Personal and health-related information collected from participants will be kept confidential and secure. Identifiable information will be anonymised to protect the privacy of participants and their families in accordance with data protection regulations.

All procedures will involve a reasonably high level of physical exertion and minimal psychological risk to participants. Any discomfort or distress experienced by the children will be addressed promptly throughout the study. Efforts will be made to ensure that participants are recruited equitably, avoiding any form of discrimination based on gender, socioeconomic status or other factors. The research team will consider the different needs and abilities of children to promote inclusivity, ensure that the findings have potential clinical relevance and utility, and contribute to advancing diagnostic tools for children's health. On completion of the study, the results will be communicated transparently and in an accessible manner to the participants and their families.

By addressing these ethical considerations, the study will uphold the principles of respect for persons, beneficence and justice, ensuring that the research is both scientifically rigorous and ethically sound.

Contributors TJ: guarantor, conceptualisation, writing—original draft preparation, review and editing, and funding acquisition. IK: project administration. TV: methodology, writing—original draft preparation, review and editing. JM: resources. EM: methodology, writing—review and editing. MS: methodology, writing—original draft preparation, review and editing, and funding acquisition. MVN: conceptualisation and supervision. During the preparation of this work the authors used ChatGPT to improve readability and language. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

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