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Relationship between physical fitness, body composition indicators and physical activity components in Spanish schoolchildren

DOCTORAL THESIS BY COMPENDIUM OF PUBLICATIONS

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Relación entre la condición física, indicadores de composición corporal y componentes de la actividad física en escolares españoles

TESIS DOCTORAL POR COMPENDIO DE PUBLICACIONES

Yesenia García Alonso

Abril 2023

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> ***“Our youth should also be educated with***
> ***music and physical education”.***
>
> Aristotle

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List of Abbreviations

AU	Arbitrary Units
BMI	Body Mass Index
CI	Confidence Interval
CRF	Cardiorespiratory fitness
CSBQ	Children's Social Behavior Questionnaire
EI	Echo-intensity
EQ	Equation
HGS	Handrip strength
IMAT	Intramuscular Adipose Tissue
KG	Kilograms
KGF	Kilograms of force
MD	Mean Difference
MVPA	Moderate-to-Vigorous Physical Activity
PA	Physical Activity
RHGS	Relative Handrip strength
SARS-Cov-2	Severe Acute Respiratory Coronavirus 2
SAT	Subcutaneous Adipose Tissue
SB	Sedentary Behaviour
SD	Standard deviation
SE	Standard Error
SES	Socioeconomic status
SLJ	Standing Long Jump
T	Tertil
TPA	Total Physical Activity
US	Ultrasound
VIF	Variance Inflation Factor
WHO	World Health Organization

Declaration and list of publications

This doctoral thesis is a compendium of three articles that have been published in international peer-reviewed journals and one article that is under review for publication.

Yesenia García Alonso, as a PhD student is the author/main author of all the articles and, has been the main collaborator in the conception and design of the studies, acquisition, analysis and interpretation of the data, writing of the articles and final approval for publication.

List of publications

Alonso-Martínez AM, Ramírez-Vélez R, García-Alonso Y, Izquierdo M, García-Hermoso A. Physical Activity, Sedentary Behavior, Sleep and Self-Regulation in Spanish Preschoolers during the COVID-19 Lockdown. Int J Environ Res Public Health. 2021 Jan 15;18(2):693. Doi: http://doi.org/10.3390/ijerph18020693

García-Alonso Y, García-Hermoso A, Izquierdo M, Legarra-Gorgoñon G, Ramírez-Vélez R, Alonso-Martínez AM. Relationship between parents' and children's objectively assessed movement behaviours prior to and during the COVID-19 pandemic. Pediatr Obes. Doi: http://doi.org/10.1111/ijpo.12923

García-Alonso Y, García-Hermoso A, Alonso-Martínez AM, Legarra-Gorgoñon G, Izquierdo M, Ramírez-Vélez R. Associations between physical fitness components with muscle ultrasound parameters in prepuberal children. Int J Obes (Lond). Doi: http://doi.org/10.1038/s41366-022-01066-7

García-Alonso Y, Alonso-Martínez AM, García-Hermoso A, Legarra-Gorgoñon G, Izquierdo M, Ramírez-Vélez R. Reference Values for Muscle Ultrasound Parameters in Children. *Under review in the journal: Frontiers in Nutrition, section Clinical Nutrition.*

Papers presented in congress

Poster: “Asociación entre los componentes de la condición física y la calidad muscular en niños españoles”, VII Simposio EXERNET, 22-23 October 2021, Cuenca, España.

Poster: “Actividad física, sedentarismo y sueño en preescolares antes y durante la pandemia COVID-19: el rol de las familias”, VII Simposio EXERNET, 22-23 October 2021, Cuenca, España.

Declaración y lista de publicaciones

Esta tesis doctoral es un compendio de tres artículos que han sido publicados en revistas internacionales revisadas por pares y un artículo que está bajo revisión para su publicación.

Yesenia García Alonso, como estudiante de doctorado es la autora/autora principal de todos los artículos y ha sido el principal colaborador en la concepción y diseño de los estudios, adquisición, análisis e interpretación de los datos, redacción de los artículos y aprobación final para su publicación.

Listado de publicaciones

Alonso-Martínez AM, Ramírez-Vélez R, García-Alonso Y, Izquierdo M, García-Hermoso A. Physical Activity, Sedentary Behavior, Sleep and Self-Regulation in Spanish Preschoolers during the COVID-19 Lockdown. Int J Environ Res Public Health. 2021 Jan 15;18(2):693. Doi: http://doi.org/10.3390/ijerph18020693

García-Alonso Y, García-Hermoso A, Izquierdo M, Legarra-Gorgoñon G, Ramírez-Vélez R, Alonso-Martínez AM. Relationship between parents' and children's objectively assessed movement behaviours prior to and during the COVID-19 pandemic. Pediatr Obes. Doi: http://doi.org/10.1111/ijpo.12923

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García-Alonso Y, Alonso-Martínez AM, García-Hermoso A, Legarra-Gorgoñon G, Izquierdo M, Ramírez-Vélez R. Reference Values for Muscle Ultrasound Parameters in Children. *Bajo revision en la revista: Frontiers in Nutrition, section Clinical Nutrition.*

Comunicaciones presentadas a congresos

Poster: “Asociación entre los componentes de la condición física y la calidad muscular en niños españoles”, VII Simposio EXERNET, 22-23 octubre de 2021, Cuenca, España.

Poster: “Actividad física, sedentarismo y sueño en preescolares antes y durante la pandemia COVID-19: el rol de las familias”, VII Simposio EXERNET, 22-23 octubre de 2021, Cuenca, España.

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Al Doctor Antonio García-Hermoso, por tu acompañamiento y apoyo durante estos tres años. Por tu soporte bibliográfico y procedimental y sobre todo por tu sentido del humor que ha hecho muchísimo más fácil el día a día en el despacho.

A la Doctora Alicia Mª Alonso-Martínez, sin duda la persona más especial que me ha regalado esta tesis doctoral. Gracias por invitarme a colaborar en 2019 en el Observatorio de Actividad Física, confiar en mí y darme la oportunidad de formarme en el mundo de la investigación. Sin duda has sido la "culpable" de que esta tesis doctoral se haya desarrollado. Gracias por no soltarme de la mano y pese a no compartir despacho todos los días te he sentido muy cerca. Gracias por ser para mí un gran ejemplo como docente, investigadora y lo que es más importante como persona.

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Summary

This doctoral thesis is based on a longitudinal research project of the E-FIT group (Physical Exercise, health, and quality of life) called (in Spanish) "Observatorio de Actividad Física en población escolar https://observatorioactividadfisica.es". This project was financed by the Department of Education of the Government of Navarre (BDNS identification: 448415) and has been carried out from the Public University of Navarre and Navarrabiomed (Spain). The research seeks to know the relationship between the physical condition of the school population in the Early Childhood Education stage and some healthy habits. The objectives of the study were to promote healthy habits, reduce sedentarism, and to promote an increase in the quantity and quality of physical activity (PA) in the child population.

This Doctoral Thesis is composed on four studies that have been published in international scientific journals. The first study (Chapter 1) aimed to examine the effects of the COVID-19 lockdown on PA, sedentary time, sleep and self-regulation; and to determine whether PA and sleep are related to self-regulation problems during the lockdown. In the second study (Chapter 2), the main objective was to compare levels of PA, sedentary behaviour (SB), and sleep time in children prior to and during the COVID-19 pandemic and to determine the association between changes in moderate-to-vigorous PA (MVPA), total PA (TPA), SB, and sleep time between parents with their children. In the third study (Chapter 3), the main purpose was to evaluate the association between physical fitness components with muscle US parameters in prepuberal children. In the last study (Chapter 4), the purpose was to establish age-specific normal ranges of muscle US parameters (i.e., Echo-intensity (EI) , Subcutaneous Adipose Tissue (SAT) and Intramuscular Adipose Tissue (IMAT) values in children aged between four and eleven years of age without underlying metabolic disease.

The most relevant methodology and results are summarized below:

Chapter 1: **Physical Activity, Sedentary Behavior, Sleep and Self-Regulation in Spanish Preschoolers during the COVID-19 Lockdown**

Background: A better understanding of the effects of the lockdown on lifestyle behaviors may help to guide the public health response to COVID-19 at a national level and to update the global strategy to respond COVID-19 pandemic.

Objective: The aim of the study was to examine the effects of the COVID-19 lockdown on device-measured PA, sedentary time, sleep and self-regulation; and to determine whether PA and sleep are related to self-regulation problems during the lockdown.

Methods: PA, sedentary time and sleep were assessed using accelerometry in the week in which the Spanish national state of alarm was declared (n = 21). Parents reported preschooler's self-regulation difficulties (internalizing and externalizing) before (n = 268) and during the lockdown (n = 157) by a validated questionnaire.

Results: Preschoolers showed a decrease in total PA (mean difference [MD] = - 43.3 min per day, 95% CI - 68.1 to - 18.5), sleep efficiency (MD = - 2.09%, 95% CI - 4.12 to - 0.05), an increase in sedentary time (MD = 50.2 min per day, 95% CI 17.1 to 83.3) internalizing (MD = 0.17, 95% CI 0.06 to 0.28) and externalizing (MD = 0.33, 95% CI 0.23 to 0.44) problems. Preschoolers who met the World Health Organization recommendations for PA had lower internalizing scores than non-active peers (MD = - 1.28, 95% CI - 2.53 to - 0.03).

Conclusions: Our findings highlight the importance of meeting PA recommendations to reduce psychosocial difficulties during a lockdown situation.

Chapter 2: Relationship between parents' and children's objectively assessed movement behaviours prior to and during the COVID-19 pandemic

Background: The coronavirus disease 2019 (COVID-19) pandemic could have provoked undesirable harmful effects on movement behaviours among children.

Objective: To compare levels of PA, SB, and sleep time in children prior to and during the COVID-19 pandemic (after the lockdown) and to determine the association between changes in moderate-to-vigorous PA (MVPA), total PA (TPA), SB, and sleep time between mothers and fathers with their children.

Methods: A total of 110 children (aged 4–7 years) and their parents (63 mothers and 52 fathers) wore GENEActiv accelerometers for 6 days (4 weekdays and 2 weekend days) prior to the pandemic and 1 year into the pandemic to assess SB, MVPA, TPA, and sleep time.

Results: Children performed more MVPA on weekdays ($p = 0.002$), had higher SB ($p = 0.001$), and slept fewer hours during the pandemic than before ($p < 0.001$). Likewise, children performed more weekend day MVPA and TPA ($p < 0.001$) during the pandemic and slept less than prior to the pandemic ($p = 0.002$). On weekdays, an increase in mother's MVPA and TPA (categorized as tertiles) was associated with higher increased on MVPA ($p = 0.030$) and TPA in their children ($p = 0.023$), respectively. On weekends, an increase in mother's MVPA was also associated with higher increases in MVPA ($p = 0.011$) in their children.

Conclusion: During the pandemic, children got more MVPA, more SB, and slept fewer hours than before. Changes in PA seem to be associated with mother's behaviours, especially during weekdays.

Chapter 3: **Associations between physical fitness components with muscle ultrasound parameters in prepuberal children**

Background: Muscle US is a convenient technique to visualize normal and pathological muscle tissue as it is non-invasive and real-time. This technique is related to several physical performance parameters and body composition components in adults; however, this relationship remains unexplored in early aged.

Objective: We aimed to evaluate the association between physical fitness components with muscle US parameters in prepuberal children.

Methods: A sample of 282 prepuberal children aged 5–9 years (144 boys) participated in the study. A trained sonographer obtained six B-mode images from femoral rectus for muscle thickness, SAT and area of the muscle of interest, were captured, and muscle US parameters (EI: EI uncorrected, EI correct equations) and intramuscular adipose tissue (IMAT) were extracted. Lean muscle tissue has low EI, whereas intramuscular fat and connective tissue have high EI. Physical fitness components (cardiorespiratory fitness, upper and lower muscle strength, speed-agility, and overall fitness levels) were also evaluated. Children were categorized as fit or unfit for each specific fitness test.

Results: After adjustment for sex and age, higher physical fitness components and overall fitness (z-score) levels were negatively associated with EI, IMAT, and SAT (cardiorespiratory fitness β range = −0.264 to −0.298; upper-muscular strength β range = −0.389 to −0.457; and lower-muscular strength β range = −0.202 to −0.279; and speed-agility β range = −0.257 to −0.302). Children categorized as fit according to four physical fitness components had lower EI uncorrected, EI correct equation 1–2, IMAT, and SAT than unfit children for each respective tests (all Ps < 0.001).

Conclusion: Physical fitness components are inversely associated with EI, IMAT, SAT after adjusting for potential confounders, including sex and age, in prepuberal children.

The present study strengthens the idea that muscle and adiposity parameters is affected by physical fitness even from early childhood.

Chapter 4: Reference values for muscle ultrasound parameters in Spanish children

Background: Quantitative musculoskeletal diagnostic US has been proposed as a clinically viable means of characterizing muscle structure.

Objective: This study aimed to define age-specific normal ranges of EI, subcutaneous adipose thickness (SAT) and IMAT values in typically developing children.

Methods: We recruited 497 children (288 boys and 209 girls) aged 4-11 years (mean age 7.39 years). Muscle US parameters (EI uncorrected, EI corrected equations 1-3, SAT and IMAT) from rectus femoris cross-sectional area by 2D B-mode US were measured. Percentile values and reference curves were computed for each parameter using the Lambda, Mu, and Sigma method (LMS). We then generated smoothed age-specific and sex-specific percentile and curves of EI, SAT and IMAT are expressed by sex and age from 4 to 11 year.

Results: Our results showed an increase in EI uncorrected, and EI corrected equations 1-3 across ages. IMAT increased with age in both sexes, with girls showing a greater increase in this body compartment than boys. In contrast, SAT increases in both sexes, with a more pronounced increase observed in girls.

Conclusion: In summary, this study established the first muscle ultrasound parameters: EI, EI corrected equation 1-3, SAT and IMAT reference curves for childhood and pre-pubescent growth. These curves can be used in association with functional measures to identify body composition abnormalities during growth. Future research should aim to validate these curves and determine their utility in clinical settings.

Resumen

Esta Tesis Doctoral se basa en un proyecto de investigación longitudinal del grupo E-FIT (Ejercicio físico, salud y calidad de vida) denominado "Observatorio de Actividad Física en población escolar https://observatorioactividadfisica.es”. Este proyecto ha sido financiado por el Departamento de Educación del Gobierno de Navarra (BDNS identificación: 448415) y se ha llevado a cabo desde la Universidad Pública de Navarra y Navarrabiomed (España). La investigación busca conocer la relación entre la condición física de la población escolar en la etapa de Educación Infantil y algunos hábitos saludables. Los objetivos del estudio fueron promover hábitos saludables, reducir el sedentarismo y promover un aumento en la cantidad y calidad de la actividad física (AF) en la población infantil.

Esta Tesis Doctoral se compone de cuatro estudios publicados en revistas científicas internacionales. El primer estudio (Capítulo 1) tuvo como objetivo examinar los efectos del confinamiento por COVID-19 sobre la AF, el tiempo sedentario, el sueño y la autorregulación; y determinar si la AF y el sueño están relacionados con problemas de autorregulación durante el confinamiento. En el segundo estudio (Capítulo 2), el objetivo principal fue comparar los niveles de AF, comportamiento sedentario (CS) y tiempo de sueño en niños antes y durante la pandemia COVID-19 y determinar la asociación entre los cambios en la AF moderada- vigorosa (AFMV) y la AF total (AFT), CS, y el tiempo de sueño entre los padres y sus hijos. En el tercer estudio (Capítulo 3), el objetivo principal fue evaluar la asociación entre los componentes de la aptitud física con los parámetros de ultrasonido muscular en niños prepúberes. En el último estudio (Capítulo 4) el propósito fue establecer rangos normales específicos por edad de los valores de EI, TAS y TAI en niños de entre cuatro y once años sin enfermedad metabólica subyacente.

A continuación, se resume la metodología y los resultados más pertinentes:

Capítulo 1: **Actividad física, comportamiento sedentario, sueño y autorregulación en preescolares españoles durante la pandemia del COVID-19**

Antecedentes: Una mejor comprensión de los efectos del confinamiento en los estilos de vida puede ayudar a guiar la respuesta de salud pública sobre el COVID-19 a nivel nacional y actualizar la estrategia global para responder a la pandemia del COVID-19.

Objetivo: El objetivo del estudio fue examinar los efectos del confinamiento por COVID-19 sobre la AF medida por dispositivo (AF), el tiempo sedentario, el sueño y la

autorregulación; y determinar si la AF y el sueño están relacionados con problemas de autorregulación durante el confinamiento.

Métodos: AF, tiempo sedentario y sueño se evaluaron mediante acelerometría en la semana en que se declaró el estado de alarma nacional español (n = 21). Los padres reportaron dificultades de autorregulación de los preescolares (internalización y externalización) antes (n = 268) y durante el confinamiento (n = 157) mediante un cuestionario validado.

Resultados: Los preescolares mostraron una disminución de la AF total (diferencia media [DM] = - 43,3 min por día, IC del 95% - 68,1 a - 18,5), eficiencia del sueño (DM = - 2,09%, IC del 95% - 4,12 a - 0,05), un aumento de conducta sedentaria (DM = 50,2 min por día, IC del 95%: 17,1 a 83,3) (DM = 0,17, IC del 95%: 0,06 a 0,28) y problemas de externalización (DM = 0,33, IC del 95%: 0,23 a 0,44). Los preescolares que cumplieron con las recomendaciones de la Organización Mundial de la Salud para la AF tuvieron puntuaciones internalizantes más bajas que los compañeros no activos (MD = - 1.28, IC 95% - 2.53 a - 0.03).

Conclusiones: Nuestros hallazgos destacan la importancia de cumplir con las recomendaciones de la AF para reducir las dificultades psicosociales durante una situación de confinamiento.

Capítulo 2: **Relación entre el comportamiento de los padres y el de los hijos en los movimientos evaluados objetivamente antes y durante la pandemia COVID-19**

Antecedentes: La pandemia del coronavirus 2019 (COVID-19) podría haber provocado efectos nocivos indeseables en los comportamientos de movimiento de los niños.

Objetivo: Comparar niveles de AF, CS y tiempo de sueño en niños antes y durante la pandemia del COVID-19 (después del confinamiento) y determinar la asociación entre cambios en la AFMV, AFT, CS, y el tiempo de sueño entre madres y padres con sus hijos.

Métodos: Un total de 110 niños (de 4 a 7 años) y sus padres (63 madres y 52 padres) usaron acelerómetros GENEActiv durante 6 días (4 días entre semana y 2 días en fin de semana) antes de la pandemia y 1 año después de la pandemia para evaluar CS, AFMV, AFT y tiempo de sueño.

Resultados: Los niños realizaron más AFMV entre semana (p = 0,002), tuvieron mayor CS (p = 0,001) y durmieron menos horas durante la pandemia que antes (p < 0,001). Asimismo, los niños realizaron más AFMV y AFT durante el fin de semana (p < 0,001) durante la pandemia, y durmieron menos que antes de la pandemia (p = 0,002). En días laborables, un aumento en la AFMV de la madre y la AFT (categorizado como terciles) se asoció con un mayor aumento en el AFMV ($p = 0.030$) y la AFT en sus hijos ($p = 0.023$), respectivamente. Los fines de semana, un aumento en el AFMV de la madre también se asoció con mayores aumentos en el AFMV ($p = 0.011$) en sus hijos.

Conclusión: Durante la pandemia, los niños tuvieron más AFMV, más CS y durmieron menos horas que antes. Los cambios en la AF parecen estar asociados con el comportamiento de la madre, especialmente durante los días laborables.

Capítulo 3: **Asociaciones entre los componentes de la aptitud física con los parámetros de ultrasonido muscular en niños prepúberes**

Antecedentes: El ultrasonido muscular es una técnica conveniente para visualizar el tejido muscular normal y patológico, ya que es no invasivo y en tiempo real. Esta técnica está relacionada con varios parámetros de rendimiento físico y componentes de la composición corporal en adultos; sin embargo, esta relación sigue siendo inexplorada a temprana edad.

Objetivo: Nuestro objetivo fue evaluar la asociación entre los componentes de la aptitud física con los parámetros de ultrasonido muscular en niños prepúberes.

Métodos: Participaron en el estudio 282 niños prepúberes de 5 a 9 años (144 niños). Un ecografista entrenado obtuvo seis imágenes en modo B del recto femoral para calcular el grosor muscular y el tejido adiposo subcutáneo (TAS). El área del músculo de interés fue capturada y se extrajeron los parámetros de ultrasonido muscular (intensidad de eco: eco-intensidad (EI) sin corregir, ecuaciones corregidas de EI y el tejido adiposo intramuscular (TAI). El tejido muscular magro tiene baja EI, mientras que la grasa intramuscular y el tejido conectivo tienen alta EI. También se evaluaron los componentes de la condición física (condición cardiorrespiratoria, fuerza muscular superior e inferior, velocidad-agilidad y niveles de condición física general). Los niños fueron clasificados como sanos o no sanos para cada prueba de condición física específica.

Resultados: Después del ajuste por sexo y edad, los componentes de condición física más altos y los niveles de aptitud general (puntuación-z) se asociaron negativamente con EI, TAS y TAI (rango de aptitud β cardiorrespiratoria = 0.264 a 0.298; rango de fuerza β superior = 0.389 a 0.457; y rango de fuerza β inferior = 0.202 a 0.279; y rango de velocidad-agilidad β = 0.257 a 0.302). Los niños clasificados como sanos de acuerdo con cuatro componentes de condición física tenían una IE inferior no corregida, ecuación corregida 1-2 EI, TAS y TAI que los niños no sanos para cada prueba respectiva (todos Ps < 0.001).

Conclusión: Los componentes de la condición física se asocian inversamente con EI, TAI, TAS después de ajustar por posibles factores de confusión, incluyendo el sexo y la edad, en niños prepúberes. El presente estudio refuerza la idea de que los parámetros musculares y adiposos se ven afectados por la condición física incluso desde la primera infancia.

Capítulo 4: Valores de referencia para los parámetros de ultrasonido muscular en niños españoles

Antecedentes: La ecografía cuantitativa de diagnóstico musculoesquelético (US) se ha propuesto como un medio clínicamente viable para caracterizar la estructura muscular.

Objetivo: Este estudio tuvo como objetivo definir rangos normales específicos por edad de los valores de eco-intensidad (EI), tejido adiposo subcutáneo (TAS) y tejido adiposo intramuscular (TAI) en niños con desarrollo típico.

Métodos: Se reclutaron 497 niños (288 niños y 209 niñas) de 4 a 11 años (edad media 7,39 años). Se midieron los parámetros de US muscular (EI no corregido, ecuaciones EI corregidas 1-3, TAS y TAI) del área transversal del recto femoral por 2D modo-B US. Los valores de los percentiles y las curvas de referencia se calcularon para cada parámetro utilizando el método Lambda, Mu y Sigma (LMS). Después, generamos percentiles y curvas de EI, TAS y TAI específicos para cada edad y sexo, expresados de 4 a 11 años.

Resultados: Nuestros resultados mostraron un aumento en la EI sin corregir, y en la EI corregidas ecuaciones 1-3 en todas las edades. TAI aumentó con la edad en ambos sexos, mostrando en las niñas un mayor aumento en este parámetro que los niños. Por el contrario, el TAS aumenta en ambos sexos, con un aumento más pronunciado en las niñas.

Discusión: En resumen, este estudio estableció los primeros parámetros de US muscular: EI ecuación corregida, EI 1-3, curvas de referencia de TAS y TAI para crecimiento infantil y prepúber. Estas curvas se pueden utilizar en asociación con medidas funcionales para identificar anormalidades de composición corporal durante el crecimiento. Futuras investigaciones deben tener como objetivo validar estas curvas y determinar su utilidad en entornos clínicos.

General introduction

1. Obesity and body composition indicators

The global trend towards obesity has been increasing over the last 50 years[1] (Figure 1) and the prevalence of childhood obesity has increased considerably in the recent years [2]. Childhood obesity is an important public health problem, also in Spain. Data from the 2019 wave indicate that 23.3% of schoolchildren are overweight, and 17.3% are obese[3].

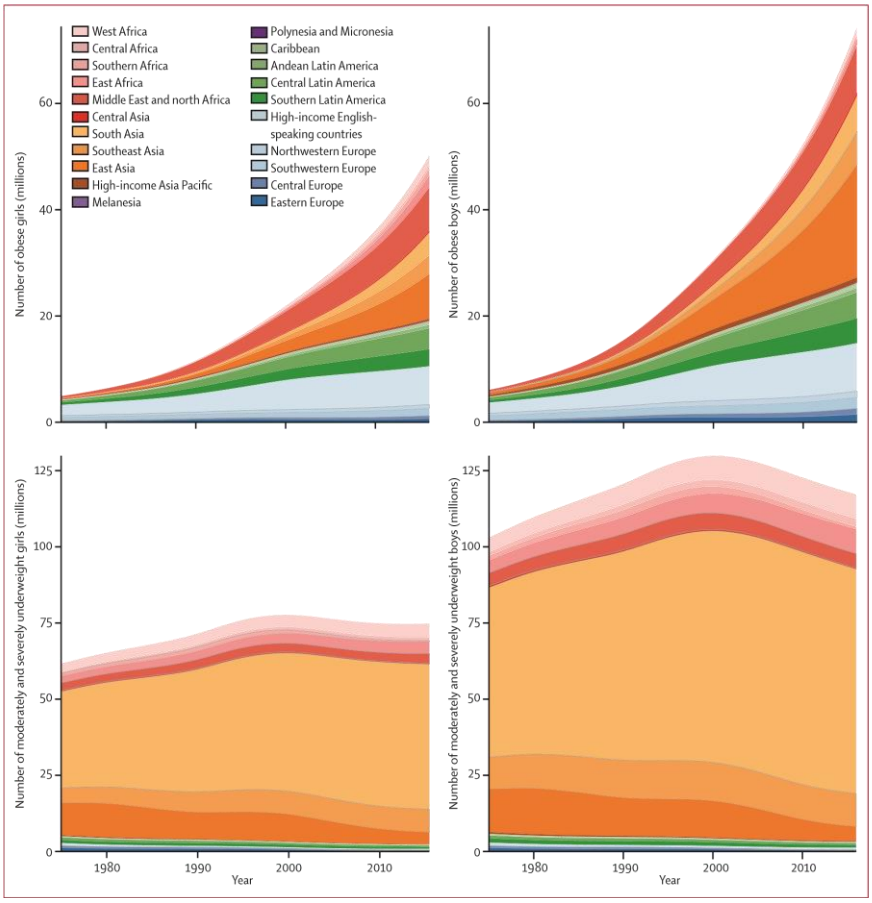
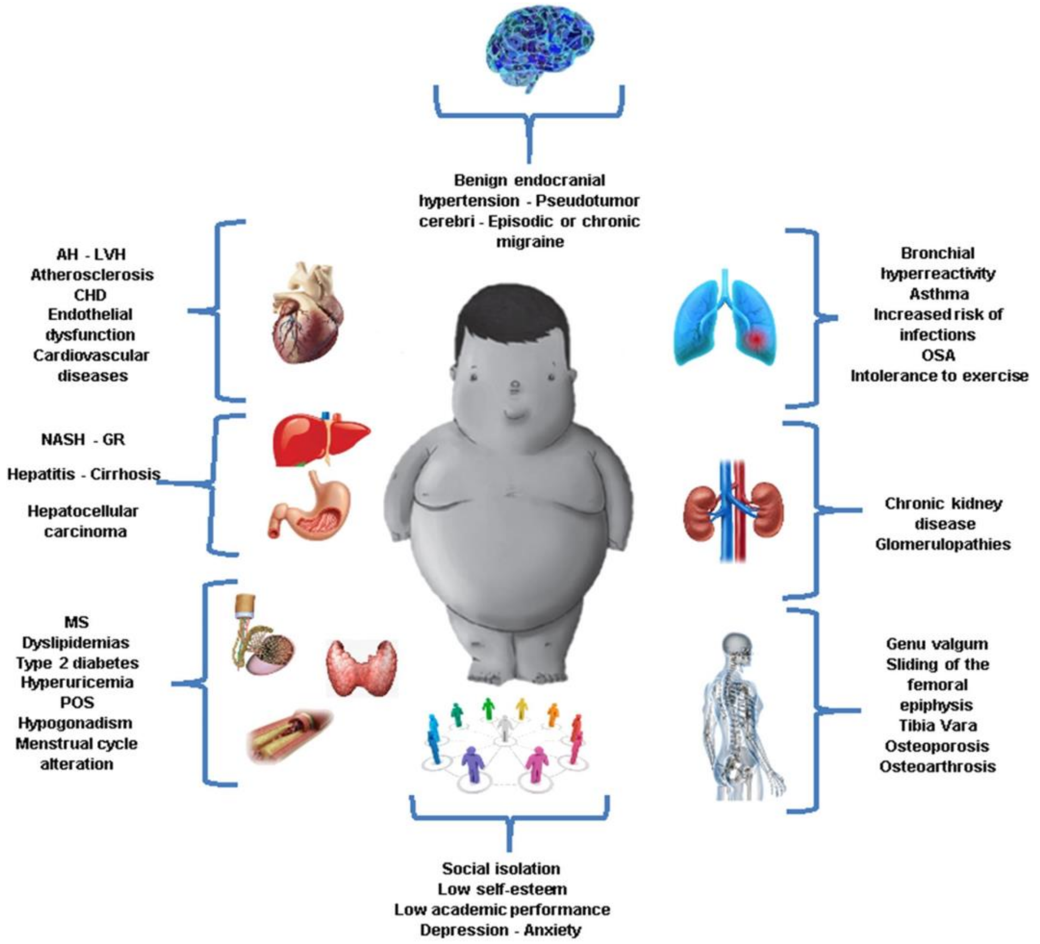


Figure 1. Trends in the number of children and adolescents with obesity and with moderate and severe underweight by region [1]

Obesity is currently considered a global epidemic and its implications in mortality and morbidity in a pediatric patient and in adulthood are increasingly important. Besides, obesity in children present many comorbidities like arterial hypertension, coronary heart disease, gastroesophageal reflux, left ventricle hypertrophy, metabolic syndrome, non-alcoholic hepatic steatosis, obstructive sleep apnea or polycystic ovary syndrome [4-6] (Figure 2).



AH: Arterial hypertension; CHD: Coronary heart disease; GR: Gastroesophageal reflux; LVH: Left ventricle hypertrophy; MS: Metabolic syndrome; NASH: Non-alcoholic hepatic steatosis; OSA: Obstructive Sleep Apnea; POS: Polycystic ovary syndrome.

Figure 2. Comorbidities observed in children and adolescents with obesity [6]

There is a growing body of evidence showing that childhood obesity is a major health problem in children achieving epidemic proportion. Thus, these findings reinforce the importance of assessing anthropometric variables like examine the percentage of total body fat in children and adolescent [7].

Several types of specific adipose tissue depots such as subcutaneous, visceral abdominal and ectopic fat, which includes intramuscular, hepatic, and pancreatic fat fractions, undergo major changes in obesity [8]. Among these different types of adipose tissue, IMAT has a close relationship with increased cardiometabolic risk and type 2 diabetes in children and adolescents [9]. High IMAT levels also impair muscle functionality resulting in decreased muscle mass, function, power, and disability [10].

The presence of IMAT has been linked to impaired strength and physical function in a range of conditions, from injury to aging to metabolic disease [11]. Myosteatosis, which refers to the excessive deposition of fat within muscles, is a form of ectopic fat deposit that results from a positive energy balance and negatively impacts muscle quality [12]. High levels of myosteatosis have also been associated with reduced activation of quadriceps muscles in older adults [13]. This pathological phenomenon not only affects muscle strength and mobility, but also overall survival and prognosis related to underlying diseases [13].

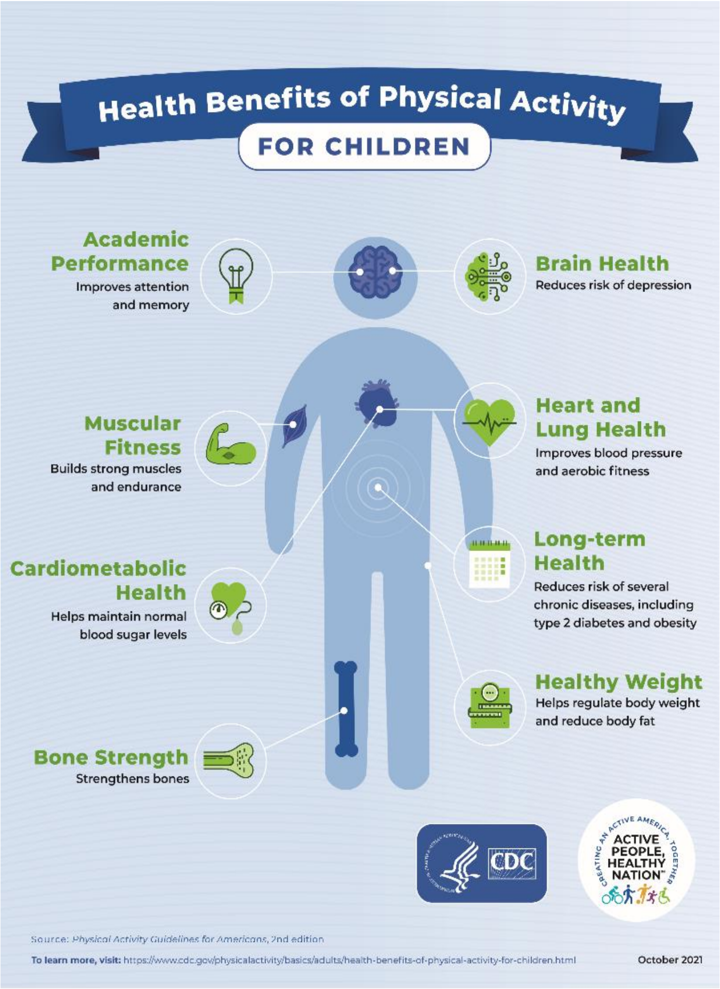
Imaging modalities such as US can detect and analyze the changes in muscle mass associated with aging and the accompanying myosteatosis, which is an increase in IMAT. US has several unique characteristics that are advantageous in routine clinical setting easy availability in the clinic, simultaneous evaluation of arms and legs, non-invasive nature, and no contraindications -and make this modality a promising diagnostic tool on muscle change before it becomes clinically apparent. In this context, EI is being increasingly implemented by investigators in the fields of exercise physiology and rehabilitation science as a measure of skeletal muscle composition or quality, which is assessed using the grayscale of US imaging. For instance, muscle EI can be useful in the clinical and sports context to identify metabolic disorders and to gauge training muscle performance [14]. The ability of EI to identify alterations in muscle composition across the lifespan provides an effective and practical means to assess age-related risks associated with reduced muscle quality [15].

In this context, muscle quality and body mass are important factors in clinical outcomes[16]. Muscle quality can be quantified through different muscle US parameters, including thickness, SAT, IMAT and EI. These parameters can provide insight into glucose metabolism, oxidative damage, protein metabolism, IMAT, capillary density, structural composition, contractility, and fatigability. Muscle quality has been reported to be significantly associated with metabolic health, [17-18] risk of cardiovascular events [19], and overall mortality [20]. Multiple factors, including composition, metabolism, fat infiltration, fibrosis, and neural activation, can influence muscle quality. Poor muscle strength, rather than low muscle mass, has been identified as a major determining factor for functional decline. Obesity and physical inactivity are independent risk factors for poor muscle strength [21]. Núñez et al. reported that a higher percentage of muscle mass and better muscle quality in quadriceps (i.e., lower EI values) are strongly associated with adverse clinical outcomes [21]. Thus, understanding the factors that influence muscle quality and assessing it using US parameters can have important implications for clinical outcomes in children. Given the rapidly growing population at risk in Spain [22] and the strong association between muscle US parameters and conditions such as sarcopenia or pediatric dynapenia [23]assessing muscle quality is critical for disease prevention [24].

2. Physical Fitness

Other powerful marker of physical and mental health in children and adolescents is physical fitness. Evidence from meta-analyses suggest that high cardiorespiratory fitness (CRF) and muscular fitness levels during childhood and adolescence are associated with health benefits such as cognitive and motor development, skeletal health, and positive cardiometabolic and weight-related outcomes later in life [25-26].

Previous studies have reported associations between muscle strength and limb muscle EI in healthy young adults [27-28], and between adipose tissue depots with physical fitness components in youth [28-29] however, this relationship has not been studied in children. Muscle quality is commonly affected with aging by the accumulation of non-contractile tissues in muscle mass, and this condition is reflected on US images with increasing EI values and results in a decrease in physical function [30].



Source: *Physical Activity Guidelines for Americans*, 2nd edition

To learn more, visit: https://www.cdc.gov/physicalactivity/basics/adults/health-benefits-of-physical-activity-for-children.html

October 2021

Figure 3. Heath benefits of PA [35].

PF is a powerful health marker in children, particularly in relation to muscle strength and cardiorespiratory capacity. For example, Steffl et al. showed that weak handgrip strength (HGS) was associated with an increased metabolic risk profile in children [32] and it has been linked to T2D and other cardiometabolic risk factors in older adults [33]. Likewise, in a systematic review and meta-analysis García-Hermoso et al. found changes in cardiorespiratory fitness (CRF) are negatively correlated with body mass index (BMI), skinfold thickness, and obesity later in life [34] . These findings reinforce the importance of assessing PF in children.

3. Physical activity components

For all the above, physical inactivity in children and adolescents is also highly prevalent [35]. World Health Organization (WHO) recommends in its PA and SB guidelines for children aged 3 and 4 years to practice at least 180 minutes of PA of which at least 60 are moderate or vigorous PA; no more than 60 minutes of screen time and 10 to 13 hours of quality sleep [36]. For children 5 years and older, it is recommended to practice at least 60 minutes of MVPA; reduce the screen time and sleep at least 6 hours of quality [36].

According to the Global Matrix 4.0, which highlighted that the global situation regarding the PA of children and adolescents, remains a serious public health concern, with only a small proportion (27%– 33%) meeting the recommended amount of MVPA required for ongoing health and well-being[37]. Conversely, the practice of PA from early ages has many health benefits. PA Guidelines for Americans (2nd edition) [31] [38] (Figure 3) and the WHO [39]concludes benefits in academic performance, muscular fitness, cardiometabolic health, bone strength, brain health, hearth and lung health and healthy weight. Therefore, it is very important to evaluate PA domains objectively.

Health Benefits Associated with Regular Physical Activity in Children and Adolescents
• Improved bone health (ages 3 through 17 years) • Improved weight status (ages 3 through 17 years) • Improved cardiorespiratory and muscular fitness (ages 6 through 17 years) • Improved cardiometabolic health (ages 6 through 17 years) • Improved cognition (ages 6 to 13 years) • Reduced risk of depression (ages 6 to 13 years)

Table 1. Health Benefits Associated with Regular PA in Children and Adolescents (Adapted to Piercy KL et al.) [38]

4. An exceptional situation: the COVID-19 pandemic

PA domains and physical fitness were seriously affected by the coronavirus 2 (SARS-CoV-2, COVID-19) specially when the Government of Spain declared a national state of alarm and stablished a mandatory home “lockdown”. This situation and its subsequent "the new normal" resulted in mass destruction of civil life, relationships and opportunities for movement were greatly affected.

Several studies published about healthy lifestyles during confinement have shown adverse collateral effects of the COVID-19 lockdown on physical health [40] in children. U.S. children performed less PA and engaged in more sedentary behavior during the early COVID-19 period as compared to before the pandemic [41] and several studies have reported serious changes in the mental health of children and adolescents during the COVID-19 quarantine [42]. For example, research carried out in China informed an increase in depressive, anxiety and stress symptoms during the COVID-19 lockdown [42,43].

Furthermore, the COVID-19 pandemic is related to changes in the quantity and nature of PA, SB, and sleep among children and youth [45]. Specifically, there is a growing body of evidence demonstrating the significant impact of the COVID-19 lockdown on these movement behaviours in Spanish preschoolers [45,46]. The COVID-19 pandemic has made children spend more time with their families, especially on weekdays, and the influence of parents on children's PA could be stronger than usual on weekends due to the above-mentioned restrictions. Along this line, several studies have looked for associations of PA between preschoolers and their parents, prior to the pandemic [47,48]. One study indicated that most mothers reported infrequent co-participation in PA with their children[48]. By contrast, Xu et al. [48] found that SB and PA levels of parents can strongly influence those of their preschool children, with maternal influence stronger during the weekdays and paternal influence stronger on the weekends. Although this information is currently not available for the current pandemic, the aforementioned longitudinal survey by Okely et al.[50] highlights the important role parents play in supporting their children to participate in healthy levels of movement behaviours during the pandemic.

The present Doctoral Thesis has two aims. First, evaluate and develop normative values of muscle US parameters and its association with physical fitness. Second, evaluate the impact of COVID-19 on PA and self-regulation in Spanish preschoolers. We hypothesize that intramuscular and subcutaneous fat affects muscle quality and physical condition and the situation derived from the COVID-19 pandemic has negatively affected the diminutions of the PA of children.

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Aims and layouts of the thesis

Chapter 1: Physical Activity, Sedentary Behavior, Sleep and Self-Regulation in Spanish Preschoolers during the COVID-19 Lockdown

Research aim: The aim of the study was to examine the effects of the COVID-19 lockdown on device-measured PA, sedentary time, sleep and self-regulation; and to determine whether PA and sleep are related to self-regulation problems during the lockdown.

Hypothesis: We hypothesized that unfavorable changes in activity behaviors occurred in preschoolers during a non-school lockdown period.

Chapter 2: Relationship between parents' and children's objectively assessed movement behaviours prior to and during the COVID-19 pandemic

Research aim: To compare levels of PA, sedentary SB, and sleep time in children prior to and during the COVID-19 pandemic (after the lockdown) and to determine the association between changes in moderate-to-vigorous PA (MVPA), total PA (TPA), SB, and sleep time between mothers and fathers with their children.

Hypothesis: We hypothesized that children would have less PA and sleep time during the pandemic and that parents would influence their movement behaviour.

Chapter 3: Associations between physical fitness components with muscle ultrasound parameters in prepuberal children

Research aim: We aimed to evaluate the association between physical fitness components with muscle US parameters in prepuberal children.

Hypothesis: We hypothesized that muscle composition is directly related to physical fitness components, and that higher levels of physical fitness are associated with lower EI, IMAT, and SAT in prepuberal children.

Chapter 4: Reference values for muscle ultrasound parameters in Spanish children

Research aim: to establish age-specific normal ranges of EI, SAT and IMAT values in children aged between four and eleven years of age without underlying metabolic disease.

Chapter 1

Physical Activity, Sedentary Behavior, Sleep and Self-Regulation in Spanish Preschoolers during the COVID-19 Lockdown

1. Introduction

The coronavirus 2 (SARS-CoV-2, COVID-19) is a severe acute respiratory syndrome that first emerged in late 2019. Countries have faced challenges with how to handle the crisis in different ways. Specifically, in March 2020, the Government of Spain declared a national state of alarm to curb the spread of the severe acute respiratory syndrome coronavirus 2 and established a mandatory home "lockdown" from 14 March to 26 April.

Accordingly, the coronavirus disease 2019 (COVID-19) pandemic disrupted life for all, with the closure of non-essential businesses and schools. For schoolchildren, this limited the opportunities for movement (i.e., children no longer had access to school-based physical activities such as physical education, recess, and walking to/from school) and social life, disrupting daily schedules and routines. Many children and adolescents were temporarily deprived of institutional, educational environments, social contact with peers and, possibly, adequate cognitive, affective and physical stimuli for their age.

Recent studies published about healthy lifestyles during confinement have shown adverse collateral effects of the COVID-19 lockdown on physical health [1] in children and adolescents. It has been shown that U.S. children performed less physical activity and engaged in more sedentary behavior during the early COVID-19 period as compared to before the pandemic [2]. Another study in Spanish children and adolescents (3 to 16 years old) also reported a reduction in physical activity levels and increased both screen exposure and sleep time [3]. Specifically, these authors suggested that preschoolers (i.e., 3 to 4 years old) reduced their total physical activity (92 min per day) and increased screen time exposure by 2.2 h per day. In contrast, another study showed that during the pandemic Swedish preschooler's physical activity, time spent outside on weekdays and weekend days, and screen time significantly increased [4]. However, it should be noted that in Sweden, preschools, playgrounds, and parks remained open, and children's organized sports and activities continued.

Several studies have reported serious changes in the mental health of children and adolescents during the COVID-19 quarantine [5]. For example, research carried out in China informed an increase in depressive, anxiety and stress symptoms during the COVID-19 lockdown [6, 7]. A recent narrative review analyzed the impact of COVID-19 and lockdown on the mental health of children and adolescents and suggested that young children show more clinginess, poor appetite, inattentiveness, and significant separation problems [8]. In another study published in Spain, when the families described the observed changes in the psychological well-being of children aged three years old, they mentioned greater difficulties in self-regulation [9]. Self-regulation refers to the ability to control one's thoughts, behaviors, emotional reactions, and social interactions, even when impulses and urges run contrary to proximal or distal goals [10] and is recognized as an indicator of positive child development [11].

Regarding sleep patterns, studies have shown inconclusive results in the youth population. Overall, the pandemic seems to significantly disturb normal sleep patterns and nightmares for children [8]. The above-mentioned study among Spanish youth reported different results according to age group, showing an increase of sleep time of 0.6 h per day among adolescents (13 to 16 years old) but a reduction of 0.4 h per day in preschoolers [3]. Pietrobelli et al. also showed an increase of 0.65 h per day of sleep time among obese Italian children [1].

A better understanding of the effects of the lockdown on physical activity, sleep and mental health may help to develop suitable strategies as part of the COVID-19 pandemic responses. In this regard, the compulsory movement restriction meant the prohibition of the movement of children outside their home for many weeks in a row, with no certainty about potentially damaging consequences on their health and well-being. Therefore, the purpose of this study was two-fold: to examine the effects of the COVID-19 lockdown on physical activity, sedentary time, sleep and self-regulation in Spanish preschoolers; and to determine whether device-measured PA and sleep are related to self-regulation difficulties during lockdown.

2. Materials and Methods

2.1. Design and Population Study

The present study was conducted in a cohort of preschoolers aged 4 to 6 years old from three schools in Pamplona (Spain). Data from baseline assessments (from September to December 2019) and from the second evaluation (from March to April 2020) were included in the study. The Ethics Committee of the Public University of Navarra approved the study protocol (PI-020/19). Before the enrollment in the study, all parents or legal guardians were informed about the purpose of the project and signed informed consent.

2.2. Procedures

2.2.1. Physical Activity, Sedentary Behavior and Sleep

Objectively measured physical activity, sedentary time, and sleep were collected using a wrist-worn GENEActiv tri-axial accelerometer attached to the preschooler's non dominant wrist over six consecutive days [12]. Raw data were sampled at 87.5 Hz and then reintegrated into 1-s epochs using the GGIR package in R (version 1.10-7) (R

Foundation for Statistical Computing, Vienna, Austria), which auto-calibrated the recorded accelerometer signals [13]. Total physical activity was defined as total recorded counts/wear time. The accelerometer counts for light, moderate and vigorous physical activity were coded using previously validated specific cut-points for preschool-aged children [14]. To meet the study inclusion criteria, preschoolers must have worn the monitor for 600 min during awake time and an average sleep time 200 min, each of the six days recorded. We used the World Health Organization (WHO) recommendations of physical activity (180 min per day of total physical activity including 60 min per day of moderate to vigorous physical activity) and sleep (10 to 13 h per day) [15] to determine its compliance.

In this study, the van Hees et al. [16] sleep algorithm was used to detect sleep and wake between self-reported bed time and get uptime. This method is based on the variability of the orientation of the accelerometer and classifies each five seconds epoch as either sleep or wake. For the purpose of this study, we used sleep duration (i.e., the difference between sleep onset and offset) and efficiency (i.e., the percent of minutes scored as sleep between onset and offset). This device has been shown to correlate well with polysomnography [16].

For the present purpose, we have used data from 21 children (57.1% boys) who wore the accelerometers in the week in which the state of alarm was declared. For analyses, we used the three days prior to lockdown compared to the three days during the lockdown.

2.2.2. Self-Regulation

We used the child self-regulation and behavior questionnaire (CSBQ), which evaluates subscales of cognitive self-regulation, behavioral self-regulation, and emotional self-regulation, as well as sociability, prosocial behavior, externalizing problems and internalizing problems. Each item requests the respondent to evaluate the general frequency of target behaviors on a scale from 1 (not true) to 5 (certainly true). All subscales contain at least 5 items and have been shown to be reliable in preschoolers [10]. Following recommendations for low-risk and general populations, we employed the two subscale model of the questionnaire [17]: externalizing and internalizing problems. Externalizing behaviors include problems such as attention difficulties, self-regulation deficits, antisocial behaviors, aggression, delinquency, and other "undercontrolled" behaviors. Internalizing behaviors include problems such as social withdrawal, loneliness, sense of inferiority, depression, shyness, anxiety, somatic complaints, and other "overcontrolled" behaviors [18].

For the present study, parents reported preschoolers' psychosocial difficulties before (n = 268, first assessment) and during (n = 157, from March to April 2020) the COVID-19 lockdown using an online questionnaire that included the CSBQ [10]. This scale presents a high level of reliability (all Cronbach's a > 0.80).

2.2.3. Confounders

Potential confounders identified in previous literature were included in the analyses: Maternal education level was recorded by asking mothers about the highest level of education, dichotomized as university education and below. Maternal education is a key predictor of other resources within the family that strongly predict children's wellbeing[19]. Socioeconomic status was measured according to the level of average income per family unit. Socioeconomic factors seem to be lifestyle determinants during the COVID-19 lockdown in children and adolescents [20]. Finally, the students were weighed and measured in light clothing and barefoot. These measurements were used to calculate the BMI (kg/m2), which was computed as the weight (kg) divided by the square of the height (m2). BMI seems to be an important factor related to physical activity levels during the COVID-19 lockdown [1].

2.3. Statistical Analysis

The data are presented as means (standard deviations, SD) or absolute and relative prevalence (n [%]). A test of normality of distribution and equality of variance between groups using the Shapiro–Wilk test and Levene's test were used. All assumptions were met, and an analysis of covariance was employed to evaluate differences before and during the COVID-19 lockdown in physical activity (total and moderate to vigorous physical activity), sedentary time, sleep (duration and efficiency), and self-regulation parameters (externalizing and internalizing scores). Also, we determined differences in self-regulation parameters according to compliance with the WHO recommendations of physical activity and sleep [15]. These analyses were adjusted for age, sex, monthly family income, maternal education, body mass index, and baseline values. Since physical activity and sedentary behavior are codependent, both physical activity parameters (i.e., total and moderate-to vigorous physical activity) were additionally adjusted by sedentary time, as well as for sedentary time by total physical activity. Results were analyzed with SPSS (version 26.0) (SPSS Inc., Chicago IL, USA), and a $p < 0.05$ was considered statistically significant.

3. Results

Table 1 summarizes the characteristics of the sample of preschoolers. There were differences in baseline characteristics between preschoolers that participated or not in the lockdown evaluation only in the number of boys (p = 0.002) and in maternal education (p < 0.001).

Table 1.1. Characteristics of the whole sample of preschoolers participating in the study before the lockdown, and differences in baseline characteristics between preschoolers that participated or not in the lockdown evaluation.

	Whole Sample (n = 268)	**Preschoolers Not Participating in the Lockdown Evaluation (n = 123)**	**Preschoolers Participating in the Lockdown Evaluation (n = 145)**	**p**
Sociodemographic characteristics				
Age, years	4.28 (0.80)	4.27 (0.84)	4.29 (0.76)	0.890
Boys, *n* (%)	143 (53.4)	70 (56.9)	73 (46.5)	0.002
Public school, *n* (%)	28 (10.4)	18 (14.6)	10 (6.9)	0.067
Monthly family income[a], *n* (%)	102 (38.2)	45 (36.6)	58 (40.2)	0.229
Maternal education[b], *n* (%)	146 (43.6)	56 (25.1)	90 (62.1)	<0.001
Anthropometric variables				
Body weight, kg	18.90 (3.33)	19.14 (3.65)	18.64 (2.97)	0.255
Height, cm	107.74 (7.17)	107.88 (7.74)	107.61 (6.57)	0.771
Body mass index, kg/m^2	16.18 (1.50)	16.33 (1.58)	16.03 (1.40)	0.119
Device-measured physical activity				
Total physical activity, minutes per day	361.3 (67.1)	363.9 (69.0)	346.9 (54.6)	0.351
MVPA, minutes per day	89.0 (31.9)	88.5 (32.8)	91.6 (26.7)	0.684
Sedentary time, minutes per day	620.6 (80.3)	622.4 (82.0)	609.6 (69.4)	0.513
Total wear time, hours	152.3 (25.6)	153.3 (26.7)	146.1 (16.6)	0.233
Meeting recommendations[c], *n* (%)	113 (79.0)	95 (77.9)	18 (85.7)	0.131
Device-measured sleep				
Sleep duration, hours per day	9.43 (0.69)	9.42 (0.66)	9.51 (0.74)	0.276
Sleep efficiency, %	84 (0.04)	84 (0.04)	84.3 (4.55)	0.691
Meeting recommendations[d], *n* (%)	29 (20.3)	23 (18.9)	6 (28.6)	0.051
Self-regulation				
Internalizing problems (0–5)	1.99 (0.64)	2.17 (0.69)	1.82 (0.59)	0.112
Externalizing problems (0–5)	2.55 (0.46)	2.60 (0.45)	2.51 (0.48)	0.667

Notes: [a] More or equal than 3000 euros ( ); [b] mother with university studies; [c] _180 min/day of total physical activity including _60 min/day of moderate-to-vigorous physical activity; [d] 10–13 h/day. MVPA, moderate-to-vigorous physical activity.

During the lockdown, preschoolers showed a decrease in total physical activity (mean difference (MD) = -43.3 min per day, 95% confidence interval (CI) -68.1 to -18.5) and sleep efficiency (MD = -2.09%, 95% CI -4.14 to -0.04), and an increase in sedentary time (MD = 50.2 min per day, 95% CI 17.1 to 83.3) and internalizing (MD = 0.17, 95% CI 0.06 to 0.28) and externalizing (MD = 0.33, 95% CI 0.23 to 0.44) problems (Table 2).

Table 1.2. Changes in physical activity, sedentary time, sleep and psychosocial parameters before and during the coronavirus disease 2019 (COVID-19) lockdown in those preschoolers participating in the two evaluations.

	Before the Lockdown	**During the Lockdown**	**Mean Differences (95% CI)**	**p***
Device-measured physical activity (*n* = 21)				
Total physical activity, minutes per day	346.9 (54.6)	303.6 (76.5)	-43.3 (-68.1 to -18.5)	0.002
MVPA, minutes per day	91.6 (26.7)	74.6 (26.0)	-17.0 (-21.7 to -12.4)	<0.001
Sedentary time, minutes per day	609.6 (69.4)	659.8 (116.6)	50.2 (17.1 to 83.3)	0.006
Device-measured sleep (*n* = 21)				
Sleep duration, hours per day	9.51 (0.74)	9.54 (1.30)	0.022 (-0.41 to 0.45)	0.914
Sleep efficiency, %	84.3 (4.55)	82.2 (4.92)	-2.09 (-4.14 to -0.04)	0.047
Self-regulation (*n* = 157)				
Internalizing problems (0–5)	1.82 (0.59)	1.99 (0.68)	0.17 (0.06 to 0.28)	0.003
Externalizing problems (0–5)	2.51 (0.48)	2.85 (0.63)	0.33 (0.23 to 0.44)	<0.001

Notes: CI, confidence interval; MVPA, moderate to vigorous physical activity. * Differences in changes were examined by adjusting for age, sex, monthly family income, maternal education, body mass index, and baseline values. Total and moderate-to-vigorous physical activity were additionally adjusted by sedentary time and sedentary time by total physical activity, respectively.

Preschoolers who met the recommendations for physical activity had lower internalizing scores than non-active peers (MD = -1.28, 95% CI -2.53 to -0.03, p = 0.046), but not for externalizing scores (MD = -0.61, 95% CI -1.96 to 0.74, p = 0.300). Regarding sleep, there were no differences between preschoolers who met or not sleep recommendations (internalizing, MD = -0.03, 95% CI -1.06 to 0.99, p = 0.940; externalizing, MD = 0.06, 95% CI -0.69 to 0.82, p = 0.839).

4. Discussion

The current study explored the effects of the COVID-19 lockdown on physical activity, sedentary behavior and sleep and its relationship with self-regulation difficulties in Spanish preschoolers. Our findings provide evidence of the negative effects of the COVID-19 lockdown on physical activity level, sedentary behavior, sleep quality and self-regulation in Spanish preschoolers. As far as we know, this is the first study that objectively examines the effect of COVID-19 home confinement on these parameters among preschoolers.

Regarding physical activity and sedentary behavior, our study reflects that preschoolers reduced their total physical activity (MD = -43.3 min per day) and increased sedentary time (MD = 50.2 min per day) during the lockdown. Previously published studies are in line with our findings, which found that children had different patterns of activity than what was seen before COVID-19. For example, changes in physical activity and sedentary behavior were reported by parents and legal guardians of children living in the U.S. using an online survey [2]. Among Spanish youth, the lockdown substantially reduced physical activity levels (MD = -102.5 min per week) and increased daily hours of screen time (MD = 2.9 h per day); suggesting that restrictive mobility measures with the closure of schools and high schools had played an important role in these lifestyle behaviors worsening [3]. With higher time spent at home, it can be expected that screen time could reach higher levels than before the COVID-19 lockdown. Therefore, our findings support objectively the hypothesis that unfavorable changes in activity behaviors occurred in preschoolers during a non-school lockdown period. Contrariwise, another study in 100 Swedish preschoolers reported an increased in physical activity and time spent outside on weekdays and weekend days during the lockdown, but also increased screen time used (MD = 30 min per day) [4].

These results could be due to the that preschools, playgrounds, and parks in Sweden remained open and children’s organized sports and activities continued and; therefore, preschools changed their routines to have children outside as much as possible. This study also revealed that active play indoors does not seem to replace active play outdoors, resulting in a net decline in reported play-based activity [4].

Because children and adolescents were experiencing changes regarding their usual daily habits, it also appears reasonable to find different sleep patterns. However, the results of this issue are inconclusive. For example, a recent narrative meta-analysis suggested that the pandemic seems to significantly disturb normal sleep patterns and nightmares for children [8]. The study mentioned above among Spanish youth reported different results according to age group, showing an increase of sleep time of 0.6 h per day among adolescents (13 to 16 years old) but a reduction of 0.4 h per day in preschoolers (3 to 4 years old) [3]. Pietrobelli et al. also showed an increase of 0.65 h per day of sleep time among obese Italian children [1]. Consistent with the above-mentioned review, the present research found that preschoolers slightly reduced their sleep efficiency (i.e., the percent of minutes scored as sleep between onset and offset), but not sleep duration. The changes in daily routines, including the lack of social activities with other children, have probably contributed to the sleep quality impairments [21]. Because sleep is a critical part of health for youths, children and families should adjust their sleep schedules to be well-rested and to have appropriate levels of energy to start their day [22].

Self-regulation is defined as psychological conduct that comprises a series of important competencies, such as the ability to control inner states or responses towards thoughts, attention, emotions or even performance [11]. The present study showed that during the lockdown, preschoolers had an increase in internalizing and externalizing problems.

Behavioral and emotional problems at this age may potentially set a child on a course of maladaptation [18], and more specifically on a pathway to internalizing (i.e., antisocial behaviors) or externalizing problems (i.e., anxious or depressed behaviors). In accordance with the present research, recent studies have suggested that the pandemic situation entailed a substantial impact on mental health [23]. This finding was also reported by Giménez-Dasí et al. [9]. In this study, families reported overall greater difficulties in emotional regulation (i.e., he/she is more irritable, has more mood swings) in their children aged three years old during the six weeks of strict confinement experienced in Madrid, Spain.

Our study also shows that preschoolers who met the recommendations for physical activity had lower internalizing scores than non-active peers. Therefore, we also highlight the importance of meeting physical activity recommendations in the early years, as it seems to influence aspects related to broad areas of mental health [24]. This result may be explained by the fact that increased physical activity was associated with higher mental health among children and adolescents [24]. In this aspect, it also has recently been shown that children who meet the physical activity guidelines have higher life satisfaction, positive affect [25] and self-regulation [26] compared to inactive peers. The current results are supported by another study [27] showing that engaging in physical activity, particularly vigorous physical activity, had a beneficial association with preschoolers internalizing problems one year later. It is plausible that the activity of higher intensities may be associated with neurochemical pathways that underpin psychosocial factors that may lead to fewer emotional problems during childhood [28].

As far as we are aware, this is the first study to examine physical activity and sleep patterns of preschoolers using objective-measures with accelerometers during the COVID-19 lockdown. Despite efforts of objectively examining physical activity and sleep via actigraphy, there are a number of methodological issues that also need to be considered. A first limitation is that it is possible that changes in behavior outcomes occurred in the initial week of the lockdown, as there were serious alterations in daily life and families struggled to adapt to their new reality. However, because the lockdown continued for several weeks, it is possible that preschoolers returned to normalcy, including their routine of sleep and physical activity. Second, the small sample size with accelerometry data and a short time of track are other important limitations. Third, subjects at such age are dependent on parents’ decisions regarding their lifestyle. Finally, the geographic/urban environment could influence physical activity and sleep patterns during the early years [20].

5. Conclusions

In conclusion, recognizing these lifestyle and psychological well-being changes are critical because they may have a lasting impact on preschoolers' physical and mental health and may help guide future interventions, perhaps by physical activity promotion. Therefore, adopting healthy movement behaviors may help to mitigate the negative effects on preschool children of this pandemic and its lockdown.

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Chapter 2

Relationship between parents' and children's objectively assessed movement behaviours prior to and during the COVID-19 pandemic

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Chapter 3

Associations between physical fitness components with muscle ultrasound parameters in prepuberal children

1. Introduction

There is a growing body of evidence showing that childhood obesity is a major health problem in children achieving epidemic proportions [1]. Several types of specific adipose tissue depots such as subcutaneous, visceral abdominal and ectopic fat, which includes intramuscular, hepatic, and pancreatic fat fractions, undergo major changes in obesity [2]. Among these different types of adipose tissue, intramuscular adipose tissue (IMAT) has a close relationship with increased cardiometabolic risk and type 2 diabetes in children and adolescents [3]. High IMAT levels also impair muscle functionality resulting in decreased muscle mass, function, power, and disability [4].

Imaging modalities such as ultrasound (US) can detect and analyze the changes in muscle mass associated with aging and the accompanying myosteatosis, which is an increase in IMAT. US has several unique characteristics that are advantageous in routine clinical setting easy availability in the clinic, simultaneous evaluation of arms and legs, non-invasive nature, and no contraindications -and make this modality a promising diagnostic tool on muscle change before it becomes clinically apparent. In this context, echo intensity (EI) is being increasingly implemented by investigators in the fields of exercise physiology and rehabilitation science as a measure of skeletal muscle composition or quality, which is assessed using the grayscale of US imaging. The technique is economical, easily accessible and highly reproducible, and has been widely used for clinical diagnosis and musculoskeletal tissue research [5–7]. For instance, muscle EI can be useful in the clinical and sports context to identify metabolic disorders and to gauge training muscle performance [8]. The ability of EI to identify alterations in muscle composition across the lifespan provides an effective and practical means to assess age-related risks associated with reduced muscle quality [7].

On the other hand, physical fitness is considered a powerful marker of physical and mental health in children and adolescents [9, 10]. Evidence from meta-analyses suggest that high cardiorespiratory fitness (CRF) and muscular fitness levels during childhood and adolescence are associated with health benefits such as cognitive and motor development, skeletal health, and positive cardiometabolic and weight-related outcomes later in life [9, 10].

Previous studies have reported associations between muscle strength and limb muscle EI in healthy young adults [7, 11], and between adipose tissue depots with physical fitness components in youth [11, 12]; however, this relationship has not been studied in children. Muscle quality is commonly affected with aging by the accumulation of non-contractile tissues in muscle mass, and this condition is reflected on US images with increasing EI values and results in a decrease in physical function [13]. For example, Lee and Arslanian [14] suggested that CRF is associated with lower visceral and abdominal subcutaneous adipose tissue (SAT) in children and adolescents. Other studies suggest physiological relations between IMAT and physical performance [7, 15]. In addition, lipotoxicity is defined as cellular dysfunction resulting from the excessive and ectopic accumulation of lipids in the cell. Both IMAT mass and lipotoxicity have been associated with decreased muscle strength, and functional performance in adults [16, 17]. For these reasons, it could be interesting to explore relations between muscle EI, deposition of adipose tissue, and fitness status.

Given the increasing prevalence of childhood overweight and obesity in some Mediterranean countries such as Spain [18], and the general decline (or stabilization) in physical fitness in recent years [19, 20], it would seem important to explore the link between physical fitness, adipose tissue depots and muscle quality in early childhood. In this context, the present study aimed to evaluate the relationship between physical fitness components (i.e., CRF, muscle fitness, and speed-agility), muscle US parameters, IMAT and SAT in prepuberal children. We hypothesized that muscle composition is directly related to physical fitness components, and that higher levels of physical fitness are associated with lower EI, IMAT, and SAT in prepuberal children.

2. Materials and methods

2.1 Study design and sample

We used a cross-sectional study design from the (in Spanish) "Observatorio de Actividad Física en escolares, https://observatorioactividadfisica.es". This epidemiological study tracks children longitudinally to examine the ways participants physical fitness and changes in physical activity align with healthy lifestyles, mental health, and school performance. A sample of 282 children (144 boys and 138 girls) with mean (95% confidence interval [CI]) age 7.0. Participant recruitment locations were selected across all sites using purposive, non-randomized sampling. Two interested schools (a private school Santa María la Real-Maristas and a state school San Juan de la Cadena), and two sports centers (a private sports center S.C.D.R Anaitasuna and a football club Gazte Berriak C.F) from the Metropolitan Region of Pamplona, Spain. Between April and June 2021, parents/ guardian of children were informed of the study objectives during meetings at the participating schools/sports centers and were invited to review the study protocol. Exclusion criteria included injury/surgery in the last month, and/or any medical limitation/restrictions on physical ability testing. Parent/guardian informed consent and childrens' informed assent was obtained. The study protocol followed the tenets of the Helsinki Declaration and was approved by the Ethics Committee of the Universidad Publica de Navarra (CENEDUCA1/2019).

2.2 Measurements

Data collection staffs had a background in fitness or physical activity assessment, and were subsequently trained by research staff from the coordinating centre (e-FIT UPNA Research Group). A self –report questionnaire was used to assess age and sex. Anthropometric measures (height, weight, and waist circumference) were collected according to the CDC-NHANES Survey protocol [21]. Height was measured in the Frankfurt position using a SECA 213® stadiometer with 1 mm precision. Weight was measured in light clothing and bare feet using a Tanita DC-430MAS® scale with 100 g precision. Waist circumference was measured in centimeters with a Seca 201® non-elastic flexible tape with 1 mm precision. Body mass index (BMI in kg/m2) was subsequently derived and BMI z-scores calculated using age- and sex-specific reference data from the World Health Organization [22]. Waist-to-height ratio was calculated as waist circumference/height.

Physical fitness was measured using the ALPHA Fitness battery [23]. CRF was measured using the Course Navette test. The number of laps was computed, and the test was conducted once. Muscle fitness was evaluated as both upper- and lower-muscular strength. Upper-muscular strength was measured by a handgrip strength (HGS) test. Children performed the test twice using a Takei 5001® analogical dynamometer, which measures isometric grip strength in kilograms of force (kgf) with an accuracy of 100 g. Lower-muscular strength was measured by the standing long jump (SLJ) test, which children performed three times, with the best results recorded. Speed agility was measured by the 4 × 10m shuttle run test. Children did the test twice and the best result (less time) was recorded. To generate the overall physical fitness variable, CRF (Course Navette - laps), relative HGS, SLJ, and speed-agility (4 × 10 test) (in this latter test, longer time indicates poorer performance, and thus the variable expressed in seconds was inverted by multiplying by −1) for the calculation of the overall fitness score (z-score). In addition, children were independently classified as fit or unfit if they achieved a result above (SLJ-fit, and speedagility-fit) or below (SLJ-unfit, and speed-agility-unfit), the sex- and agespecific 20th centile created by Kolimechkov et al. [24] As no reference standards are provided for peak oxygen uptake (VO2peak) and relative HGS in this age group, children were categorized as VO2peak (laps)-fit and relative HGS-fit or VO2peak (laps)-unfit and relative HGS-unfit according to the sex- and age-specific 20th centile [25].

All images were taken from the right side of the participants while supine for the distal third of the body between the anterior superior iliac spine and the patella; in the femoral rectus [26, 27]. The images were recorded with a portable B-mode imaging device (Esaote MyLab™50, Genova, Italy) using a linear array probe of 40mm, scanning depth of 80mm, frequency of 7.5 MHz, and gain of 70 dB, with maximum brightness and contrast. The still images were captured in the sagittal and transverse planes, and then complete images were captured with the panoramic function. Six images were taken for each participant, three transverse and three longitudinal images using clinical water-based gel layer in all of them for acoustic coupling between the transducer and the tissue [28]. The largest visible area of femoral rectus was marked by the polygon tool and the EI was determined by the standard histogram function in ImageJ® software (version 1.53k; National Institutes of Health, Bethesda, MD, USA), with a gray-scale range from 0 (black) to 255 (white). The mean and standard deviation (SD) of each histogram was computed. Using the same image that muscle US and EI were outlined on, SAT was quantified using the straight-line function at three sites (medial, midpoint, lateral) from the skin to the superficial aponeurosis and calculated as the average of the three values [28, 29]. All images were evaluated by the same examiner. At the time of the experiment, the experienced sonographer (Y.G-A) had ~2 years of experience with musculoskeletal sonography in children, adolescents, and the older adults. Representative examples of two different EI are shown in Fig. 1. Panel B (left) shows an image with an EI of 15.4 AU (arbitrary units) and with 0.3mm of SAT. Image B shows an EI of 64.5 AU 21.6mm of SAT depth. Higher EI indicates greater amounts of IMAT (worse muscle quality).

Several equations were designed to correct EI for the SAT using the following formula [29]:

Corrected echo intensity 1: EI + (SAT [cm] x 40.5278) (1)

Corrected echo intensity 2: EI - 5:0054 x adipose tissue^2 [cm] + 38.30836 x adipose tissue [cm] (2)

In addition, the percent IMAT was calculated using the following formula [29]

Girls: [0.062 x (40 x SAT [cm]) + EI] + 7.901 (1)

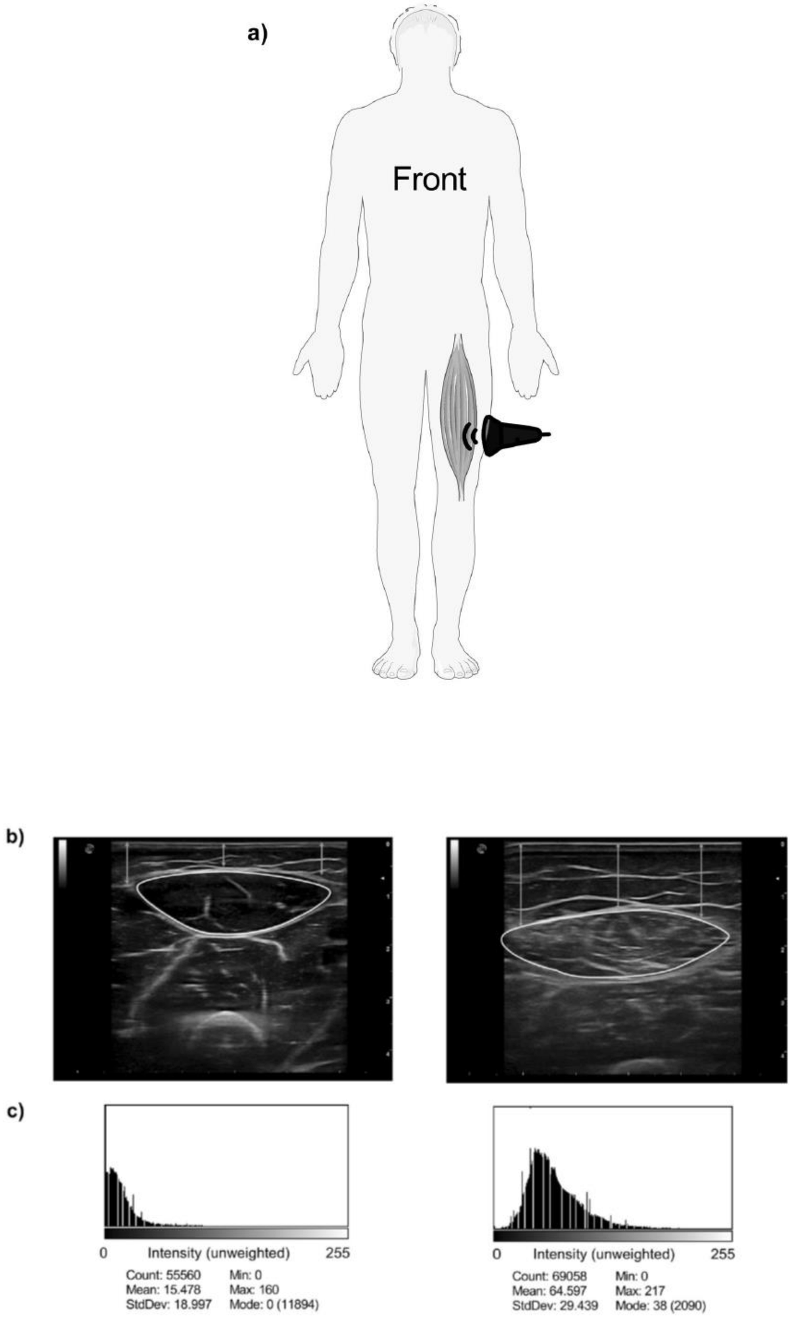
Boys: [0.144 x (40 x SAT [cm]) + EI] + 1.126 (2)

The validity of IMAT and muscle mass measurements using ultrasound has been confirmed in recent studies using magnetic resonance imaging [22, 28, 29].

2.3 Statistical analyses

A post-hoc power analysis was conducted with G*Power 3.1, using an correlation coefficient of 0.20 observed in a previous study [13] that found relations between physical performance and skeletal muscle architecture by N=[(Zα+Zβ)/C]2+3), where Zα=1.9600, Zβ=0.8416, and C=0.5 * ln [(1+r)/(1−r)] =0.2027. This showed that, given α=0.05 and 0.80 power, a sample size of 194 participants. Our final sample was 40% larger, allowing us to detect a slightly smaller effect (r=0.10). Descriptive data from qualitative variables were expressed in relative and absolute frequencies. Quantitative data were described as mean ± SD or mean (95% confidence interval). The Shapiro–Wilk test was used to determine the normality of the distribution. Preliminary analyses showed not significant interactions between sex, ultrasound parameters and physical fitness score (all Ps > 0.1); therefore, all analyses were performed jointly for the entire sample. Linear regression analysis was performed to evaluate the associations of physical fitness (independent variables: CRF z-score, Relative HGS z-score, SLJ z-score, speed agility z-score, and overall fitness z-score) with muscle quality (EI, EI corrected equation 1–2, IMAT), and SAT as continuous dependent variables in z-score due non-normally distributed variables). To monitor for a multicollinearity effect, the variance inflation factor (VIF) was calculated. A model was considered ill conditioned if the VIF was higher than 10. Transformed data as z-score are presented in tables and figures, unless otherwise indicated. Differences in muscle ultrasound parameters (i.e., EI, EI corrected equation 1–2, IMAT, and SAT) between fit and unfit children [for each physical fitness test: VO2peak (laps)- fit vs. VO2peak (laps)-unfit, HGS-fit vs. HGS-unfit, SLJ-fit vs. SLJ-unfit, and Speed agility-fit vs. Speed agility-unfit], were expressed as median and standard error and assessed by analysis of covariance (ANCOVA) adjusted for sex and age. All analyses were performed using the Statistical Package for the Social Sciences (IBM SPSS Statistics 26 version for WINDOWS; SPSS Inc., Chicago), and the level of significance was set at $\alpha \leq 0.05$.

Figure 3.1. Illustrative representation of the experimental model (upper panel). Panel a, b. US image with representation of region of interest of the rectus femoris muscle showing the subcutaneous adipose tissue measured at three points in green (middle panel). Panel c. EI mean value is shown in the grayscale histogram (bottom panel). Examples show of a lower EI (better muscle quality) and a small subcutaneous adipose tissue level (left bottom panel). A big echo intensity (worse muscle quality) and big a subcutaneous adipose tissue as shown in right (bottom panel). The EI was reported in arbitrary units (a.u.) ranging from 0 to 255.



3. Results

The descriptive characteristics of the children by sex and the full sample are shown in Table 1. The final sample included 282 participants (mean ± SD age 7.0 ± 0.4 years). Muscle parameters (EI corrected equation 1–2, IMAT, and SAT) were higher and physical fitness components (CRF in laps, HGS, SLJ, and overall fitness z-score) lower in girls than in boys.

Table 2 shows the association between physical fitness components and muscle quality. After adjustment for sex and age, higher levels of physical fitness components and overall fitness (z-score) levels were negatively associated with higher muscle US parameters (EI uncorrected, EI correct equation 1–2, IMAT, and SAT) (P < 0.001), Table 2.

Figure 2 depicts the differences in muscle ultrasound parameters between children classified as fit or unfit according to physical fitness components. VO^{2}peak (laps)-fit children had significantly lower EI$_{\text{uncorrected}}$ Fig. 2a), EI$_{\text{corrected equation 1}}$ Fig. 2c), EI$_{\text{corrected equation 2}}$, Fig. 2e), SAT (Fig. 2g), and IMAT (Fig. 2i) than their VO2peak (laps)-unfit peers (all Ps < 0.002).

Differences in EI$_{\text{uncorrected}}$, EI$_{\text{corrected equation 1–2}}$, and in IMAT, and SAT between fit and unfit children according to relative HGS are also shown in Fig. 2(b, d, f, h, j). In the same line, children categorized as fit according to both SLJ (all Ps < 0.001, Fig. 3(a, c, e, g, i) and speed-agility tests (b, d, f, h, j) (all Ps < 0.001, Fig. 3 had lower EI$_{\text{uncorrected}}$, EI$_{\text{correct equation 1–2}}$, IMAT, and SAT than unfit children for the respective tests.

Finally, the relationships between muscle ultrasound parameters and overall physical fitness groups (low, medium, and high) are shown in Fig. 4. Significant differences were found between the lower and the higher overall physical fitness (z-score) groups with EI$_{\text{uncorrected}}$, EI $_{\text{corrected equation 1–2}}$, IMAT, and SAT (all Ps < 0.001) levels in children. Similarly, there were significant differences in medium physical fitness (z-score) group and EI $_{\text{uncorrected}}$ (P=0.020), EI $_{\text{corrected equation 1}}$ (P=0.011), EI $_{\text{corrected equation 2}}$ (P=0.011), IMAT (P=0.014), and SAT (*P*=0.035).

Table 3.1. Descriptive characteristics of full sample, boys and girls participating in the study.

Variables	**Full sample (n= 282)**	**Boys (n = 144)**	**Girls (n= 138)**
Anthropometrics parameters			
Age (years)	7.0 (6.8; 7.2)	7.3 (7.0; 7.5)	6.6 (6.4; 6.8)
Height (cm)	122.8 (121.6; 123.9	124.5 (122.8; 126.2)	120.4 (118.9; 121.9
Weight (kg)	25.9 (25.2; 26.6)	26.7 (25.7; 27.7)	24.8 (23.9; 25.8)
Body mass index (kg/m2)	18.0 (17.3; 18.7)	18.0 (17.0; 18.9)	18.0 (16.9; 19.1)
Body mass index (z-score)	0.69 (0.57; 0.80)	0.70 (0.55; 0.85)	0.67 (0.49; 0.85)
Waist circumference (cm)	55.5 (54.2; 56.8)	56.1 (54.5; 57.6)	54.7 (52.6; 56.9)
Waist-to-height ratio	0.45 (0.44; 0.46)	0.45 (0.44; 0.46)	0.45 (0.44; 0.47)
Muscle ultrasound parameters			
EI uncorrected (au)	31.5 (30.3; 32.7)	30.9 (29.3; 32.4)	32.5 (30.6; 34.3)
EI corrected equation 1 (au)	58.5 (56.3; 60.7)	55.5 (52.8; 58.3)	62.6 (59.1; 66.0
EI corrected equation 2 (au)	57.6 (55.5; 59.8)	54.8 (52.0; 57.5)	61.6 (58.2; 65.0)
IMAT (%)	38.3 (37.0; 39.7)	35.5 (33.8; 37.2)	42.2 (40.3; 44.2)
SAT (cm)	6.6 (6.3; 6.9)	6.0 (5.7;6.4)	7.4 (6.9; 7.9)
Physical fitness components			
CRF (laps)	35.5 (33.5; 37.5)	39.2 (36.0; 42.3)	31.6 (29.4; 33.9)
Absolute HGS (kgf)	11.0 (10.6; 11.3)	11.6 (11.0; 12.1)	10.1 (9.7; 10.5)
Relative HGS (kgf/kg)	0.42 (0.42; 0.43)	0.43 (0.42; 0.44)	0.41 (0.40; 0.42)
SLJ (cm)	118.1 (115.8; 120.3)	122.4 (119.1; 125.7)	112.1 (109.5; 114.7)
Speed agility (s)	13.8 (13.6; 14.0)	13.8 (13.5; 14.0)	13.9 (13.6; 14.1)
Overall fitness (z-score)[a]	−0.31 (−0.61; −0.01)	0.01 (−0.44; 0.47)	−0.65 (−1.04; −0.26)

Values are means and 95% CI. Boldfaced values: $P < 0.05$. [a]To account for scale differences between the two fitness tests, the children's fitness scores were standardized for age and sex by conversion to z scores (mean deviation of an individual score/standard deviation) to understand the distribution of the scores in this sample.

CRF cardiorespiratory fitness, *EI* echo-intensity, *IMAT* intramuscular adipose tissue, SAT subcutaneous adipose tissue, *SLJ* standing long jump.

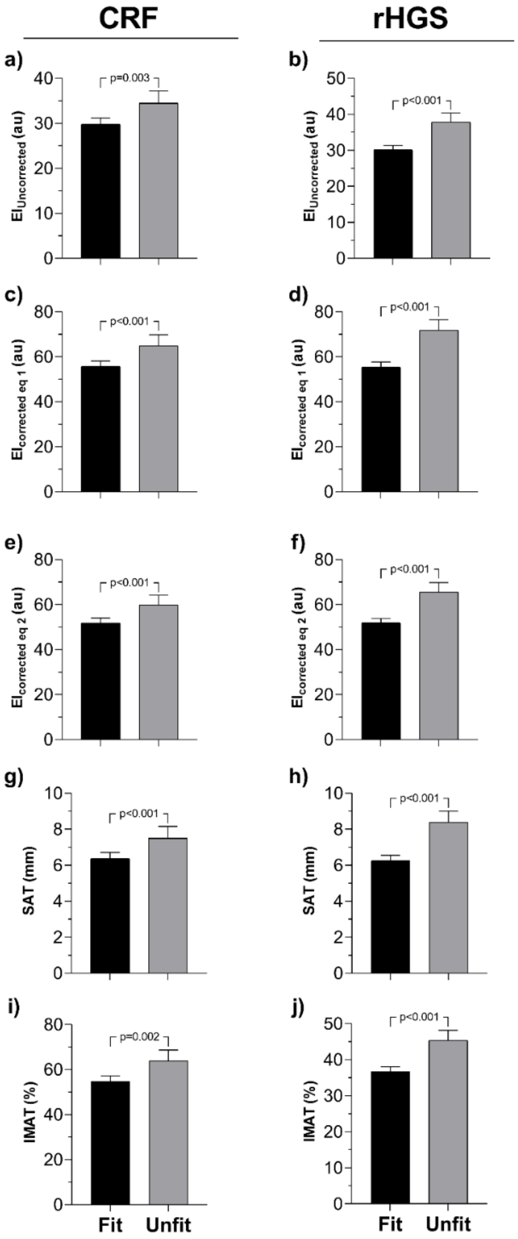
Table 3.2. Associations between physical fitness components and muscle US parameters.

Physical fitness components	**EI $_{\text{uncorrected}}$ (z-score)**		**EI $_{\text{correct equation 1}}$ (z-score)**		**EI $_{\text{correct equation 2}}$ (z-score)**		**IMAT (z-score)**		**SAT (z-score)**	
	β (SD)	P value	β (SD)	P value	β (SD)	P value	β (SD)	P value	β (SD)	P value
CRF (z-score)	−0.264 (0.05)	<0.001	−0.298 (0.05)	<0.001	−0.273 (0.05)	<0.001	−0.298 (0.05)	<0.001	−0.284 (0.06)	<0.001
Relative HGS (z-score)	−0.389 (0.05)	<0.001	−0.457 (0.05	<0.001	−0.416 (0.05)	<0.001	−0.456 (0.05)	<0.001	−0.421 (0.05)	<0.001
SLJ (z-score)	−0.202 (0.04)	<0.001	−0.261 (0.04	<0.001	−0.279 (0.03)	<0.001	−0.261 (0.04)	<0.001	−0.224 (0.04)	<0.001
Speed agility (z-score)	−0.257 (0.06)	<0.001	−0.302 (0.06)	<0.001	−0.277 (0.05)	<0.001	−0.301 (0.06)	<0.001	−0.278 (0.06)	<0.001
Overall fitness (z-score)	−0.820 (0.13)	<0.001	−0.922 (0.14)	<0.001	−0.789 (0.13)	<0.001	−0.922 (0.14)	<0.001	−0.885 (0.14)	<0.001

Values are expressed as unstandardized coefficients (β) and standard deviation (SD). Analysis was adjusted for sex and age.

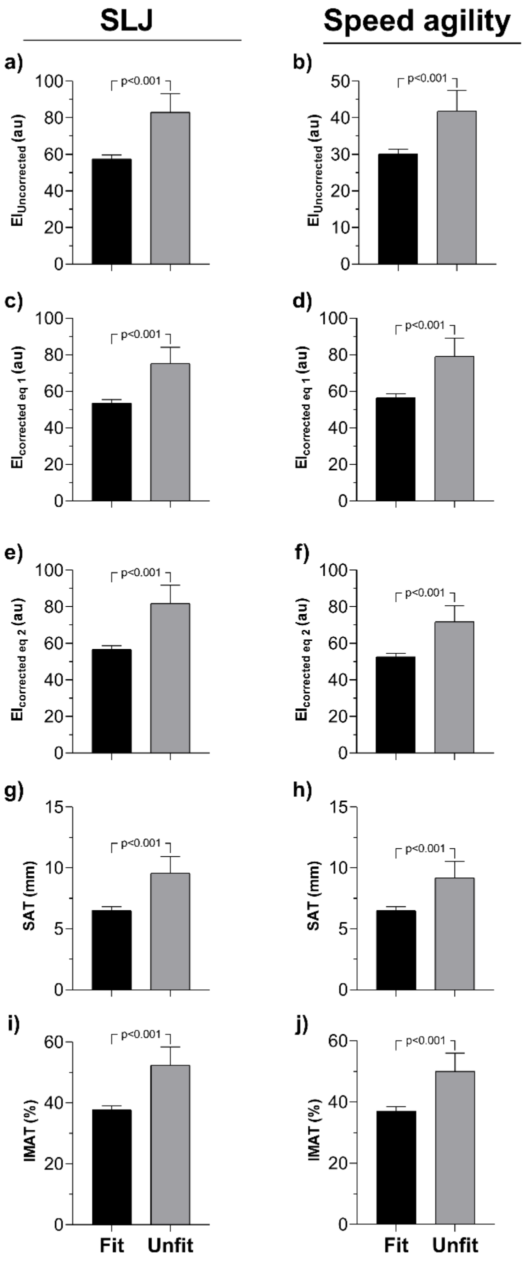
CRF cardiorespiratory fitness, *EI* echo-intensity, *IMAT* intramuscular adipose tissue, SAT subcutaneous adipose tissue, *SLJ* standing long jump.

Figure 3.2. Differences in muscle quality and fat fractions of echointensity uncorrected, echo-intensity corrected equation 1–2, IMAT, and SAT of VO2peak(laps)-fit vs. VO2peak(laps)-unfit and relative HGS-fit vs. relative HGS-unfit prepuberal children. Values are expressed as median and standard error.



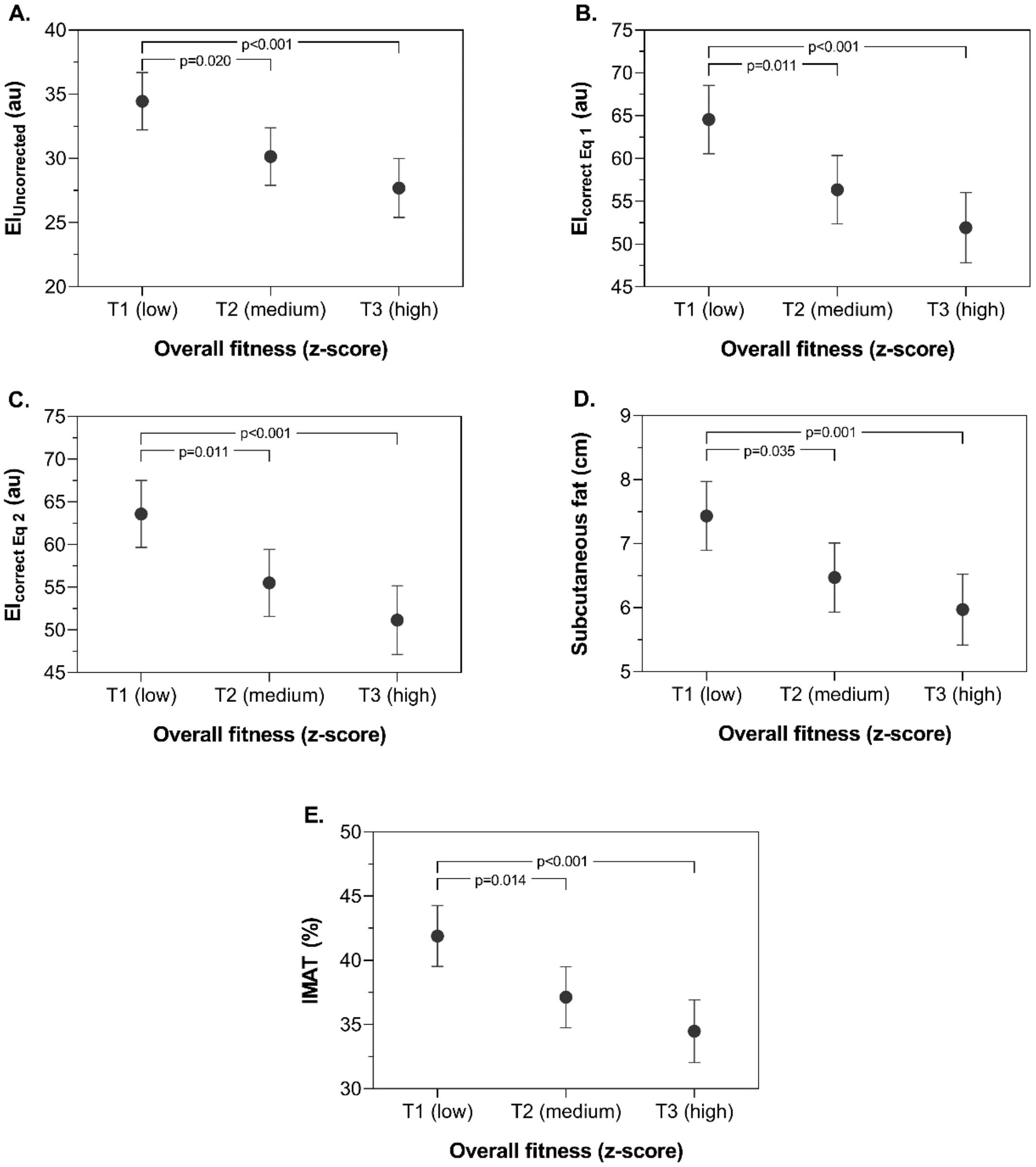
Differences in pooled raw data obtained from sonograms uncorrected (a, b); pooled raw data obtained from sonograms with correction factor 1 (Equation 1) (c, d); pooled raw data obtained from sonograms with correction factor 2 (Equation 2) (e, f); pooled raw data obtained from sonograms for subcutaneous adipose tissue (g, h); and pooled raw data obtained from sonograms for the intramuscular adipose tissue "proxy" estimation (I, j). As no reference standards are provided for peak oxygen uptake (VO2peak) and relative HGS in this age group, children were categorized as VO2peak (laps)-fit and relative HGS-fit or VO2peak (laps)-unfit and relative HGS-unfit according to the sexand age-specific 20th centile [25]. ANCOVA analysis was adjusted for sex and age.

Figure 3.3. Differences in muscle quality and fat fractions of echointensity uncorrected, echo-intensity corrected equation 1–2, IMAT, and SAT of SLJ-fit vs. SBJ-unfit and speed-agility-fit vs. speed-agility-unfit prepuberal children.



Values are expressed as median and standard error. Differences in pooled raw data obtained from sonograms uncorrected (a, b); pooled raw data obtained from sonograms with correction factor 1 (Equation 1) (c, d); pooled raw data obtained from sonograms with correction factor 2 (Equation 2) (e, f); pooled raw data obtained from sonograms for subcutaneous adipose tissue (g, h); and pooled raw data obtained from sonograms to the intramuscular adipose tissue “proxy” estimation (I, j). Children were independently classified as fit or unfit if they achieved a result above (SLJ-fit, and speed-agility-fit) or below (SBJ-unfit, and speedagility- unfit), the sex-and age-specific 20th centile according by Kolimechkov et al. [24]. ANCOVA analysis was adjusted for sex and age.

Figure 3.4. Associations between overall physical fitness (z-score) with muscle quality parameters and SAT in prepuberal children.



Differences in pooled raw data obtained from sonograms uncorrected (a); pooled raw data obtained from sonograms with correction factor 1 (Equation 1) (b); pooled raw data obtained from sonograms with correction factor 2 (Equation 2) (c); pooled raw data obtained from sonograms for subcutaneous fat thickness (d); and pooled raw data obtained from sonograms to the intramuscular adipose tissue "proxy" estimation (e). ANCOVA analysis was adjusted for sex and age.

4. Discussion

The main finding of this study is that physical fitness components are inversely associated with EI, IMAT, and SAT after adjusting for potential confounders, including sex and age, in prepuberal children. Despite the observational nature of the study, our findings contribute to the current knowledge by suggesting that physical fitness components have a beneficial influence on muscle US parameters, even from early childhood. To our knowledge, the present study advances the limited architecture muscle and overall physical fitness literature among prepuberal children.

Ultrasonography appears to be an effective technique to analyze muscle architecture and quality in a non-invasive and safe manner. EI, SAT, and IMAT have been considered strong predictors of functional and clinical outcomes [30] and, accordingly, critical methodological issues have been addressed to improve the EI interpretation, such as the control of image depth, rest duration and participant position, probe tilt, and correct EI values for SAT [31]. Given that the composition of a muscle contributes to its function, it would be expected that the EI metric would associate with functional performance. There is limited information regarding the magnitude of the relationships between EI and physical fitness in young populations. For example, a recent study showed significant correlations between muscle thickness and EI in the vastus lateralis and rectus femoris and several measurements of athletic performance and isometric strength in 12-year-old children [32]. In the similar context, García et al. [33] examined the relationship between quantitative ultrasound of the quadriceps and the vertical jump in schoolage children and found correlations between the EI and the percentage of muscle fat of the components evaluated of the quadriceps. These findings provide a plausible support for the conceptual premise that physical fitness favors higher muscle quality from early childhood.

With respect to adipose tissue depots, inverse associations VO2peak with visceral adiposity tissue [12] and with abdominal SAT [11] have been reported in children and adolescent populations. Although there is no available evidence on the influence of physical fitness on IMAT in prepuberal children, a previous study performed in adults with metabolic disorders [34] reported that CRF (treadmill duration) was lower in participants in the highest quartile of IMAT (evaluated by abdominal computed tomography). Because increased IMAT has been found to be associated with functional decline and metabolic disorders [35], it is important to promote physical exercise, which has been shown to be beneficial in reducing IMAT [16]. Regarding the EI of the muscle, there is an increase in intramuscular connective tissue, IMAT and a decrease in the number of capillaries in aging process [36, 37]. These changes will contribute to greater isolation of each capillary from the adjacent muscle fiber and reduced blood supply to muscle fibers [37], which may explain the possible increase in muscle EI in children with lower physical fitness levels. Although the relationship of physical fitness which is involved in muscle quality, has been proposed as possible explanation of the positive effects of metabolism on ectopic fat, its undelaying mechanisms remain to be identified [38]. On the contrary, the role of high physical activity/fitness levels, and the improvement of glucose and lipid metabolism are some of the mechanisms proposed for the benefits of skeletal, and other ectopic fat depots [39]. According to Radaelli et al. [40], physical activity decreases muscle EI, indicating a greater amount of contractile tissue. Studies reveal age-related changes in musculoskeletal composition, such as an increase in IMAT, replacing contractile tissue, and reducing muscle mass, leading to an increase in the EI of the muscles of the upper and lower extremities [41].

Although we do not have an extensive literature with which to compare our results, it would appear that all the findings in the different published populations go in the same direction. Thus, it would be safe to conclude that better physical fitness levels are inversely associated with EI, SAT, and IMAT in healthy children. The present study strengthens the idea that promotion of physical fitness from early childhood is important for muscle quality [33]. Accordingly, it would seem to be important to achieve the recommendations of the World Health Organization [42] about physical activity, sedentary behavior, physical fitness components and screen time to prevent muscle deterioration. Although studies with larger sample sizes will be required to further understand the role of physical fitness in changes SAT, IMAT, or EI parameters, our results indicate that US is a valuable tool for evaluating muscle composition as it relates to a prepuberal children's fitness and health status.

The present study has some limitations. First, due to the crosssectional nature of the study, our ability to establish causal relationships is limited. Second, our study only examined the muscle quality of a single muscle group and, therefore, further research is required to confirm our findings in other muscle groups. Third, the selection of specific cut-off points to determine fit and unfit levels could affect the overall results. We selected recommendation of Tomkinson et al. [25]. Fourth, it is important to note that the results of this study are specific to the US device and the calibration equations being used. As manufacturer settings differ among ultrasound devices, applying the calibration equations with muscle EI and IMAT obtained with other ultrasound devices can result in inaccurate estimation of ectopic fat. Thus, care should be taken when applying the calibration equations used in this study. Lastly, although the present sample of participants varied in their sex, anthropometry, lifestyles status, and socioeconomical status, they were all prepuberal children free of disease, illness, and injury. Future studies are encouraged to identify muscle quality parameters across a longitudinal observational (i.e., physical activity levels or domains, trajectories from childhood into midlife, health status, and/or nutritional status) to identify muscle quality abnormalities and target subsequent intervention and monitor physical fitness/body composition progress. Nevertheless, these results should be interpreted with caution. In contrast, the study has some strength point like safety, portability, and low cost. Besides it is an ideal examination technique for evaluation of muscle quality parameters and for its application in children.

5. Conclusion

In summary, the main finding in this study is that physical fitness components are related to better muscle US parameters in prepuberal children. Given the fact that the origins of some cardiometabolic disorders can be found from early life, and physical fitness tracks from childhood to adulthood [43], it would be important to improve muscle architecture during childhood to reduce ectopic fat, which could mitigate cardiometabolic risk during adulthood [44]. Therefore, developing health and educational policies that encourage global physical fitness during childhood should be a priority.

Author contributions: AGH, RRV, and MI researched and analyzed data, and wrote the paper. YGA, GLG, and AMAM researched data and revised the paper. All authors approved the final paper. YGA and AMAM take primary responsibility for the integrity of the data and the accuracy of the data analysis.

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Chapter 4

Reference Values for Muscle Ultrasound Parameters in Children

1. Introduction

Ultrasound (US) has been favored as a first-line imaging modality for children due to its noninvasive nature, low-cost, and easily accessible modality[1]. The term muscle quality—despite its ambiguity—has emerged as a useful construct that allows researchers to explore facets skeletal muscle function deficits beyond age-related declines of lean body mass[1]. In this context, muscle quality and body mass are important factors in clinical outcomes[2]. While muscle size plays a role in strength and physical functioning, physiological adaptations that are separate from skeletal muscle size can occur in response to strength training and chronic disuse[3].

Muscle quality can be quantified through different muscle US parameters, including thickness, subcutaneous adipose thickness (SAT), intramuscular adipose tissue (IMAT) and echo-intensity (EI). These parameters can provide insight into glucose metabolism, oxidative damage, protein metabolism, intramuscular adipose tissue, capillary density, structural composition, contractility, and fatigability. Muscle quality has been reported to be significantly associated with metabolic health[4-5], risk of cardiovascular events[6], and overall mortality[7]. Multiple factors, including composition, metabolism, fat infiltration, fibrosis, and neural activation, can influence muscle quality. Poor muscle strength, rather than low muscle mass, has been identified as a major determining factor for functional decline. Obesity and physical inactivity are independent risk factors for poor muscle strength[8]. Núñez et al. reported that a higher percentage of muscle mass and better muscle quality in quadriceps (i.e., lower EI values) are strongly associated with adverse clinical outcomes[9]. Thus, understanding the factors that influence muscle quality and assessing it using US parameters can have important implications for clinical outcomes in children.

The presence of intramuscular adipose tissue (IMAT) has been linked to impaired strength and physical function in a range of conditions, from injury to aging to metabolic disease[10]. Myosteatosis, which refers to the excessive deposition of fat within muscles, is a form of ectopic fat deposit that results from a positive energy balance and negatively impacts muscle quality[11]. High levels of myosteatosis have also been associated with reduced activation of quadriceps muscles in older adults [12]. This pathological phenomenon not only affects muscle strength and mobility, but also overall survival and prognosis related to underlying diseases[12].

Given the rapidly growing population at risk in Spain[13] and the strong association between muscle US parameters and conditions such as sarcopenia or pediatric dynapenia[14], assessing muscle quality is critical for disease prevention [15]. For instance, García-Alonso et al.[16] observed a relationship between physical fitness components and muscle ultrasound parameters (EI, EI corrected equation 1-3, SAT, and IMAT) in prepubertal children's rectus femoris muscle. With regard to subcutaneous adipose tissue (SAT), regarding SAT, Chmid-Zalaudek et al.[17] studied SAT measured via ultrasound in children and adolescents demonstrated that those with excess adiposity, as determined by DXA (%body fat), had significantly higher levels of cardio-metabolic risk factors. In this context, quantitative musculoskeletal diagnostic US has been proposed as a viable method for characterizing muscle structure[18]. Reference data is necessary, as a first step, to identify individuals with low muscle quality and/or high SAT and IMAT across the age spectrum. While use of the US device has become routine practice in adults, the question remains of whether both the technique and the diagnostic cutoff values for adults can be applied to youth. Moreover, published muscle quality data from healthy youth remain scarce[19]. Nevertheless, population-specific data are valuable in reducing the risk of misclassifying the muscle quality phenotype among children, as sociodemographic, genetic, and lifestyle factors influence body composition.

Accordingly, the purpose of the present study was to establish age-specific normal ranges of EI, SAT and IMAT values in children aged between four and eleven years of age without underlying metabolic disease. These reference values may assist in identifying target populations for primary prevention and guiding population health programs, policies and priorities.

2. Materials and methods

2.1 Study design and simple

Using a cross-sectional study design from the “Observatorio de Actividad Física en escolares, https://observatorioactividadfisica.es”, we examined muscle US parameters in Spanish children. The sample included 497 children aged 4-11 (288 boys and 209 girls, mean age 7.39). Participants were enrolled from four interested schools (a private school, Santa María la Real-Maristas; and three state schools, San Juan de la Cadena, El Lago de Mendillorri and Garcia Galdeano), two sports centers (a private sports center, S.C.D.R Anaitasuna; and a football club, Gazte Berriak C. F) and a health center (C.S Iturrama) from the Metropolitan Region of Pamplona, Spain. This sample of the population was chosen due to the lack of muscle US parameters studies (independently of anthropometric values). Parents/guardians of children were informed of the study objectives during meetings and were invited to review the study protocol. Exclusion criteria included injury/surgery in the last month and/or any medical limitation/restrictions on physical ability testing. Informed consent was obtained from the parents/guardians and the children. Evaluations took place from December 2021 to June 2022. The study protocol was completed in accordance with the Helsinki Declaration and was approved by the Ethics Committee of the Universidad Pública de Navarra (CENEDUCA1/2019).

2.2 Measurements

The data collection staff had a background in physical fitness and physical activity assessment and were trained by research staff from the coordinating center (e-FIT UPNA Research Group). Age and sex were assessed using a self-report questionnaire. Anthropometric measures (height, weight, and waist circumference) were collected following the CDC-NHANES survey protocol[20]. Height was measured in the Frankfurt position using a SECA 213® stadiometer with 1-mm precision. Body mass was measured in light clothing and bare feet using a Tanita DC-430MAS® scale with 100 g precision. Waist circumference was measured in centimeters with a Seca 201® nonelastic flexible tape with 1-mm precision. Body mass index (BMI in kg/m^2) was subsequently derived, and BMI z-scores were calculated using age- and sex-specific reference data from the World Health Organization[21]. The waist-to-height ratio was calculated as waist circumference/height.

In terms of cross-sectional US imaging, all images were captured from the right side of the participants while they were in a supine position, covering the distal third of the body between the anterior superior iliac spine and the patella in the femoral rectus muscle[22-23]. The images were taken with a portable B-mode imaging device (Esaote MyLab™50, Genova, Italy) using a linear array probe of 40 mm, scanning depth of 80 mm, frequency of 7.5 MHz, and gain of 70 dB, with maximum brightness and contrast. The still images were captured in the sagittal and transverse planes, and then complete images were captured with the panoramic function. For each participant, six images were taken, three transverse and three longitudinal images, using a clinical water-based gel layer in all of them for acoustic coupling between the transducer and the tissue[24]. The polygon tool was used to mark the largest visible area of the femoral rectus, and the EI was determined by the standard histogram function in ImageJ® software (version 1.53k; National Institutes of Health, Bethesda, MD, USA), with a grayscale range from 0 (black) to 255 (white). The mean and standard deviation (SD) of each histogram were computed. Using the same image that muscle US and EI were outlined on, SAT was quantified using the straight-line function at three sites (medial, midpoint, lateral) from the skin to the superficial aponeurosis and calculated as the average of the three values[24-25]. The same examiner evaluated all images. At the time of the experiment, the experienced sonographer (Y.G-A) had ~3 years of experience with musculoskeletal sonography in children, adolescents, and older adults.

Several equations were designed to correct EI for the SAT using the following formula[25]:

(i) Corrected echo intensity 1: EI + (SAT [cm] x 40.5278)
(ii) Corrected echo intensity 2: EI – 5.0054 x adipose tissue^2 [cm] + 38.30836 x adipose tissue[cm]
(iii) Corrected echo intensity 3: EI + 39.2297 x adipose tissue [cm]

A correction factor has been used to correct the influence of SAT in EI [25].

In addition, the percent IMAT was calculated using the following formula[25]:

(i) Girls: [0.062 x (40 x SAT [cm]) + EI] + 7.901
(ii) Boys: [0.144 x (40 x SAT [cm]) + EI] + 1.126

The validity of IMAT and muscle mass measurements using ultrasound has been confirmed in recent studies in adult population using magnetic resonance imaging [27-25]. IMAT is not a direct measurement, but is a variable that is calculated from the EI and SAT.

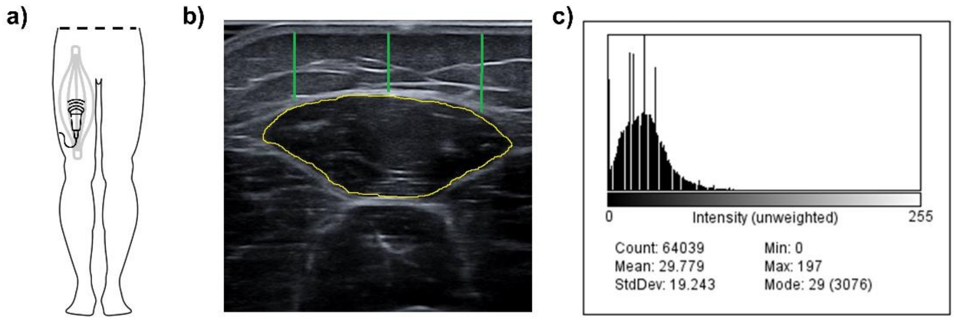

Figure 4.1. Illustrative representation of the experimental model. Figure 1a represents the area where the ultrasound is performed (rectus femoris of quadriceps). Figure 1b represents the selection of the area of interest (yellow) and SAT (green). Figure 1c represents the histogram. The EI was reported in arbitrary units (a.u.) ranging from 0 to 255.

2.3 Statistical analyses

Outlier analysis was performed to verify that all values were within a physiologically possible range. Smoothed age-specific and sex- specific percentiles and curves were developed by Cole and Green[28]. The least mean squares (LMS) technique estimates 3 parameters: median (M), coefficient of variation (S), and power in the Box–Cox transformation (L). These three parameters vary as a function of independent variables, and worm plots were used to assess goodness of fit. Normality was assessed using Kolmogorov–Smirnov tests. We included in the analysis smoothed LMS curves for the 3rd, 10th, 25th, 50th, 75th, 90th and 97th percentiles of all parameters. All data are presented as the mean 95% CI. Student's t test was used to determine whether significant differences were found between the descriptive characteristics using IBM SPSS 25.0. The level of statistical significance was set at $P < 0.05$.

3. Results

Table 1 displays the descriptive characteristics of the full sample of 497 children, consisting of 288 boys and 209 girls aged 4-11 years, with a mean age of 7.39 (±1.93) years. The table presents the primary anthropometric parameters, with a significant age difference observed between boys and girls. It also shows the muscle US parameters, with significant differences in the $\text{EI}_{\text{corrected equation 1-3,}}$ IMAT and SAT observed between boys and girls.

Table 4.1. Descriptive characteristics of full sample, boys and girls participating in the study

Variables	**Full sample (*n*= 497)**	**Boys (*n*= 288)**	**Girls (*n*= 209)**
Anthropometrics parameters			
Age (years)**	7.39 (7.22; 7.56)	7.64 (7.41; 7.88)	7.04 (6.81; 7.28)
Height (cm)*	124.87 (123.70;126.04)	126.63 (125.03;128.23)	122.46(120.78;124.13)
Weight (kg)*	27.24 (26.51;27.96)	28.19 (27.21;29.16)	25.94(24.87;27.00)
Body mass index (kg/m^2)	17.08(16.87;17.28)	17.19(16.92;17.46)	16.93(16.61;17.25)
Body mass index (z-score)	-0.00(-0.08;0.09)	0.04(-0.06;0.16)	-0.05(-0.06;0.16)
Waist circumference (cm)*	58.30(57.67;58.92)	58.90(58.08;59.73)	57.47(56.52;58.42)
Waist-to-height ratio	0.46(0.46;0.47)	0.46(0.46;0.47)	0.47(0.46;0.47)
Muscle ultrasound parameters			
$\text{EI}_{\text{uncorrected}}$ (au)*	44.82(43.61;46.03)	43.13(41.45;44.81)	47.15(45.48;48.82)
$\text{EI}_{\text{corrected equation 1}}$ (au)**	73.08(71.42;74.75)	68.95(66.76;71.14)	78.78(76.42;81.14)
$\text{EI}_{\text{corrected equation 2}}$ (au)**	68.88(67.37;70.39)	65.28(63.25;67.30)	73.85(71.74;75.95)
$\text{EI}_{\text{corrected equation 3}}$ (au)**	72.20(70.56.06;73.84)	68.14(65.97;70.31)	77.80(75.47;80.13)
IMAT (%)**	51.68(50.36;53.00)	48.17(46.43;49.92)	56.51(54.68;58.34)
SAT (mm)**	6.44(6.17;6.72)	6.01(5.68;6.34)	7.04(6.59;7.49)

Values are means and 95% CI. *P<0.05; **P<0.01. EI, echo-intensity; IMAT, intramuscular adipose tissue.

The LMS reference curves (using 3rd, 10th, 25th, 50th, 75th, 90th and 97th percentiles) derived for uncorrected EI, corrected $\text{EI}_{\text{equation 1-3}}$, IMAT and SAT for boys and girls respectively are summarized in **Supplemental Tables S1-S6** and Figures 1-2.

In regards to uncorrected EI for children, we found similar trends were for boys and girls. In both, the EI decreases as age increases, with the most pronounced curve in boys. Smoothed age-specific and sex-specific percentiles obtained from the EI corrected equation 1-3 are presented in Table S2 (Figure 1- C, D), Table S3 (Figure 1- E, F), and Table S4 (Figure 2 - A, B).

The distribution is slightly different during the years for both boys and girls. In boys, the corrected equations go down over the years, while in girls the values remain relatively stable. The results of IMAT (Table S5, Figure 3- C, D) tend to decrease with a steeper decline in boys. In contrast, SAT (Table S6, Figure 3-E, F) increases in both sexes, with a much more pronounced increase in girls.

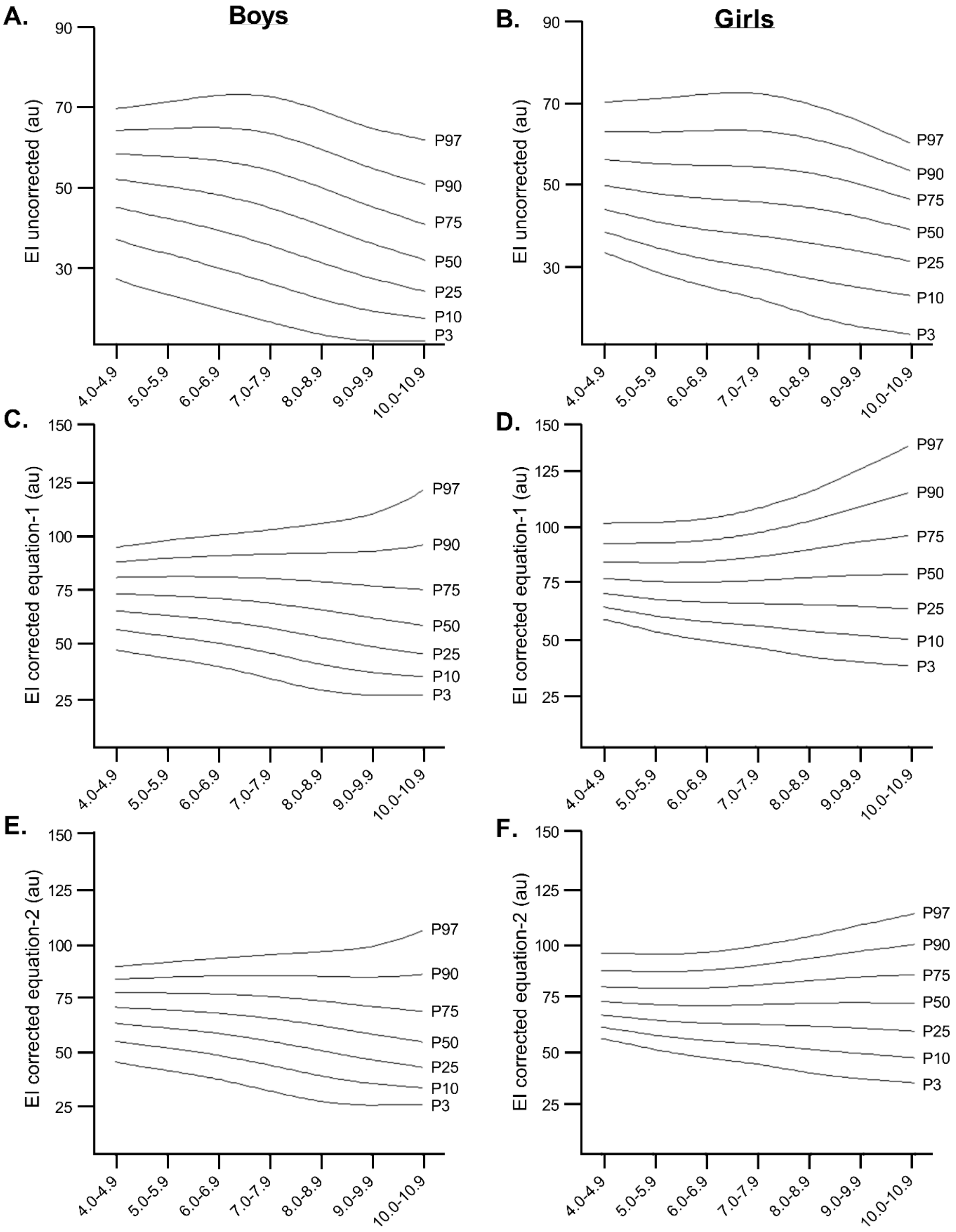

Figure 4.2. Centile's age- and sex-specific of EI uncorrected (Panel A, B), echo intensity corrected equation 1 (Panel C, D), and echo intensity corrected equation 2 (Panel E, F) in boys and girls.

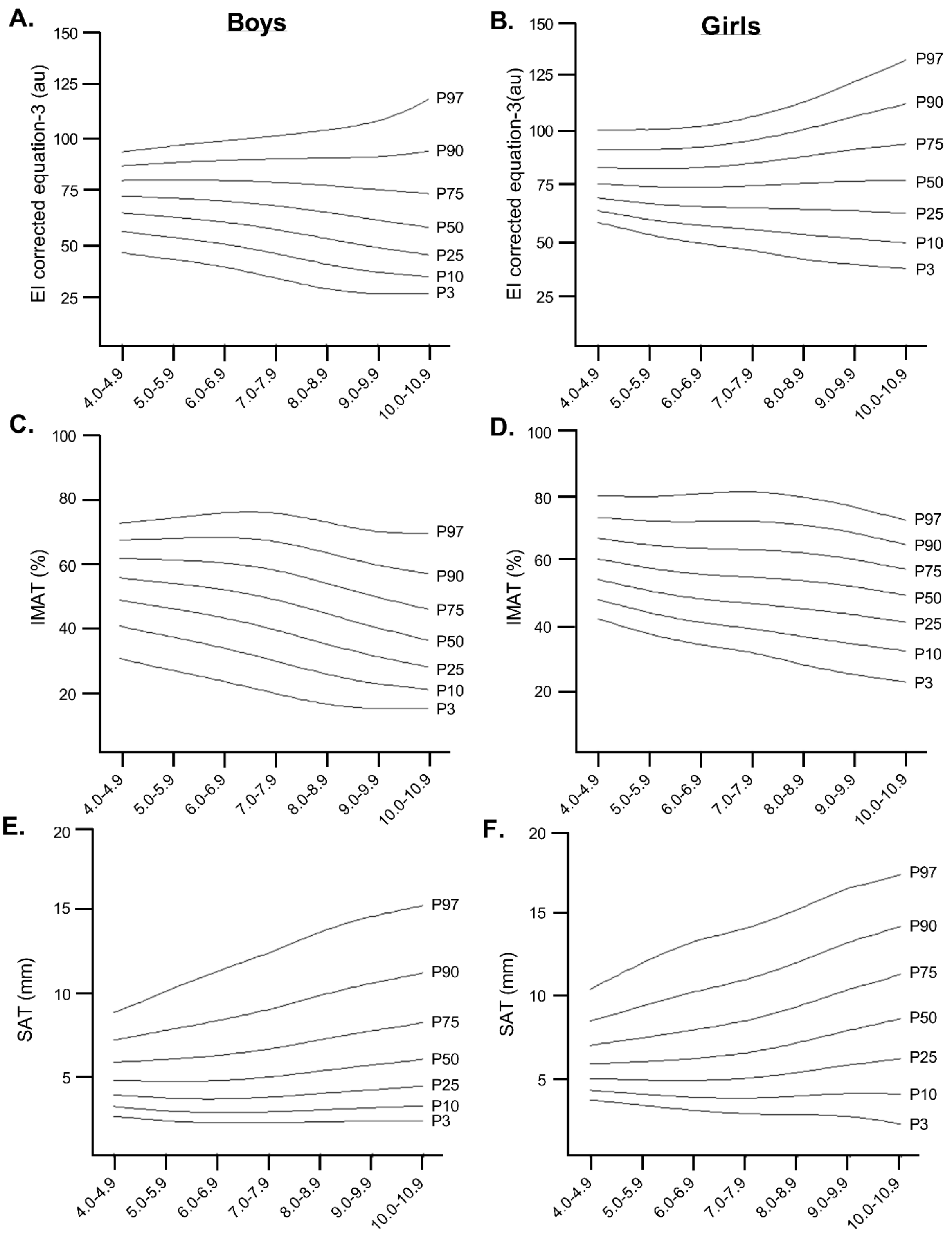

Figure 4.3. Centile's age- and sex-specific of EI corrected equation 3 (Panel A, B), IMAT (Panel C, D) and SAT (Panel E, F) in boys and girls.

4. Discussion

The accuracy of ultrasound (US) in assessing skeletal muscle and its potential to predict clinical outcomes has been postulated in previous studies[29-30]. Our study highlights the use of EI, EI corrected equation 1-3 SAT and IMAT, in elementary school children, providing reference values for normal muscle US parameters in healthy youth. These values can guide pediatricians who wish to apply the technique and can also be a valuable resource in the clinical assessment of muscle function and for comparisons with studies from other countries, as recommended by the European Working Group on Sarcopenia in Older People (EWGSOP2)[31] and the SARCUS study (SARCopenia through UltraSound) [32].

US has the potential to become an imaging-based tool for screening and diagnosing skeletal muscle US parameters, comparable to computed tomography and magnetic resonance imaging, which quantify body composition on the tissue level, and dual-energy X-ray absorptiometry, which assesses the chemical level. Among qualitative measures, muscle EI provides helpful information about the presence of inflammation, fibrosis, and adipose tissue infiltration[33]. In our study, boys in the 50th percentile have muscle EI values of 52.13 au in the range of 4.0-4.9 years, increasing to 32.15 a.u. in the range of 10.0-10.9. For girls, the 50th percentile, leaves with 50.32 au in the range of 4-4.9 years, achieving 39.56 a.u. in the range of 10.0-10.9. However, girls have higher EI values when SAT is taken into account, indicating a worsening of their muscle quality [32]. Although there is no established cut-off for defining low quality muscle mass in the pediatric population, the lowest EI percentiles computed in our study (3rd and 10th percentile) could be used as an indicative of the low lean mass phenotype.

During early childhood, there is a consistent pattern of sexual dimorphism and muscle mass quantity that aligns with previous findings from ethnically diverse populations, likely due to hormonal influences[36]. This trend also applies to SAT, with girls showing an increase in SAT percentage as they age, from 5.94 in the 4-4.9 age group to 8.69 in the 10-10.9 age group. These results could be linked to early adiposity rebound, which is becoming more prevalent and occurs earlier in girls than boys, as shown in a recent systematic review and meta-analysis[23]. Investigating the correlation between these muscle US parameters and early adiposity rebound could provide an effective marker of obesity in children and help tailor nutritional and exercise interventions to improve treatment of metabolic diseases associated with sarcopenia in early life[37].

During pre and pubertal growth spurts, there is a dramatic activation of growth hormone and sex steroids that leads to rapid increases in skeletal muscle and lean mass. Therefore, tracking the development of lean mass during adolescence can be a useful tool in identifying potential interventions for metabolic diseases associated with sarcopenia early in life[11]. The evaluation of muscle US parameters is critical for this purpose. It can provide insight into the extent of subcutaneous adiposity and the presence of inflammation, fibrosis, and adipose tissue infiltration, which are significant factors in muscle quality assessment [36].

Previous studies have increasingly integrated the concepts of muscular strength, peak force and body size to assess muscle performance and provide an estimate of muscle quality[34]. In older adults, there appears to be a correlation between EI and muscle strength, gate speed, and sit-to-stand test[35]. Among children, Garcia-Alonso et al.[16] shown that there are associations between physical fitness components and muscle ultrasound parameters in prepuberal children. In addition, the increase in muscle US parameters may partly be related to the improvement of the physical fitness and vice versa. Future studies combining these percentiles with functional data (e.g., muscle strength) are required for defining dynapenia and/or low-quality muscle mass among children and adolescents[11].

Although our analyses were robust, several limitations should be considered. First, our sample size of 497 children is representative of only a subsample of the Spanish population, and it may be influenced by factors such as region, socioeconomic status, and lifestyle. Second, the study design did not involve following up with the same group over time, so we cannot confirm if the observed trends will persist over time. Third, the formula used to evaluate IMAT was designed for adults, and future studies could explore alternative methods, such as DXA, for assessing IMAT in children. Fourth, the lack of data on dietary patterns limits our ability to interpret the contribution of diet to body composition development. Finally, the calculation of SAT was limited to the femoral rectus, which could have influenced the results. Despite these limitations, our study contributes to our understanding of how muscle quality varies by sex and age in children. Despite these limitations, our study contributes to our understanding of how muscle quality varies by sex and age in children. We used the highly accurate and reproducible US technique to measure muscle quality, which is considered a reliable method for assessing body composition in all age groups[18]. Additionally, the LMS analysis is a popular method to obtain smoothened centile curves for cross-sectional data [38]. Therefore, our charts provide valuable information to researchers studying adolescents in this geographical region and may assist healthcare providers in identifying abnormalities in body composition development during youth.

In summary, we have presented age- and sex-specific reference data for muscle US parameters that are unique to children. Future studies should consider combining echo-intensity or IMAT percentile data from different regions in Spain to establish charts that can be used countrywide. This would allow for the identification of the risk of low-quality mass and the provision of targeted treatments, such as nutritional and exercise interventions, and the initiation of sports in schools through public policies. These measures can prevent associated outcomes, such as pediatric dynapenia, and promote healthy development in children.

Conflict of Interest

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

Author Contributions

YGA, AMAM, MI: study concept and design; GLG, YGA, AMAM: data collection; RRV: statistical analysis; GLG, YGA, AMAM, RRV, AGH: interpretation of data: GLG, YGA, AMAM, RRV, AGH: drafting of the manuscript. All authors: have critically revised and approved the final version.

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SUPPLEMENTARY MATERIAL

Table 1. Smoothed age-specific and sex-specific percentile of echo intensity uncorrected (au) in boys and girls

Sex / age group	N	L	S	P3	P10	P25	P50 (M)	P75	P90	P97
Boys (*n*=288)										
4.0−4.9	38	1.82	0.19	27.54	37.24	45.19	52.13	58.39	64.14	69.49
5.0−5.9	38	1.50	0.23	23.71	33.76	42.48	50.38	57.70	64.58	71.11
6.0−6.9	35	1.22	0.27	20.34	30.25	39.48	48.25	56.68	64.84	72.78
7.0−7.9	56	1.03	0.31	16.95	26.40	35.74	45.00	54.20	63.34	72.44
8.0−8.9	24	0.93	0.34	13.83	22.52	31.46	40.61	49.90	59.33	68.87
9.0−9.9	53	0.77	0.37	12.30	19.59	27.55	36.09	45.11	54.57	64.42
10.0−10.9	44	0.49	0.38	12.26	17.82	24.44	32.15	40.93	50.80	61.76
Girls (*n*= 208)										
4.0−4.9	35	0.42	0.18	33.83	38.90	44.40	50.32	56.68	63.49	70.75
5.0−5.9	25	0.58	0.22	29.16	35.11	41.53	48.39	55.70	63.44	71.60
6.0−6.9	29	0.67	0.25	25.50	32.23	39.46	47.15	55.28	63.82	72.76
7.0−7.9	66	0.79	0.27	22.64	30.13	38.03	46.30	54.89	63.77	72.92
8.0−8.9	24	1.05	0.29	18.69	27.58	36.31	44.94	53.48	61.94	70.35
9.0−9.9	19	1.21	0.29	15.74	25.34	34.22	42.64	50.72	58.54	66.15
10.0−10.9	10	1.30	0.29	13.88	23.35	31.76	39.56	46.92	53.95	60.72

L, power in the Box–Cox transformation for ‘correcting’ the skewness; M, median; P, percentile; S, coefficient of variation.

Table 2. Smoothed age-specific and sex-specific percentile of echo intensity corrected equation-1 (au) in boys and girls

Sex / age group	N	L	S	P3	P10	P25	P50 (M)	P75	P90	P97
Boys (*n*=288)										
4.0–4.9	38	1.58	0.16	47.86	57.16	65.64	73.53	80.95	88.00	94.73
5.0–5.9	38	1.31	0.18	44.15	54.15	63.60	72.62	81.31	89.72	97.88
6.0–6.9	35	1.16	0.21	40.21	50.90	61.25	71.31	81.15	90.80	100.29
7.0–7.9	56	1.05	0.24	34.74	46.36	57.82	69.17	80.42	91.59	102.69
8.0–8.9	24	0.87	0.29	29.52	41.30	53.52	66.11	79.00	92.17	105.58
9.0–9.9	53	0.56	0.33	27.22	37.54	49.30	62.48	77.02	92.90	110.08
10.0–10.9	44	0.09	0.37	27.27	35.49	45.89	58.99	75.40	95.84	121.20
Girls (*n*= 208)										
4.0–4.9	35	0.10	0.14	59.21	64.67	70.68	77.32	84.65	92.75	101.72
5.0–5.9	25	0.48	0.16	53.82	60.78	68.18	76.01	84.29	93.01	102.17
6.0–6.9	29	0.77	0.18	49.93	58.26	66.87	75.75	84.87	94.23	103.80
7.0–7.9	66	0.84	0.20	46.80	56.42	66.33	76.47	86.84	97.41	108.17
8.0–8.9	24	0.85	0.23	42.90	54.14	65.76	77.71	89.95	102.45	115.19
9.0–9.9	19	0.65	0.27	40.54	52.31	65.09	78.83	93.47	108.97	125.29
10.0–10.9	10	0.45	0.31	38.69	50.46	63.95	79.21	96.27	115.18	135.95

L, power in the Box–Cox transformation for ‘correcting’ the skewness; M, median; P, percentile; S, coefficient of variation.

Table 2. Smoothed age-specific and sex-specific percentile of echo intensity corrected equation-2 (au) in boys and girls

Sex / age group	N	L	S	P3	P10	P25	P50 (M)	P75	P90	P97
Boys (*n*=288)										
4.0–4.9	38	1.90	0.15	45.73	55.33	63.62	71.03	77.80	84.08	89.95
5.0–5.9	38	1.64	0.17	41.83	52.25	61.48	69.88	77.68	85.00	91.94
6.0–6.9	35	1.44	0.20	37.70	48.85	58.97	68.37	77.23	85.67	93.75
7.0–7.9	56	1.26	0.23	32.39	44.31	55.43	65.99	76.13	85.92	95.43
8.0–8.9	24	1.04	0.28	27.57	39.38	51.05	62.61	74.09	85.49	96.83
9.0–9.9	53	0.68	0.32	25.70	35.75	46.79	58.72	71.48	84.99	99.22
10.0–10.9	44	0.17	0.35	26.03	33.76	43.32	55.02	69.23	86.37	106.88
Girls (*n*= 208)										
4.0–4.9	35	0.01	0.13	56.22	61.46	67.18	73.43	80.26	87.72	95.87
5.0–5.9	25	0.65	0.15	51.13	57.81	64.77	72.01	79.52	87.29	95.31
6.0–6.9	29	0.94	0.17	47.36	55.36	63.43	71.56	79.73	87.96	96.22
7.0–7.9	66	1.02	0.19	44.31	53.57	62.79	71.99	81.15	90.30	99.42
8.0–8.9	24	1.09	0.22	40.18	51.21	62.02	72.64	83.13	93.49	103.74
9.0–9.9	19	0.95	0.25	37.51	49.20	61.01	72.93	84.95	97.05	109.23
10.0–10.9	10	0.77	0.27	35.68	47.27	59.57	72.47	85.93	99.88	114.30

L, power in the Box–Cox transformation for 'correcting' the skewness; M, median; P, percentile; S, coefficient of variation.

Table 4. Smoothed age-specific and sex-specific percentile of echo intensity corrected equation-3 (au) in boys and girls

Sex / age group	N	L	S	P3	P10	P25	P50 (M)	P75	P90	P97
Boys (*n*=288)										
4.0–4.9	38	1.74	0.16	46.36	56.26	65.01	72.96	80.33	87.22	93.74
5.0–5.9	38	1.41	0.18	43.08	53.42	62.98	71.98	80.53	88.73	96.63
6.0–6.9	35	1.21	0.21	39.48	50.28	60.62	70.59	80.28	89.73	98.97
7.0–7.9	56	1.07	0.24	34.28	45.82	57.18	68.38	79.46	90.45	101.34
8.0–8.9	24	0.88	0.29	29.21	40.83	52.88	65.27	77.96	90.92	104.10
9.0–9.9	53	0.56	0.33	26.91	37.08	48.66	61.62	75.90	91.49	108.35
10.0–10.9	44	0.10	0.37	26.90	35.00	45.24	58.11	74.19	94.18	118.92
Girls (*n*= 208)										
4.0–4.9	35	-0.16	0.13	58.80	64.11	69.98	76.48	83.70	91.72	100.65
5.0–5.9	25	0.45	0.16	53.30	60.11	67.37	75.09	83.27	91.90	101.01
6.0–6.9	29	0.76	0.18	49.28	57.50	66.00	74.77	83.80	93.06	102.55
7.0–7.9	66	0.84	0.20	46.08	55.62	65.42	75.46	85.71	96.16	106.79
8.0–8.9	24	0.86	0.23	42.14	53.31	64.82	76.63	88.69	100.98	113.48
9.0–9.9	19	0.67	0.27	39.75	51.45	64.10	77.63	91.99	107.13	123.00
10.0–10.9	10	0.48	0.30	37.89	49.58	62.92	77.91	94.56	112.90	132.92

L, power in the Box–Cox transformation for ‘correcting’ the skewness; M, median; P, percentile; S, coefficient of variation.

Table 5. Smoothed age-specific and sex-specific percentile of IMAT (%) in boys and girls

Sex / age group	N	L	S	P3	P10	P25	P50 (M)	P75	P90	P97
Boys (*n*=288)										
4.0–4.9	38	1.96	0.17	31.19	41.10	49.11	56.02	62.20	67.83	73.05
5.0–5.9	38	1.63	0.21	27.46	37.79	46.57	54.41	61.60	68.30	74.60
6.0–6.9	35	1.33	0.24	23.98	34.32	43.69	52.43	60.71	68.62	76.23
7.0–7.9	56	1.13	0.28	20.27	30.34	39.99	49.33	58.45	67.38	76.16
8.0–8.9	24	0.99	0.31	16.92	26.26	35.64	45.05	54.48	63.93	73.40
9.0–9.9	53	0.76	0.34	15.49	23.21	31.62	40.60	50.09	60.04	70.40
10.0–10.9	44	0.37	0.37	15.60	21.40	28.38	36.65	46.27	57.33	69.92
Girls (*n*= 208)										
4.0–4.9	35	0.85	0.15	42.60	48.61	54.74	60.96	67.29	73.70	80.19
5.0–5.9	25	0.77	0.18	38.02	44.50	51.21	58.13	65.25	72.55	80.02
6.0–6.9	29	0.68	0.21	34.74	41.56	48.76	56.31	64.21	72.42	80.95
7.0–7.9	66	0.73	0.22	32.30	39.62	47.33	55.40	63.80	72.51	81.51
8.0–8.9	24	1.01	0.24	28.59	37.19	45.76	54.30	62.83	71.33	79.82
9.0–9.9	19	1.19	0.24	25.57	34.94	43.87	52.46	60.80	68.93	76.88
10.0–10.9	10	1.29	0.24	23.27	32.81	41.60	49.88	57.77	65.37	72.72

L, power in the Box–Cox transformation for ‘correcting’ the skewness; M, median; P, percentile; S, coefficient of variation.

Table 6. Smoothed age-specific and sex-specific percentile of SAT (mm) in boys and girls

Sex / age group	N	L	S	P3	P10	P25	P50 (M)	P75	P90	P97
Boys (*n*=288)										
4.0–4.9	38	-0.06	0.30	2.68	3.25	3.95	4.82	5.90	7.24	8.90
5.0–5.9	38	-0.14	0.36	2.40	2.99	3.76	4.76	6.08	7.82	10.17
6.0–6.9	35	-0.17	0.40	2.28	2.89	3.71	4.81	6.31	8.40	11.34
7.0–7.9	56	-0.16	0.42	2.28	2.93	3.82	5.03	6.71	9.07	12.46
8.0–8.9	24	-0.13	0.44	2.34	3.06	4.04	5.39	7.26	9.92	13.72
9.0–9.9	53	-0.07	0.45	2.39	3.18	4.26	5.74	7.79	10.64	14.64
10.0–10.9	44	0.00	0.46	2.41	3.29	4.48	6.09	8.29	11.27	15.32
Girls (*n*= 208)										
4.0–4.9	35	-0.43	0.25	3.76	4.34	5.05	5.94	7.07	8.53	10.47
5.0–5.9	25	-0.29	0.31	3.42	4.10	4.96	6.07	7.52	9.45	12.07
6.0–6.9	29	-0.11	0.36	3.12	3.91	4.93	6.26	8.00	10.30	13.34
7.0–7.9	66	0.09	0.40	2.90	3.85	5.06	6.61	8.57	11.05	14.17
8.0–8.9	24	0.25	0.41	2.85	3.98	5.42	7.22	9.42	12.10	15.30
9.0–9.9	19	0.42	0.43	2.74	4.14	5.88	7.98	10.46	13.33	16.61
10.0–10.9	10	0.67	0.44	2.28	4.10	6.25	8.69	11.37	14.29	17.42

L, power in the Box–Cox transformation for ‘correcting’ the skewness; M, median; P, percentile; S, coefficient of variation.

Chapter 5

General discussion

The present doctoral thesis has two aims. First, evaluate and develop normative values of muscle US parameters and its association with physical fitness. Second, evaluate the impact of COVID-19 on physical activity and self-regulation in Spanish preschoolers.

Muscle ultrasound parameters in schoolchildren (Study 3 and Study 4)

The main finding of study 3 is that physical fitness components are inversely associated with EI, IMAT, and SAT after adjusting for potential confounders, including sex and age, in prepuberal children. Despite the observational nature of the study, our findings contribute to the current knowledge by suggesting that physical fitness components have a beneficial influence on muscle US parameters, even from early childhood. To our knowledge, this study advances the limited architecture muscle and overall physical fitness literature among prepuberal children.

Although we do not have an extensive literature with which to compare our results, it would appear that all the findings in the different published populations go in the same direction. Thus, it would be safe to conclude that better physical fitness levels are inversely associated with EI, SAT, and IMAT in healthy children. This study strengthens the idea that promotion of physical fitness from early childhood is important for muscle quality [1]. Accordingly, it would seem to be important to achieve the recommendations of the World Health Organization [2] about physical activity, sedentary behavior, physical fitness components and screen time to prevent muscle deterioration.

The study 4 highlights the use of EI, EI corrected equation 1-3 SAT and IMAT, in elementary school children, providing reference values for normal muscle US parameters in healthy youth. These values can guide pediatricians who wish to apply the technique and can also be a valuable resource in the clinical assessment of muscle function and for comparisons with studies from other countries, as recommended by the European Working Group on Sarcopenia in Older People (EWGSOP2)[3] and the SARCUS study (SARCopenia through US) [4].

Among qualitative measures, muscle EI provides helpful information about the presence of inflammation, fibrosis, and adipose tissue infiltration [5].However, girls have higher EI values when SAT is taken into account, indicating a worsening of their muscle quality[4]. These results could be linked to early adiposity rebound, which is becoming more prevalent and occurs earlier in girls than boys, as shown in a recent systematic review and meta-analysis [6]. Investigating the correlation between these muscle US parameters and early adiposity rebound could provide an effective marker of obesity in children and help tailor nutritional and exercise interventions to improve treatment of metabolic diseases associated with sarcopenia in early life [7].

In addition, the increase in muscle US parameters may partly be related to the improvement of the physical fitness and vice versa. Future studies combining these percentiles with functional data (e.g., muscle strength) are required for defining dynapenia and/or low-quality muscle mass among children and adolescents [8].

Impact of COVID-19 in schoolchildren (Study 1 and Study 2)

The study 1 explored the effects of the COVID-19 lockdown on physical activity, sedentary behavior and sleep and its relationship with self-regulation difficulties in Spanish preschoolers. Our findings provide evidence of the negative effects of the COVID-19 lockdown on physical activity level, sedentary behavior, sleep quality and self-regulation in Spanish preschoolers. As far as we know, this is the first study that objectively examines the effect of COVID-19 home confinement on these parameters among preschoolers.

Regarding physical activity and sedentary behavior, our study reflects those preschoolers reduced their total physical activity and increased sedentary time during the lockdown. Previously published studies are in line with our findings, which found that children had different patterns of activity than what was seen before COVID-19. For example, changes in physical activity and sedentary behavior were reported by parents and legal guardians of children living in the U.S. using an online survey [9].

Self-regulation is defined as psychological conduct that comprises a series of important competencies, such as the ability to control inner states or responses towards thoughts, attention, emotions or even performance[10]. The study 1 showed that during the lockdown, preschoolers had an increase in internalizing and externalizing problems. Behavioral and emotional problems at this age may potentially set a child on a course of maladaptation [11], and more specifically on a pathway to internalizing (i.e., antisocial behaviors) or externalizing problems (i.e., anxious or depressed behaviors). In this study, families reported overall greater difficulties in emotional regulation (i.e., he/she is more irritable, has more mood swings) in their children aged three years old during the six weeks of strict confinement experienced in Madrid, Spain.

Study 1 also shows that preschoolers who met the recommendations for physical activity had lower internalizing scores than non-active peers. Therefore, we also highlight the importance of meeting physical activity recommendations in the early years, as it seems to influence aspects related to broad areas of mental health [12]. This result may be explained by the fact that increased physical activity was associated with higher mental health among children and adolescents [12]. In this aspect, it also has recently been shown that children who meet the physical activity guidelines have higher life satisfaction, positive affect [13] and self-regulation [14] compared to inactive peers.

Study 2 compared the levels of PA, SB, and sleep time in children aged 4–7 years prior to and during the pandemic, and we examined for relationships between these behaviors and those of their parents. Our main results are that, during the pandemic, the levels of MVPA and SB increased in children during weekdays, and sleep time decreased. Much the same was true at the weekend. Interestingly, an increase in mother's MVPA during this period was positively related with higher increases in MVPA in their children for both weekdays and weekends.

Contrary to expectations and the aforementioned findings, our study shows that children increased their PA and SB and decreased their sleep time during the pandemic. This finding is consistent with that of Hurter et al. [15] who showed that PA levels increased significantly upon return to school and after the COVID-19 lockdown. There are several possible explanations for our changes. One explanation might be due to information about healthy habits received by the families after the first assessments; specifically, the 68.1% of the families report that after the first evaluation they did or tried to do more PA with their children. Furthermore, restrictions and social distancing made children spend more time with their families, which could have resulted in higher PA levels. In addition, perimeter closures reduced mobility to interprovincial movements and, consequently, families could have made many excursions around Navarre to nature sites. Second, we hypothesize that the increase of SB in our sample may be due to the change in school stage from early childhood education to primary education, which involves a change in school routines and increased sedentary activities at desks.

Regarding the relationships between parents and children's movement behaviour during the pandemic, our results indicate that the PA of children is related to their mother's behaviour, mainly MVPA. Although it has been hypothesized that this relationship may vary with environmental conditions[16], and that some young children are naturally physically active without parental intervention [17], our results have been reported in recent studies. Specifically, our findings show that an increase in mother's TPA was positively related with higher increases in TPA in their children on weekdays. A possible explanation for this might be that the majority of mothers were walking or cycling together with their child as the main form of transport to the school, as suggested by Hnatiuk et al [18].Therefore, the relationships observed between PA of children and their mothers might reflect the changes in behaviour before and during the COVID19 pandemic observed in the present study.

Practical applications and future perspectives

The present doctoral thesis has two aims. First, evaluate and develop normative values of muscle US parameters and its association with physical fitness. Second, evaluate the impact of COVID-19 on physical activity and self-regulation in Spanish preschoolers.

This thesis proposes innovative evaluation strategies. For the evaluation of muscle quality, we used musculoskeletal US, being the first to develop cutting points of parameters of muscle US parameters in children and associate them to physical condition. In addition, for the evaluation of physical activity we have used accelerometers, which has allowed us to record an objective evaluation of the domains of physical activity.

The intervention of this doctoral thesis has been framed in the Project "Observatorio de Actividad Física en Navarra" and has been carried out in different educational centers, sports and health center. The proposal was to evaluate the relationship between physical condition, body composition indicators and physical activity components in Spanish schoolchildren. Therefore, future training programs could be designed to enhance BMC for the benefit of children's fitness and overall health.

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Chapter 6

Conclusions

CONCLUSIONS

Chapter 1: Physical Activity, Sedentary Behavior, Sleep and Self-Regulation in Spanish Preschoolers during the COVID-19 Lockdown

Recognizing these lifestyle and psychological well-being changes are critical because they may have a lasting impact on preschoolers' physical and mental health and may help guide future interventions, perhaps by PA promotion. Therefore, adopting healthy movement behaviors may help to mitigate the negative effects on preschool children of this pandemic and its lockdown.

Chapter 2: Relationship between parents' and children's objectively assessed movement behaviours prior to and during the COVID-19 pandemic

During the pandemic after the COVID-19 lockdown, children got more MVPA, more SB, and slept fewer hours than before, which indicates that children's movement behaviours were slightly affected by the COVID-19 pandemic. Increases in the PA levels of children could be associated with their mother' PA levels in this period, especially on weekdays. Future studies are needed to confirm whether these movement behaviours are maintained over time, without social restrictions.

Chapter 3: Associations between physical fitness components with muscle ultrasound parameters in prepuberal children

The main finding in this study is that physical fitness components are related to better muscle US parameters in prepuberal children. Given the fact that the origins of some cardiometabolic disorders can be found from early life, and physical fitness tracks from childhood to adulthood, it would be important to improve muscle architecture during childhood to reduce ectopic fat, which could mitigate cardiometabolic risk during adulthood. Therefore, developing health and educational policies that encourage global physical fitness during childhood should be a priority.

Chapter 4: Reference values for muscle ultrasound parameters in Children

We have presented age- and sex-specific reference data for muscle US parameters that are unique to children. Future studies should consider combining EI or IMAT percentile data from different regions in Spain to establish charts that can be used countrywide. This would allow for the identification of the risk of low-quality mass and the provision of targeted treatments, such as nutritional and exercise interventions, and the initiation of sports in schools through public policies. These measures can prevent associated outcomes, such as pediatric dynapenia, and promote healthy development in children.

CONCLUSIONES

Capítulo 1: Actividad Física, conducta sedentaria, sueño y auto-regulación en preescolares españoles durante el confinamiento del COVID-19

Reconocer cambios en el estilo de vida y el bienestar psicológico es fundamental porque pueden tener un impacto duradero en la salud física y mental de los preescolares y pueden ayudar a guiar futuras intervenciones, quizás mediante la promoción de la actividad física. Por lo tanto, la adopción de comportamientos de movimiento saludables puede ayudar a mitigar los efectos negativos en los niños preescolares de esta pandemia y su confinamiento.

Capítulo 2: Relación entre los comportamientos de movimiento de padres e hijos evaluados objetivamente antes y durante la pandemia COVID-19

Durante la pandemia, después del confinamiento por el COVID-19, los niños tuvieron más AFMV, más CS y durmieron menos horas que antes, lo que indica que los comportamientos de movimiento de los niños se vieron ligeramente afectados por la pandemia del COVID-19. Los aumentos en los niveles de AF de los niños podrían estar asociados con los niveles de AF de su madre en este período, especialmente en días laborables. Se necesitan estudios futuros para confirmar si estos comportamientos de movimiento se mantienen a lo largo del tiempo, sin restricciones sociales.

Capítulo 3: Asociaciones entre los componentes de la condición física con los parámetros de ultrasonido muscular en niños prepúberes.

El principal hallazgo de este estudio fue que los componentes de la condición física están relacionados con mejores parámetros musculares en niños prepúberes. Dado que los orígenes de algunos trastornos cardiometabólicos se pueden encontrar desde los primeros años de vida, y las pistas de aptitud física desde la infancia hasta la edad adulta, sería importante mejorar la arquitectura muscular durante la infancia para reducir la grasa ectópica, que podrían mitigar el riesgo cardiometabólico durante la edad adulta. Por lo tanto, el desarrollo de políticas de salud y educación que fomenten la condición física global durante la infancia debe ser una prioridad.

Capítulo 4: Valores de referencia para los parámetros de ultrasonido muscular en niños

Se presentan datos de referencia específicos por edad y sexo para los parámetros musculares (US) exclusivos de los niños. En futuros estudios se debería considerar la posibilidad de combinar datos de intensidad de eco-intensidad o TAI de diferentes regiones de España para establecer gráficos que puedan utilizarse en todo el país. Esto permitiría identificar el riesgo de masa de baja calidad y la provisión de tratamientos específicos, como intervenciones nutricionales y de ejercicio, y el inicio de deportes en las escuelas a través de políticas públicas. Estas medidas pueden prevenir los resultados asociados, como la dinapenia pediátrica, y promover el desarrollo saludable en los niños.

Appendix

Relevant papers