INTERNATIONAL DOCTORAL THESIS

EFFECTS OF EXERCISE ON HEPATIC STEATOSIS IN CHILDREN: ROLE OF FITNESS

Author of the Thesis: María Medrano Echeverría

Supervisor of the Thesis: Idoia Labayen Goñi

Public University of Navarra, Department of Health Sciences

Institute for Innovation & Sustainable Development in Food Chain (IS-FOOD)

TESIS DOCTORAL INTERNACIONAL

EFECTOS DEL EJERCICIO FÍSICO EN LA ESTEATOSIS HEPÁTICA EN NIÑOS: PAPEL DE LA CONDICIÓN FÍSICA

Autora de la Tesis: María Medrano Echeverría

Directora de la Tesis: Idoia Labayen Goñi

Universidad Pública de Navarra, Departamento de Ciencias de la Salud

Instituto de Innovación y Desarrollo Sostenible en la Cadena Alimentaria (IS-FOOD)

A todos vosotros.

A quienes somos, fuimos y seremos.

This International Doctoral Thesis is presented as a *compendium* of five studies. The complete references of the articles are the following:

Study I. Medrano M, Labayen I, Ruiz JR, Rodríguez G, Breidenassel C, Castillo M, Pedrero R, Widhalm K, Kafatos A, Manios Y, Molnar D, González-Gross M, Ortega FB, Moreno LA. Cardiorespiratory fitness, waist circumference and liver enzyme levels in European adolescents: The HELENA cross-sectional study. Published in the *Journal of Science and Medicine in Sport*, 2017;20(10):932-936.

Impact factor: 3.929. 1st Decile. Thematic area of the journal: Sport Sciences.

Study II. Medrano M, Arenaza L, Hidalgo-Migueles J, Rodríguez-Vigil B, Ruiz JR, Labayen I. Associations of physical activity and fitness with hepatic steatosis, liver enzymes and insulin resistance in children. Submitted.

Study III. Medrano M, Cadenas-Sánchez C, Álvarez-Bueno C, Cavero-Redondo I, Ruiz JR, Ortega FB, Labayen I. Evidence-based exercise recommendations to reduce hepatic fat content in youth- a systematic review and meta-analysis. Published in *Progress in Cardiovascular Diseases*, 2018;61(2):222-231.

Impact factor: 6.754. 1st Quartile. Thematic area of the journal: Cardiac & Cardiovascular Systems.

Study IV. Labayen I, Medrano M, Arenaza L, Maíz E, Osés M, Martínez-Vizcaíno V, Ruiz JR, Ortega FB. Effects of exercise in addition to a family-based lifestyle intervention program on hepatic fat in children with overweight. Published in *Diabetes Care*. Epub ahead of print. doi: 10.2337/dc19-0351.

Impact factor: 15.270. 1st Decile. Thematic area of the journal: Endocrinology & Metabolism.

Study V. Medrano M, Arenaza L, Ramírez-Vélez R, Ortega FB, Ruiz JR, Labayen, I. Prevalence of responders for hepatic fat, overall and abdominal adiposity and gamma-glutamyl transferase in response to a lifestyle intervention with or without supervised exercise in children with overweight/obesity: secondary analyses of the EFIGRO randomized controlled trial. Submitted.

Esta Tesis Doctoral Internacional se presenta como un compendio de cinco artículos. Las referencias completas de los artículos son las siguientes:

Estudio I. Medrano M, Labayen I, Ruiz JR, Rodríguez G, Breidenassel C, Castillo M, Pedrero R, Widhalm K, Kafatos A, Manios Y, Molnar D, González-Gross M, Ortega FB, Moreno LA. Cardiorespiratory fitness, waist circumference and liver enzyme levels in European adolescents: The HELENA cross-sectional study. Publicado en *Journal of Science and Medicine in Sport*, 2017;20(10):932-936.

Factor de impacto: 3.929. 1er Decil. Área temática de la revista: Ciencias del Deporte.

Estudio II. Medrano M, Arenaza L, Hidalgo-Migueles J, Rodríguez-Vigil B, Ruiz JR, Labayen I. Associations of physical activity and fitness with hepatic steatosis, liver enzymes and insulin resistance in children. Pendiente de evaluación.

Estudio III. Medrano M, Cadenas-Sánchez C, Álvarez-Bueno C, Cavero-Redondo I, Ruiz JR, Ortega FB, Labayen I. Evidence-based exercise recommendations to reduce hepatic fat content in youth- a systematic review and meta-analysis. Publicado en *Progress in Cardiovascular Diseases*, 2018;61(2):222-231.

Factor de impacto: 6.754. 1º Cuartil. Área temática de la revista: Sistemas Cardiaco y Cardiovascular.

Estudio IV. Labayen I, Medrano M, Arenaza L, Maíz E, Osés M, Martínez-Vizcaíno V, Ruiz JR, Ortega FB. Effects of exercise in addition to a family-based lifestyle intervention program on hepatic fat in children with overweight. Publicado en *Diabetes Care*. Epub ahead of print. doi: 10.2337/dc19-0351.

Factor de impacto: 15.270. 1er Decil. Área temática de la revista: Endocrinología y Metabolismo.

Estudio V. Medrano M, Arenaza L, Ramírez-Vélez R, Ortega FB, Ruiz JR, Labayen, I. Prevalence of responders for hepatic fat, overall and abdominal adiposity and gamma-glutamyl transferase in response to a lifestyle intervention with or without supervised exercise in children with overweight/obesity: secondary analyses of the EFIGRO randomized controlled trial. Pendiente de evaluación.

AUTORIZACIÓN DE LA DIRECTORA DE TESIS A PRESENTAR LA TESIS DOCTORAL EN MODALIDAD DE "COMPENDIO DE PUBLICACIONES":

VºBº Directora de Tesis

Fdo.: Idoia Labayen Goñi

Directora de tesis

- INFORME MOTIVADO DE LA COMISIÓN ACADÉMICA DEL PROGRAMA DE DOCTORADO EN :

Pamplona, de de

Vº Bº

Fdo.:

Coordinador del P.D.

The author of the Thesis **Ms. María Medrano Echeverría** performed the present International Doctoral Thesis as a beneficiary of a grant "Ayuda de Formación de Profesorado Universitario (FPU14/03329)" as part of the program "Formación y Movilidad dentro del Programa Estatal de Promoción del Talento y su Empleabilidad" from the Spanish Ministry of Education, Culture and Sport, by resolution of 20th August 2015 by the "Formación y Movilidad dentro del Programa Estatal de Promoción del Talento y su Empleabilidad" (BOE-A-2015-9456, published on 28th August 2015).

The author of the Thesis Ms. María Medrano Echeverría performed the predoctoral research stay, which allows her to opt for the International Doctoral Thesis, thanks to the grant "Ayuda de Movilidad para Estancias Breves en otros Centros Españoles y Extranjeros (EST17/00210)" for beneficiaries of the program "Formación del profesorado universitario" by the Ministry of Science, Innovation and Universities, by resolution of 31th August 2018, by the "Secretaría de Estado de Universidades, Investigación, Desarrollo e Innovación".

La doctoranda Dña. **María Medrano Echeverría** ha realizado la presente Tesis Doctoral Internacional como beneficiaria de una beca-contrato Ayuda de Formación de Profesorado Universitario (FPU14/03329) de los subprogramas de Formación y Movilidad dentro del Programa Estatal de Promoción del Talento y su Empleabilidad del Ministerio de Educación, Cultura y Deporte, por resolución del 20 de agosto de 2015 de la Formación y Movilidad dentro del Programa Estatal de Promoción del Talento y su Empleabilidad (BOE-A-2015-9456, publicado el 28 de agosto 2015).

La doctoranda Dña. María Medrano Echeverría realizó la estancia predoctoral que le permite optar a la mención de Tesis Doctoral Internacional gracias a la Ayuda de Movilidad para Estancias Breves en otros Centros Españoles y Extranjeros (EST17/00210) a beneficiarios del subprograma de formación del profesorado universitario del Ministerio de Ciencia, Innovación y Universidades, por resolución del 31 de agosto de 2018, por la Secretaría de Estado de Universidades, Investigación, Desarrollo e Innovación.

RESEARCH PROJECTS

The present International Doctoral Thesis was performed mainly as a result of the following research projects:

The EFIGRO Study, "The Effect of Exercise on Hepatic Fat on Overweight Children Study", which was supported by the Spanish Ministry of Health, "Fondo de Investigación Sanitaria del Instituto de Salud Carlos III", "Fondos de la Unión Europea (FEDER), una manera de hacer Europa" (PI13/01335).
Principal investigator: Idoia Labayen Goñi
Duration: 09/10/2014 - 08/10/2015
Funding: 136.972€

Other projects related to the present International Doctoral Thesis:

The PREDIKID Study "The PREvention of DIabetes in KIDs", which was supported by the Spanish Ministry of Economy, Competition and Competitiveness, grant "Programa Estatal de Investigación, Desarrollo e Innovación Orientada a los Retos de la Sociedad" (DEP2016-78377-R).
Principal investigator: Idoia Labayen Goñi
Duration: 2016-2019
Funding: 108.900€

The HELENA Study "Healthy Lifestyle in Europe by Nutrition in Adolescence", which was supported by the European Community Sixth RTD Framework Program (Con- tract FOODCT-2005-007034).
Principal investigator: Luis Moreno Aznar
Duration: 2005-2008
Funding: 4.994.194,90€

Other fundings:

The research projects and the research group of the author of this Thesis also were supported by the University of the Basque Country (GIU14/21) and by "Ayudas a grupos de Investigación" from the Public University of Navarra.

The author of the present International Doctoral Thesis was additionally funded by a grant of "Ayudas a la Movilidad Internacional de Doctorandos" in the modality of "Ayudas para Asistencias a Congresos" from the Public University of Navarra, by resolution of 15th April 2019 by the Comisión de Valoración de la Escuela de Doctorado de Navarra (EDONA) (Resolución Nº 421/2019, 5th March 2019).

PROYECTOS DE INVESTIGACIÓN

La presente Tesis Doctoral Internacional se realizó principalmente como resultado de los siguientes proyectos de investigación:

Estudio EFIGRO, "Estudio del Efecto del Ejercicio Físico sobre la Grasa Hepática en Niños con Obesidad", financiado por el Ministerio de Salud, "Fondo de Investigación Sanitaria del Instituto de Salud Carlos III", "Fondos de la Unión Europea (FEDER), una manera de hacer Europa" (PI13/01335).
Investigadora principal: Idoia Labayen Goñi
Duración: 09/10/2014 - 08/10/2015
Cuantía económica: 136.972€

Otros proyectos relacionados con la presente Tesis Doctoral Internacional:

Estudio PREDIKID, "Prevención de la Diabetes en Niños", financiado por el Ministerio de Economía, Industria y Competitividad, beca "Programa Estatal de Investigación, Desarrollo e Innovación Orientada a los Retos de la Sociedad" (DEP2016-78377-R).
Investigadora principal: Idoia Labayen Goñi
Duración: 2016-2019
Cuantía económica: 108.900€

Estudio HELENA, "Healthy Lifestyle in Europe by Nutrition in Adolescence", financiado por la "European Community Sixth RTD Framework Program" (Contract FOODCT-2005-007034).
Investigador principal: Luis Moreno Aznar
Duración: 2005-2008
Cuantía económica: 4.994.194,90€

Otras fuentes de financiación:

Los proyectos de investigación en los que ha participado y el grupo de investigación al que pertenece la doctoranda también han recibido financiación por parte de la Universidad del País Vasco (GIU14/21) y de las "Ayudas a grupos de Investigación" de la Universidad Pública de Navarra.

La autora de esta Tesis Doctoral Internacional adicionalmente ha recibido una Ayuda a la Movilidad Internacional de Doctorandos en la modalidad de Ayudas para asistencias a Congresos de la Universidad Pública de Navarra, por resolución del 15 de abril 2019 de la Comisión de Valoración de la Escuela de Doctorado de Navarra (EDONA) (Resolución Nº 421/2019, del 5 de marzo de 2019).

AGRADECIMIENTOS

Como siempre, llega el final del camino. Esta vez llega el final de un camino de 4 años que ha sido esta tesis doctoral. Un camino que, aunque a veces sea solitario, no se puede andar solo. Por eso quiero agradecer a todas y cada una de las personas que habéis dado, aunque sea, un paso a mi lado.

En primer lugar, quiero agradecer a Idoia por haber marcado la trayectoria de este camino y haberme ayudado día a día a recorrerlo. No sólo me has aportado los conocimientos científicos necesarios para llegar con éxito al destino, sino que has sido un gran modelo a nivel humano; enseñándome mediante el ejemplo, una serie de valores como: la generosidad, la honestidad, el trabajo en equipo, la equidad, la comprensión, el esfuerzo y la constancia, el compañerismo, la capacidad de superarse y seguir siempre adelante, por muchas cuestas y piedras que nos encontremos por el camino. Gracias por tu dedicación, tu paciencia y la confianza depositada en mí. No solo has sido una directora de tesis excepcional, sino que te considero una madre científica. Aparte de la paciencia, toda ciencia necesitaría una madre como tú.

También quiero agradecer de forma especial a Lide y Maddi el haber recorrido la mayor parte del camino juntas. Gracias chicas, sin vosotras, este camino hubiera sido mucho más duro. Habéis hecho que los días en el despacho, las sesiones o las mediciones se hayan pasado volando. Gracias por cada abrazo o sonrisa en las cuestas más arduas. Gracias por los tantos momentos bonitos vividos durante el recorrido. Además de compañeras de tesis, tengo el honor de poder llamaros amigas. Os deseo suerte para el resto del camino**.**

Gracias también a todos los profesionales o doctorandos que habéis compartido conmigo alguna de las etapas en forma de proyectos: María Amase, Alicia, Edurne, Iratxe, Lourdes, Natalia, Maitane, Santi, Clara…

Gracias todos y cada uno de los alumnos de prácticas que habéis ayudado a que las sesiones salieran adelante y por haber llenado con vuestra alegría el despacho: Aitor, Saioa, Amaia, Cristina, Leire Amantegui, Leire Plazaola, Eukene, Iker, Iñaki, Ander, Amaia, Andoni, María, Maite, Amaia, Jon…

Gracias, por supuesto, a todas las familias, a los niñas y niños, a las madres y padres por vuestra participación en los proyectos. Gracias por vuestras sonrisas y palabras de agradecimiento. Porque eso vale más que cualquier resultado significativo o paper publicado. Sois vosotros los que hacéis que recorrer este camino y dedicarse a la ciencia cobre sentido.

Gracias también a todos los médicos y pediatras, enfermeras, técnicos de laboratorio y demás profesionales que habéis participado en los proyectos, haciéndolos posibles: Beatriz, Nacho, Ainhoa, Ignacio, Eider, Zuriñe… y a todos los que habéis colaborado y estáis colaborando para que los resultados salgan a la luz.

También quiero agradecer a mis profesores el haberme inculcado el gusto por aprender, a nunca perder la curiosidad, a ser crítica, cuestionarme y querer saber más. Sois muchos y no podría poner aquí todos vuestros nombres, pero gracias de corazón. Que me

encantase la carrera y no quiera abandonar nunca la universidad también hacen que esté hoy aquí.

Los caminos a veces no se eligen, sino que es el camino el que nos elige a nosotros. Gracias a Inma por mostrarme en el mapa la posibilidad de escogerlo. Quizás nunca se me hubiera ocurrido sola. A Sara por acompañarme en mis primeros pasos científicos y ponerme en contacto con quien me abriría las puertas a hacer esta tesis doctoral. Gracias a Ilargi, Pablo, Aitor, Erreka. Aunque a veces los caminos tengan que separarse, nunca debemos olvidar de dónde venimos.

Gracias a la Universidad del País Vasco y a la Universidad Pública de Navarra por haberme permitido crecer como doctoranda en vuestras instituciones. Gracias a todas las personas que las conformáis y trabajáis cada día por mejorarlas: personal de limpieza, administrativos, conserjes, profesores, coordinadores, directores… Gracias a los alumnos, por haberme enseñado más de lo que yo os he podido enseñar a vosotros. Gracias a las becas y ayudas que me han permitido mantenerme en este camino.

Durante el recorrido he pasado por otras ciudades: Vitoria, Granada, Cuenca, Estocolmo, Bogotá… En ellas he encontrado a personas maravillosas y de las que he aprendido a todos los niveles. Gracias a Fran y a Jonatan por hacerme sentir como en casa siempre que bajo a Granada. Y por supuesto gracias a Cristina, Irene, Victoria y Celso, Jairo, Pepe, Guille, Borja, Eli, Daniela, Palma, Miguel, Luis, Esther, Mireia de Castellón, María, Fernando, los Acosta, y un largo etcétera de la enorme familia de i-MUDS. Gracias por vuestra inspiración investigadora, vuestra alegría y hacerme disfrutar de grandes momentos entre amigos. Gracias a Vicente, Mairena, Celia e Iván y a todo el equipazo de Cuenca. Gracias por vuestra calurosa acogida y vuestro cariño en una semana llena de aprendizaje. Tack a Marie, y Christine por aquella maravillosa semana en Estocolmo. Ojalá haber podido compartir más momentos del camino con vosotros. Pero estoy segura de que llegarán. Caminar solo puede hacerte llegar más rápido, pero hacerlo acompañado te hará llegar más lejos.

Gracias a todo el equipo de CEMA: a los profes Robin y Jorge, a Viviana, Reinaldo, Aleja, Chiqui, Lisbeth, Pool, Ana, Mónica, Paula… De mis pasos por Colombia durante mi estancia, me llevo otra familia investigadora y una experiencia inolvidable y dejo parte de mi allí. Hicisteis que fuera uno de los momentos más especiales de este viaje por muy lejos que me encontrase de mi casa.

Gracias a todas las personas que habéis compartido esas paradas en el camino llamados congresos. Aprender de grandes personas siempre es un lujo, pero más compartir luego momentos que demuestran que la ciencia no tiene por qué ser aburrida. A todo el equipazo de Cádiz, Cuenca, GENUD Zaragoza y Toledo, Granada, Madrid y a toda la gran familia EXERNET, gracias. Caminar juntos nos hace mejores. Gracias a Rocío, y a los chicos de Cataluña y Toledo por hacer de Orlando una experiencia mágica.

Y por último, aunque no menos importante, gracias a toda mi familia y a mis amigas, a todas las personas importantes que habéis formado parte de esto. Gracias por ser ese apoyo y esa compañía incondicional en todos los caminos, también en este; a pesar de las ausencias provocadas por recorrerlo y que no siempre sea entendido. Gracias por vuestra comprensión y vuestros ánimos en todo momento. Gracias a mi madre por haberlo dado

todo siempre por mi para que pudiera recorrer los caminos que quisiera. Gracias por tu confianza y todas las grandes lecciones inculcadas. Gracias por ser norte. A mis hermanos, por ser brújula. A mi padre. Gracias a mis tías, tíos y primos por vuestras sonrisas, por dar sentido a la palabra familia. A Inés, aunque ya no puedas dar más pasos, siempre seguiré los tuyos. A mi abuela, por ser la mejor persona que conozco e inspirarme para ser mejor cada día por querer parecerme, aunque sea solo un poco, a ti. A mi cuadrilla, a mi equipo, a mis compañeros de piso y a todas las personas que tras este camino os considero amigos. Gracias también a ti.

En definitiva, gracias a todos vosotros. Al empezar este camino, no sabía a dónde me iba a llevar. Sin embargo, esta andadura ha superado con creces cualquier expectativa. Habéis sido huella, estrella en la noche del camino. Me habéis enseñado que una sonrisa, es mucho más importante que cualquier *P* menor a 0.05. Que el efecto, el impacto realmente importante es aquel que provocas en los demás. Y no el de una variable o cualquier revista. Me habéis enseñado la importancia de hacer cada vez más grande, en este caso, el intervalo de confianza. Que los grupos más relevantes, por *random* que sean, no son los de intervención, sino los equipos, las personas que los conforman. Esa variabilidad individual de cada uno de nosotros, lo que nos hace especiales. Gracias por demostrar, sin necesidad de rechazar una hipótesis nula, la importancia de ~~una~~ ser constante, de que puedo contar con vosotros siempre sin necesidad de ninguna estadística. Gracias por demostrarme que aunque creas que has llegado al límite, siempre queda otra respuesta a la pregunta. Siempre puede haber otro *reject* más antes de un *accepted*. A veces es la última llave la que abre la puerta. Gracias por enseñarme el método para ser más feliz, aunque no siempre sepa aplicarlo. Gracias por enseñarme que la meta es el camino. Gracias por ayudarme a levantar tras cada tropiezo. Gracias a lo bueno y a lo no tan bueno. Las dificultades son las otras grandes maestras, perderse enseña más que el camino recto. Gracias a la piedra y a la ignorancia, gracias a vosotras sé todo lo que sé y sé que aún ignoro mucho más que lo aprendido. Gracias al camino por enseñarme realmente lo que significaba que la paciencia es la madre de la ciencia. El destino no estará más cerca por muchas ganas que tenga de que llegue. Porque lo que me llevará al final será mis pasos y no el camino. Se hace camino al andar. En este caso: “científico, no hay ciencia, se hace la ciencia al investigar”. Gracias a cada uno de vosotros por ser objetivo, por ser la evidencia de que quien quiere puede alcanzar sus sueños. Por ser el *brackground* de mi mochila vital. Gracias por el resultado significativo de cada una de vuestras palabras. Soy la consecuencia de vuestros pasos a mi lado. La discusión siempre ha sido para elegir la mejor senda. Sin embargo, en este camino aún no hay conclusiones. Esto no ha hecho más que empezar. Espero seguir caminando, aprendiendo y mejorando, siempre a vuestro lado.

Infinita y eternamente agradecida.

Gracias.

CONTENTS/ÍNDICE

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The prevalence of childhood overweight and obesity has increased in the last decades becoming a global *pandemia*. Children with obesity have a higher risk of developing cardiovascular diseases, type 2 diabetes, and non-alcoholic fatty liver disease (NAFLD). NAFLD is considered the most common liver disease in childhood and as the hepatic component of the metabolic syndrome. Higher levels of physical fitness and physical activity are related with healthier profiles of cardiovascular health, insulin resistance and other health conditions y children and adolescents. However, to date, limited data are available regarding the association of physical fitness and physical activity with hepatic steatosis in children. Lifestyle interventions represent the cornerstone in the management of paediatric NAFLD. However, there are no previous studies examining the effect of a supervised exercise program in addition to a familiar lifestyle intervention on percentage hepatic fat measured by accurate methods in prepubertal children. Likewise, specific exercise recommendations (regarding type, intensity, frequency and duration) to reduce hepatic steatosis have been not provided in youth, and little is known about the role of the improvements in physical fitness on percentage hepatic fat changes after an exercise intervention in children and adolescents.

Therefore, the objectives of the present International Doctoral Thesis were: **I**) to examine whether cardiorespiratory fitness is related to liver enzymes levels in European adolescents independent of waist circumference, and to test whether having a high cardiorespiratory fitness is associated with an improved liver enzyme profile in those adolescents with a high waist circumference; **II**) to explore the association of physical fitness components and physical activity with percentage hepatic fat, liver enzymes levels, insulin resistance, and cardiometabolic risk in children with overweight/obesity; **III**) to systematically analyse the effects of supervised exercise interventions on percentage hepatic fat and hepatic steatosis prevalence in children and adolescents, to investigate the most appropriate design of exercise in terms of the type, intensity, volume and frequency, for the management of paediatric hepatic steatosis, and to explore whether exists an association of improvements in cardiorespiratory fitness and musculoskeletal

fitness with the reduction of percentage hepatic fat in youth; **IV**) to determine whether a 22-week multicomponent family-based lifestyle and psycho-education program plus a supervised exercise intervention, is more effective reducing percentage hepatic fat, than the lifestyle program alone in children with overweight/obesity; and **V**) to analyse the interindividual variability and to compare the prevalence of responders regarding percentage hepatic fat, total and abdominal adiposity, and gamma-glutamyl-transferase levels among children with overweight/obesity participating in a 22-week lifestyle intervention, with or without supervised exercise.

To address these objectives, five studies were conducted in the context of two projects: the HELENA cross-sectional study and the EFIGRO study.

The conclusions of this Thesis were: **I**) high cardiorespiratory fitness levels may attenuate the deleterious consequences associated with a high waist circumference on liver enzymes in adolescents; **II**) high levels of cardiorespiratory fitness seem to have specific protective effects on hepatic fat, liver enzyme levels, insulin resistance, and cardiometabolic risk in children with overweight/obesity; **III**) supervised exercise alone reduces percentage hepatic fat in children and adolescents and, its combination with lifestyle activities, is an effective approach to reduce hepatic steatosis prevalence in youth. Both aerobic and resistance exercise at vigorous or moderate-to-vigorous intensities in sessions of at least 60 minutes and with a minimum of 3 sessions per week, focused on increasing cardiorespiratory fitness and musculoskeletal fitness, should be included in the design of exercise programs for the prevention and management of hepatic steatosis in children and adolescents; **IV**) a 22-week family-based multicomponent intervention program that included supervised exercise in addition to a lifestyle- and psycho-education program has the potential to reduce hepatic steatosis, total and abdominal fat, and insulin resistance in preadolescent children with overweight/obesity. These findings highlight the importance of including supervised exercise in addition to other lifestyle components for the management of hepatic steatosis and related health risks in children with overweight/obesity; and **V**) the addition of supervised exercise to a

22-week family-based lifestyle educational intervention seems to increase the rates of responders for percentage hepatic fat, adiposity, and gamma-glutamyl-transferase levels in children with overweight/obesity after the intervention. Improvements in cardiorespiratory fitness may explain the differences between responders and non-responders for weight and body mass index.

Altogether, the findings of this Thesis may have practical and clinical implications for the design of lifestyle interventions focused on the prevention/treatment of paediatric obesity and hepatic steatosis, as well as other related comorbidities such as type 2 diabetes and cardiovascular diseases. Specifically, exercise training, both aerobic and resistance exercise at least at moderate-to-vigorous intensity, in sessions of 60 minutes and 3 sessions per week and focused on the improvements of cardiorespiratory fitness, should be included in programs for the management of hepatic steatosis and related health risks in youth, and particularly in children with overweight/obesity.

RESUMEN

La prevalencia de sobrepeso y obesidad infantil ha aumentado en las últimas décadas llegando a convertirse en una pandemia global. Las niñas y niños con obesidad tienen un mayor riesgo de desarrollar enfermedades cardiovasculares, diabetes tipo 2 e hígado graso no alcohólico (NAFLD en sus siglas en inglés). La NAFLD está considerada como la enfermedad hepática más común en la infancia y como el componente hepático del síndrome metabólico. Mayores niveles de condición física y actividad física se asocian, entre otros aspectos relacionados con la salud, con una mejor salud cardiovascular y una menor resistencia a la insulina en niños y adolescentes. Sin embargo, hasta la fecha se dispone de muy poca información acerca de la asociación de la condición física y la actividad física con la esteatosis hepática en edad pediátrica. Las intervenciones basadas en los estilos de vida son el pilar fundamental en el manejo de la NAFLD infantil. Sin embargo, no hay estudios previos que examinen el efecto de la adición de un programa de ejercicio físico supervisado a una intervención familiar basada en los estilos de vida sobre la grasa hepática medida mediante métodos precisos en población pediátrica prepuberal. Igualmente, no hay recomendaciones específicas de ejercicio físico (en relación al tipo, intensidad, frecuencia y duración) para la reducción de esteatosis hepática en jóvenes; además, se conoce muy poco acerca del papel de la condición física en los cambios del porcentaje de grasa hepática tras una intervención de ejercicio físico en niños y adolescentes.

Por lo tanto, los objetivos principales de esta Tesis Doctoral Internacional fueron: **I**) examinar si la capacidad cardiorrespiratoria se relaciona con la concentración plasmática de enzimas hepáticas en adolescentes europeos independientemente de la circunferencia de cintura, y testar si tener una mayor capacidad cardiorrespiratoria está asociada con un mejor perfil de enzimas hepáticas en aquellos adolescentes con una mayor circunferencia de cintura; **II**) explorar la asociación de los componentes de la condición física y la actividad física con el porcentaje de grasa hepática, las concentraciones plasmáticas de enzimas hepáticas, la resistencia a la insulina y el riesgo

cardiometabólico en niñas y niños con sobrepeso u obesidad; **III**) analizar sistemáticamente el efecto del ejercicio físico supervisado en el porcentaje de grasa hepática y en la prevalencia de esteatosis hepática en niños y adolescentes; investigar el diseño de ejercicio más apropiado en términos de tipo, intensidad, volumen y frecuencia, para el manejo de la esteatosis hepática pediátrica; y explorar si existe una asociación entre las mejorar de la capacidad cardiorrespiratoria y la fuerza muscular con la reducción del porcentaje de grasa hepática en jóvenes; **IV**) determinar si un programa de intervención multicomponente de 22 semanas de duración, basado en educación familiar en estilos de vida saludable y psicoeducación que incluya además ejercicio físico supervisado, es más efectivo reduciendo el porcentaje de grasa hepática que solamente el programa educativo en niñas y niños con sobrepeso u obesidad; y **V**) analizar la variabilidad interindividual y comparar la prevalencia de respondedores en relación con el porcentaje de grasa hepática, la adiposidad total y abdominal y las concentraciones plasmáticas de gamma-glutamil-transferasa en los niñas y niños participantes en una intervención basada en educación familiar en estilos de vida saludable y psicoeducación de 22 semanas de duración, que incluya o no ejercicio físico.

Para dar respuesta a estos objetivos, se llevaron a cabo cinco estudios en el contexto de dos proyectos: el estudio trasversal HELENA y el estudio EFIGRO.

Las conclusiones de esta Tesis fueron: **I**) una elevada capacidad cardiorrespiratoria podría atenuar las consecuencias negativas de la circunferencia de la cintura sobre las concentraciones plasmáticas de las enzimas hepáticas en los adolescentes; **II**) una elevada capacidad cardiorrespiratoria podría tener efectos beneficiosos específicos sobre la grasa hepática, las concentraciones plasmáticas de las enzimas hepáticas, la resistencia a la insulina y el riesgo cardiometabólico en niñas y niños con sobrepeso u obesidad; **III**) el ejercicio físico supervisado reduce el porcentaje de grasa hepática en los menores, y conjuntamente con otras actividades basadas en los cambios del estilo de vida, es una estrategia eficaz para reducir la prevalencia de esteatosis hepática en población pediátrica. Tanto el ejercicio aeróbico como el de fuerza, a

intensidades vigorosas o de moderadas a vigorosas, en sesiones de al menos 60 minutos de duración, con un mínimo de 3 sesiones por semana, con el objetivo de aumentar la capacidad cardiorrespiratoria y la fuerza muscular, deberían ser incluidos en el diseño de los programas de ejercicio para la prevención y el tratamiento de la esteatosis hepática en niños y adolescentes; **IV**) un programa de intervención familiar multicomponente de 22 semanas de duración que incluye ejercicio físico supervisado además de un programa basado en educación familiar en estilos de vida saludable y psicoeducación, es capaz de reducir la esteatosis hepática, la adiposidad total y abdominal, y la resistencia a la insulina en niñas y niños preadolescentes con sobrepeso u obesidad; y **V**) la adición de un programa de ejercicio físico supervisado a un programa de educación familiar de 22 semanas de duración, basado en educación familiar en estilos de vida saludable y psicoeducación, parece aumentar la tasa de respondedores en cuanto al porcentaje de grasa hepática, la adiposidad y las concentraciones plasmáticas de gamma-glutamil-transferasa en niñas y niños con sobrepeso u obesidad tras la intervención. Las mejoras en la capacidad cardiorrespiratoria podrían explicar las diferencias entre respondedores y no respondedores en relación con el peso y el índice de masa corporal.

Los resultados de esta Tesis pueden tener implicaciones clínicas y prácticas a la hora de diseñar intervenciones basadas en los estilos de vida y el ejercicio físico para la prevención o el tratamiento de la obesidad y la esteatosis hepática pediátrica, así como de otras comorbilidades asociadas como la diabetes tipo 2 o las enfermedades cardiovasculares. Concretamente, el ejercicio físico supervisado, tanto el aeróbico como el de fuerza, realizado al menos a intensidad moderada a vigorosa, en sesiones de 60 minutos y 3 veces por semana, y cuyo objetivo sea el aumento de la condición física cardiorrespiratoria, debería formar parte de los programas de prevención y tratamiento de la esteatosis hepática y de los factores de riesgo cardiovascular en jóvenes, particularmente en niñas y niños con sobrepeso u obesidad.

LIST OF ABBREVIATIONS

^{1}H-MRS, Proton magnetic resonance spectroscopy

20mSRT, 20 meters shuttle run test

ALT, Alanine aminotransferase

AST, Aspartate aminotransferase

BMI, Body mass index

CRF, Cardiorespiratory fitness

CT, Computed tomography

CVD, Cardiovascular diseases

EFIGRO, The Effect of Exercise on Hepatic Fat on Overweight Children Study

FMI, Fat mass index

GGT, Gamma-glutamyl-transferase

HDL, High-density lipoprotein cholesterol

HELENA, Healthy Lifestyle in Europe by Nutrition in Adolescence Study

HOMA-IR, Homeostatic model assessment for insulin resistance

MF, Musculoskeletal fitness

MRI, Magnetic resonance imaging

MVPA, Moderate to vigorous physical activity

NAFLD, Non-alcoholic fatty liver disease

NASH, Non-alcoholic steatohepatitis

PA, Physical activity

T2D, Type 2 diabetes

TG/HDL ratio, Triglyceride-to-high density lipoprotein ratio

US, Ultrasonography

VPA, Vigorous physical activity

VO_2peak, Peak of volume of oxygen consumption

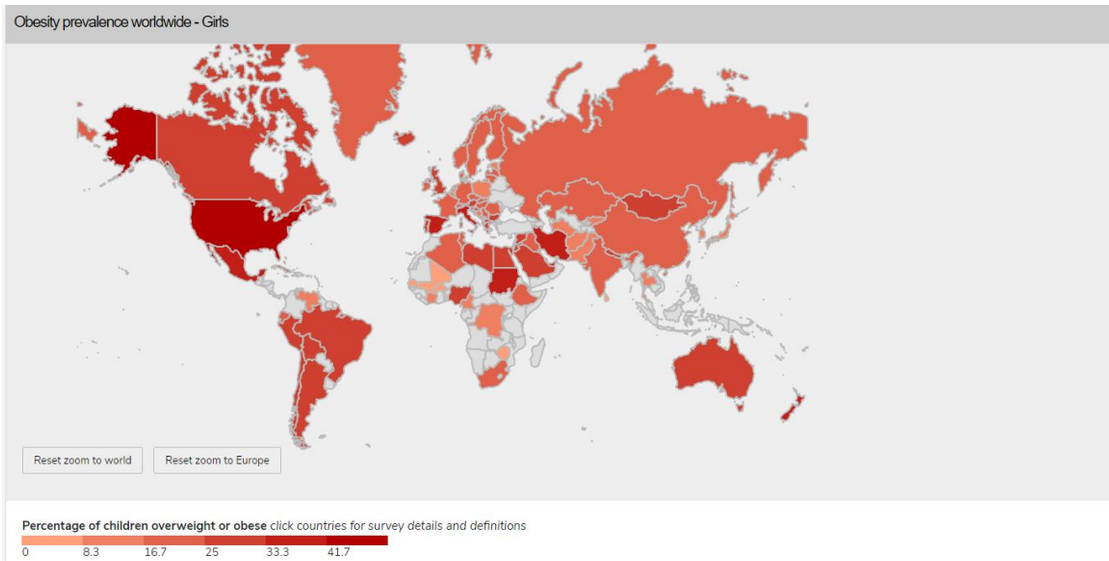
WC, Waist circumference

WHO, World Health Organization

1. GENERAL INTRODUCTION

1.1. Paediatric obesity: prevalence and health consequences

The prevalence of overweight and obesity among children and adolescents has increased in the last decades becoming a global *pandemia*[1]. According to the World Health Organization (WHO)[2], childhood obesity is one of the most serious public health concerns of this century. Over 340 million children aged 5-19 years had overweight or obesity in 2016, and the worldwide prevalence was 18%[3]. In our country, the prevalence of overweight and obesity in children and adolescents was nearly 40% (26% overweight, 12.6% obesity) in 2012, being the prevalence of 45% in children between 8 and 13 years and 25.5% in adolescents between 14 and 17 years[4]. More recently, the 2015 ALADINO Study[5] reported a prevalence of overweight and obesity of 23% and 18%, respectively in children aged 6-9 years. Approximately 60% of children with overweight before puberty will have overweight in early adulthood[6]. This problem, far from improving may worse. By 2025, it has been estimated that a total of 268 million children aged 5 to 17 years old will be overweight, 91 million will have obesity, 4 million will have type 2 diabetes (T2D), and 38 million will have hepatic steatosis[7]. Children with obesity have a higher risk of developing cardiovascular diseases (CVD), T2D, and non-alcoholic fatty liver disease (NAFLD) already in childhood[8], and later in adulthood[9].



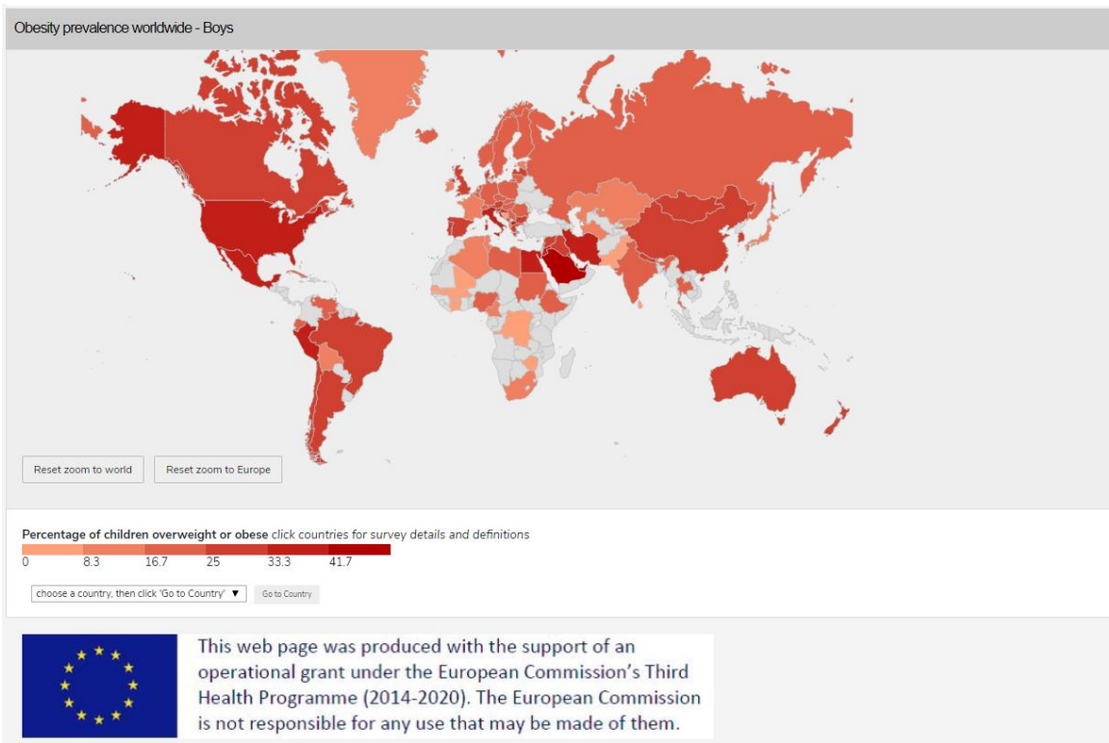


Image 1. Maps of obesity prevalence worldwide for girls and boys. Original images from the World Obesity-Global Obesity Observatory[10].

1.2. Paediatric non-alcoholic fatty liver disease and hepatic steatosis

1.2.1. Definition of paediatric non-alcoholic fatty liver disease and hepatic steatosis

Excess adiposity is associated with an accumulation of lipids in different tissues and organs, such as skeletal muscle or liver[11]. Specifically, the excessive accumulation of triglycerides in hepatocytes leads to hepatic steatosis[12]. Paediatric NAFLD is considered as a chronic hepatic steatosis in children (≤18 years) in the absence of genetic, infections, steatogenic medications use, or alcohol intake[12]. NAFLD is an umbrella term that includes a *spectrum* of conditions from a simple accumulation of lipids in hepatocytes to different grades of hepatic damage[13]. In 20-25% of cases, simple hepatic steatosis can progress to steatohepatitis (NASH)[14], which implies inflammation in addition to fat accumulation in liver cells[15]. NASH may progress to fibrosis and subsequently to cirrhosis[14] and its complications, such as liver failure[15] or hepatocellular carcinoma[16]. Several authors establish the presence of fat over 5.0% of hepatocytes to define hepatic steatosis[17], while others propose 4.85%[18] or 5.5%[19] as cut points. According to the percentage of hepatic fat content, hepatic steatosis could be classified as mild (<9%), moderate (9%-19%) or severe (>19%)[18].

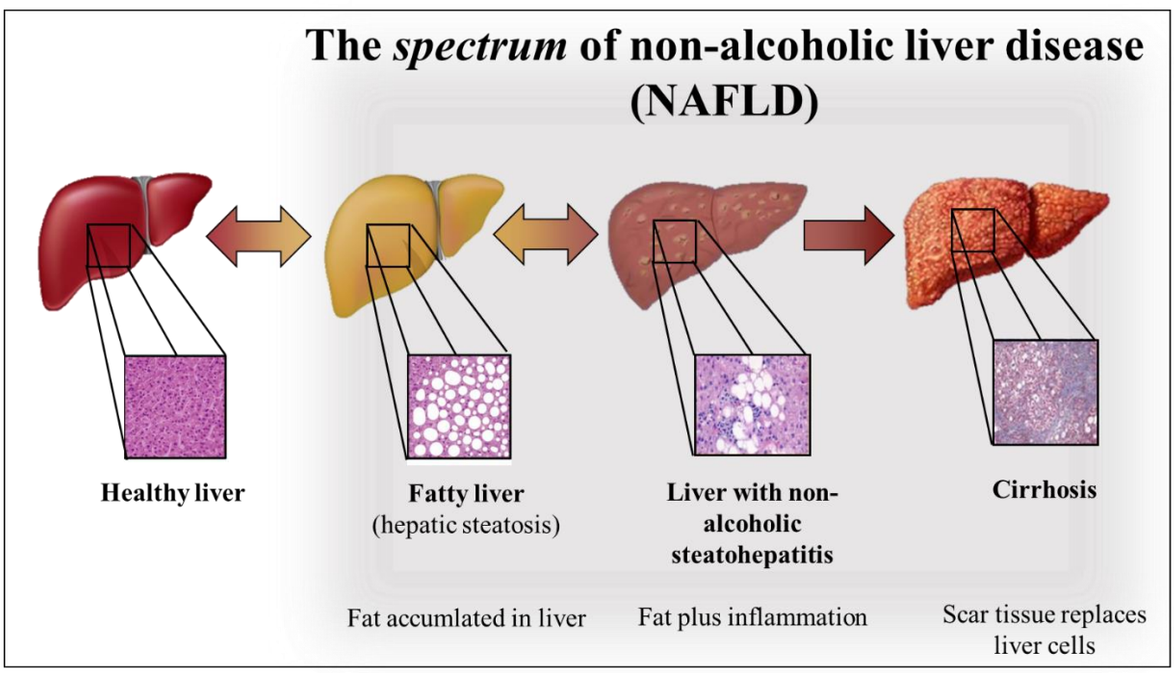


Image 2. *Sprectrum* and progression of NAFLD.

1.2.2. Prevalence of paediatric non-alcoholic fatty liver disease and hepatic steatosis

Nowadays, NAFLD is considered as the most common liver disease in childhood[20], affecting nearly 8% of general paediatric population, and 34% of children with obesity[21], which supposes a serious public health concern. It is important to note that the prevalence of NAFLD differs according to the population studied and the diagnostic tool employed[21]. Hepatic steatosis is more prevalent in older youth (0.7% of children aged 2-4 years old *vs.* 17.3% adolescents aged 15-19 years old), in males (11.1% of boys *vs.* 7.9% of girls), children with a higher body mass index (BMI) (0% of children with underweight, 5% with normal weight, 16% with overweight, and 38% with obesity), and in Hispanic (11.8%) or Asian populations (10.2%) (white populations: 8.6%, and black populations: 1.5%)[20]. It has been estimated that 23% of the children with hepatic steatosis have NASH, and that among them, 9% develop fibrosis or cirrhosis[20]. In Europe, it has been reported that the prevalence of NAFLD is between 2% and 44% in all general paediatric population, and between 36% and 44% in children and adolescents with overweight or obesity[22]. In Spain, there are no studies of NAFLD prevalence in children, but available data in adults (17-85 years) reveal a NAFLD prevalence of 25.8%[23]. In economic terms, the NAFLD represents an important economic burden in Europe, with an annual cost of about €35 billion (354-1.163€ per patient), which may increase because the incidence of NAFLD continues raising[24].

1.2.3. Diagnosis of non-alcoholic fatty liver disease

NAFLD is commonly asymptomatic until significant liver damage or other associated comorbidities appear[25]; therefore, it is often identified incidentally due to other conditions[12]. The mean age of diagnosis in youths is about 11-13 years old[26]. The early identification of NAFLD in children, before the development of fibrosis which would be

irreversible, is important[12]. Different diagnosis techniques are employed to identify NAFLD. Liver biopsy is the gold standard for the diagnostic of NAFLD/NASH and the assessment of its severity[27,28]. However, because of its invasiveness, potential complications, and high economic cost, biopsy is considerate a suboptimal tool for the diagnosis of NAFLD[28]. In consequence, other non-invasive measures, such as ultrasonography (US), computed tomography (CT), proton magnetic resonance spectroscopy (^{1}H-MRS), or magnetic resonance imaging (MRI), are employed for NAFLD detection and assessment[28]. Although other non-invasive methods have insufficient sensitivity and specificity[20], MRI is considered as an accurate method for detecting and quantifying hepatic steatosis in children[29]. High plasma concentrations of liver enzymes (alanine aminotransferase (ALT) and gamma-glutamyl-transferase (GGT)) have been proposed as surrogate biomarkers of NAFLD in youth[30]. However, it has been shown that liver enzymes alone are inadequate for the identification of hepatic steatosis, particularly in children[26].

1.2.4. Health consequences of paediatric non-alcoholic fatty liver disease

NAFLD is strongly associated with excess adiposity, insulin resistance and other metabolic risk factors such as dyslipidaemia, and elevated blood pressure[31,32]. Children with overweight/obesity and with NAFLD have higher frequency of central obesity, insulin resistance, dyslipidaemia, and hypertension than children with overweight/obesity, but with a healthy liver[31]. Among children with overweight/obesity, those with NAFLD have at least one metabolic syndrome factor, and are more likely to have metabolic syndrome than their peers without NAFLD[31]. In fact, NAFLD is considered as the hepatic component of the metabolic syndrome, due to its strong relationship with obesity, insulin resistance and dyslipidemia[31–33]. Moreover, NAFLD, specifically NASH and advanced fibrosis, increases the risk of all-cause mortality in

adults[34]. On the other hand, high plasma levels of liver enzymes (ALT, GGT and aspartate aminotransferase (AST)) are also associated with cardiovascular disease risk factors and insulin resistance in youths[30]. Likewise, the AST/ALT ratio is a hepatic biomarker inversely associated with cardiovascular health in adolescents[30] and, recently, it has been related to cardiovascular mortality and hepatic complications such as fibrosis and cirrhosis in adults[35,36]. In children and adolescents, another strong marker of cardiovascular disease risk[37], the triglyceride-to-high density lipoprotein (TG/HDL) ratio, is associated with hepatic steatosis, and also with cardiovascular risk and insulin resistance[38].

1.2.5. Prevention and treatment of paediatric hepatic steatosis: role of exercise and lifestyle interventions

The prevention and treatment of paediatric NAFLD have become a challenge for paediatric practitioners and health care systems[12]. Currently, lifestyle interventions, including improvements in dietary habits and physical activity (PA) levels or exercise, are the cornerstone in the management of paediatric NAFLD due to its association with adiposity reduction[12] and other health benefits[39]. There is evidence that sedentary lifestyle and unhealthy diets are driving the *pandemia* of obesity and its comorbidities in youth. Therefore, multidisciplinary family-based lifestyle interventions including nutritional education, psycho-education, and PA are recognized as key factors in the obesity treatment or prevention in the paediatric population, since they have been demonstrated to be effective in the improvement of weight status and adiposity, cardiovascular disease risks factors, insulin resistance, nutritional habits and physical fitness in children and adolescents[40–44]. In particular, several studies have analysed the specific role of exercise (alone or in combination with other lifestyle components) on adiposity[40], cardiovascular disease risk factors and insulin resistance[45], which should be a general and additional goal

of the treatment of NAFLD in children with overweight[12]. However, there is scarce evidence on the effects of lifestyle interventions in hepatic steatosis in youth[39,46,47]. More specifically, there are no previous studies examining the effect of a supervised exercise program in addition to a lifestyle intervention on changes in percentage hepatic fat measured by accurate methods in prepubertal children. Moreover, to date, specific exercise recommendations (regarding type, intensity, frequency or duration) to reduce hepatic steatosis in children and adolescents are not provided.

On the other hand, the vast majority of published studies only report the effect of an intervention as overall mean value[48]. Nevertheless, it exits an interindividual variability in response to the same intervention[49]; whereas some participants show an improvement in one or several outcomes (responders), others show an unchanged or even a worsened response (non-responders). In children and adolescents, previous studies examining the effect of either exercise or lifestyle interventions on changes on percentage hepatic fat only reported mean values[50,51], but not interindividual variability. In order to be as effective as possible in the prevention/treatment of obesity and obesity-related comorbidities, it would be interesting to know the most appropriate an effective design for reducing the number of children not achieving beneficial effects on health.

1.3. Physical fitness and physical activity

1.3.1. Definition and measurement of physical fitness and physical activity

Physical fitness is defined as "the capacity to perform physical activity, and makes reference to a full range of physiological and psychosocial qualities"[52]. This set of attributes is determined by a combination of physical activity and genetic, and it is related with health or performance[53]. Physical fitness can be measured using laboratory or field tests. The Alpha Fitness test battery is a compendium of different valid and reliable tests (20 meters shuttle run test (20mSRT), handgrip, standing broad jump, and 4x10 meters,

principally) created and employed to evaluate the different components (cardiorespiratory fitness (CRF), upper and lower musculoskeletal fitness (MF), and speed agility, respectively) of physical fitness related with health in youth[54,55]. The gold standard for the assessment of CRF is a direct cardiopulmonary progressive incremental test performed with metabolic gas exchange to exhaustion[56]. However, laboratory tests have a considerable cost and need highly trained professionals, making them not feasible for use with large groups of participants[57].

PA is any body movement produced by skeletal muscles that results in an energy expenditure[53], which would encompass practically all the daily activities of a person. Exercise is a subcategory of PA; it is structured, planned and repetitive and its aim is to improve or maintain physical fitness[53]. Self-reported questionnaires have been employed to assess PA in large epidemiological studies or interventions[58]. However, PA questionnaires have poor reliability and validity, especially in younger populations[59]. Therefore, accelerometry has become an objective and feasible alternative to self-report methods to measure PA levels in children and adolescents[59,60].

1.3.2. Role of physical fitness and physical activity on health

A growing body of evidence shows that physical fitness is a powerful marker of health in children and adolescents. In adults, lower levels of CRF are associated with increased all-cause mortality in both sexes[61]. In children and adolescents, CRF, MF, and speed-agility[52] have been associated with cardiovascular health[52,62], insulin resistance, and other health conditions in children and adolescents[52].

The current international PA guidelines of the WHO[63] and the "Centers for Disease Control and Prevention"[64] for the promotion of health in children and adolescents recommends 60 minutes of moderate to vigorous intensity PA (MVPA) every day or at

least 3 days per week in this population. Nevertheless, most children and adolescents over the world are inactive: they do not meet the guidelines of PA recommendations[65]. In Spain, 48.4% of children aged 9 to 12 years old and 62.6% of adolescents aged 13 to 17 years are not meeting the daily PA recommendations[66]. Physical inactivity is a health concern, because the lack of PA is associated with adiposity, cardiovascular disease risk factors and other health outcomes in children[67].

1.3.2.1. Physical fitness and physical activity: specific role on hepatic health

As mentioned above, physical fitness and PA are associated with many health factors in youth. However, to date, limited data are available regarding the association of physical fitness and PA with hepatic steatosis[68–70] or liver enzyme levels in young people[39,69–75]. Some studies have reported inverse associations of CRF with hepatic fat content[68] or ALT[71–73], while others did not[74]. Regarding PA, a longitudinal study, reported that children with higher levels of total PA and MVPA (measured by accelerometery) at ages 12 to 14 years had lower odds of liver fat (US) and lower GGT levels in the late adolescence[69]. Other study showed that children with hepatic steatosis spent 66.9% of waking hours in sedentary activities (PA assessed by questionnaire)[70].

Greater improvements in CRF levels after exercise interventions have been associated with reductions in adiposity and percentage hepatic fat in adults[76], and adolescents at risk of T2D[77]. However, there is scarce evidence supporting the relationships between CRF and MS with the reduction of percentage hepatic fat in youth. Moreover, little is known about the role of changes in physical fitness after an exercise program on interindividual variability in the response to the intervention.

2. HYPOTHESIS AND OBJECTIVES/

HIPÓTESIS Y OBJETIVOS

2.1. Hypothesis

The hypothesis of the present International Doctoral Thesis is that supervised exercise will reduce percentage hepatic fat in children, and that this reduction will be associated with improvements in physical fitness.

2.2. Objectives

The objectives of this International Doctoral Thesis are:

I. To examine whether higher cardiorespiratory fitness is related to lower liver enzyme levels in European adolescents independent of waist circumference, and to test whether having a high cardiorespiratory fitness is associated with a healthier liver enzyme profile in those adolescents with an elevated waist circumference (Study I).

II. To explore the associations of physical fitness components and physical activity levels with percentage hepatic fat, plasma liver enzyme concentrations, insulin resistance, and cardiometabolic risk in children with overweight/obesity (Study II).

III. To systematically analyse the effects of supervised exercise interventions on percentage hepatic fat and hepatic steatosis prevalence in children and adolescents; to investigate the most appropriate design of exercise in terms of the type, intensity, volume and frequency for the management of paediatric hepatic steatosis; and to explore whether improvements in cardiorespiratory fitness and musculoskeletal fitness are associated with the reduction of percentage hepatic fat in youth (Study III).

IV. To determine whether a 22-week multicomponent family-based lifestyle and psycho-education program plus a supervised exercise intervention, is more effective reducing percentage hepatic fat, than the lifestyle program alone in children with overweight/obesity. The effects of these interventions on physical fitness, fat mass,

cardiovascular and type 2 diabetes risk factors, dietary habits, physical activity and psychological well-being, are also analysed (Study IV).

V. To analyse the interindividual variability and to compare the prevalence of responders regarding percentage hepatic fat, total and abdominal adiposity, and gamma-glutamyl-transferase levels among children with overweight/obesity participating in a 22-week lifestyle intervention, with or without supervised exercise (Study V).

2.1. Hipótesis

La hipótesis de esta Tesis Doctoral Internacional es que el ejercicio físico supervisado reducirá el porcentaje de grasa hepática en niños, y que dicha reducción estará asociada a las mejoras de la condición física.

2.2. Objetivos

Los objetivos de esta Tesis Doctoral Internacional son:

I. Examinar si la condición física cardiorrespiratoria se relaciona con la concentración plasmática de las enzimas hepáticas en adolescentes europeos, independientemente de la circunferencia de cintura, y testar si tener una alta capacidad cardiorrespiratoria está asociada con un mejor perfil de enzimas hepáticas en aquellos adolescentes con una circunferencia de cintura elevada (Estudio I).

II. Explorar la asociación de la condición física y la actividad física con el porcentaje de grasa hepática, la concentración plasmática de las enzimas, la resistencia a la insulina y el riesgo cardiometabólico en niñas y niños con sobrepeso u obesidad (Estudio II).

III. Analizar de forma sistemática el efecto del ejercicio físico supervisado sobre el porcentaje de grasa hepática y la prevalencia de esteatosis hepática en niños y adolescentes; investigar cuál es el diseño más apropiado para el manejo de la esteatosis hepática pediátrica en términos de tipo, intensidad, volumen y frecuencia del ejercicio físico; y explorar si existe alguna asociación entre las mejoras de la capacidad cardiorrespiratoria y la fuerza muscular con la reducción del porcentaje de grasa hepática en los jóvenes (Estudio III).

IV. Determinar si un programa de intervención familiar multicomponente de 22 semanas de duración, basado en educación familiar en estilos de vida saludable y psicoeducación que incluya además ejercicio físico supervisado, es más efectivo reduciendo el porcentaje de grasa hepática que solamente el programa basado en el cambio de estilo de vida y psicoeducación en niñas y niños con sobrepeso u obesidad. El efecto de dichas intervenciones sobre la condición física, la masa grasa, los factores de riesgo de enfermedad cardiovascular y diabetes tipo 2, los hábitos dietéticos, la actividad física y el bienestar psicológico también será analizado (Estudio IV).

V. Analizar la variabilidad interindividual y comparar la prevalencia de respondedores en relación con el porcentaje de grasa hepática, la adiposidad total y abdominal y la concentración plasmática de gamma-glutamil-transferasa en los niñas y niños participantes en una intervención, basada en educación familiar en estilos de vida saludable y psicoeducación de 22 semanas de duración, que incluya o no ejercicio físico (Estudio V).

3. RESULTS

3.1. Study I

Published in: Journal of Science and Medicine in Sport. 2017;20(10);932-936.

Journal of Science and Medicine in Sport 20 (2017) 932–936

Contents lists available at ScienceDirect

Journal of Science and Medicine in Sport

journal homepage: www.elsevier.com/locate/jsams

Cardiorespiratory fitness, waist circumference and liver enzyme levels in European adolescents: The HELENA cross-sectional study

María Medrano[a], Idoia Labayen[a,*], Jonatan R. Ruiz [b,c], Gerardo Rodríguez[d], Christina Breidenassel[e], Manuel Castillo[f], Raquel Pedrero[g], Kurt Widhalm[h], Anthony Kafatos[i], Yannis Manios[j], Dénés Molnar[k], Marcela González-Gross[g], Francisco B. Ortega[b,c], Luis A. Moreno[l,m]

[a]Department of Nutrition and Food Science, University of the Basque Country, UPV/EHU, Spain

[b]Department of Physical Education and Sport, School of Sport Sciences, University of Granada, Spain

[c]Department of Biosciences and Nutrition at NOVUM, Unit for Preventive Nutrition, Karolinska Institutet, Sweden

[d]Department of Pediatrics, Faculty of Medicine, University of Zaragoza, Health Research Institute of Aragon (IIS Aragón), Spain

[e]Department of Nutrition and Food Science, University of Bonn, Germany

[f]Departament of Physiology, Medicine School, University of Granada, Spain

[g]Department of Health and Human Performance, Faculty of Physical Activity and Sport Sciences-INEF, Universidad Politécnica de Madrid, Spain

[h]Division of Nutrition and Metabolism, Department of Pediatrics, Medical University of Vienna, Austria

[i]University of Crete School of Medicine, Greece

[j]Department of Nutrition and Dietetics, Harokopio University, Greece

[k]Department of Pediatrics, University of Pecs, Hungary

[l]Faculty of Health Sciences, University of Zaragoza, Spain

[m]Department of Preventive Medicine, Faculty of Medicine, University of Sao Paulo, Brazil

*Corresponding author. E-mail address: idoia.labayen@ehu.eus (I. Labayen).

Abstract

Objectives: 1) To examine whether cardiorespiratory fitness (CRF) is related to liver enzyme levels independent of waist circumference (WC), and 2) To test whether having a high CRF is associated with an improved liver enzyme profile with a high WC.

Design: Cross-sectional.

Methods: CRF (20m-shuttle-run test) and WC were assessed in 811 European adolescents (48.5% males) aged 12.5 to 17.5 years. Fatty liver biomarkers included fasting serum alanine-aminotransferase (ALT), gamma-glutamyl-transpeptidase (GGT) and the aspartate-aminotransferase to ALT (AST/ALT) ratio. Participants were categorized as fit or unfit (CRF below or above 43.8 mL/kg/min and 34.6 mL/kg/min, for boys and girls, respectively) and as high or non-high WC (sex and age-specific cut-offs).

Results: CRF was associated with ALT (β=-0.106; p=0.049) and GGT levels (β=-0.225; p<0.001) and AST/ALT ratio (β=0.234; p<0.001), yet these relationships were attenuated after further controlling for WC (all p>0.1). High WC and fit adolescents had lower ALT levels (28±1 U/L *vs*. 23±2 U/L, unfit and fit respectively, p=0.018) and higher AST/ALT ratio (0.94±0.04 *vs*. 1.10±0.06, unfit and fit respectively, p=0.010) than those who were high WC but unfit.

Conclusions: The results showed that CRF is not independently associated with liver enzymes, and that WC is a stronger predictor in adolescents. These findings also suggest that high CRF may have specific protective effects on liver enzyme levels in adolescents with high WC. Exercise programs focused on increasing CRF and decreasing abdominal adiposity could be a good alternative in the treatment and prevention of obesity related fatty liver disease in adolescents.

Key words: adolescents, nonalcoholic fatty liver disease, obesity, waist circumference, fitness, alanine-aminotransferase.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most frequent liver diseases in youths and it is related to type 2 diabetes and cardiovascular diseases later in life.[1] NAFLD is associated with abdominal adiposity[2] and it is considered the hepatic manifestation of metabolic syndrome.[3] In youth, elevated levels of fatty liver biomarkers are associated with abdominal obesity and cardiovascular disease risk factors.[4] Moreover, fatty liver biomarkers track from childhood to adulthood increasing all-cause mortality risk in adults.[5] Over the last years, the prevalence of NAFLD in childhood has increased due to the raise in obesity rates.[6] High levels of alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) and low aspartate aminotransferase/alanine aminotransferase (AST/ALT) ratio are considered as surrogate markers of NAFLD.[7]

High levels of physical activity and cardiorespiratory fitness (CRF) are associated with a healthier metabolic profile.[8] Likewise, CRF is an important marker of cardiovascular health in adolescents.[9] High levels of CRF are associated with improved liver function in obese children.[10] In a previous study, our group observed that moderate to vigorous physical activity was associated with a healthier liver enzyme profile in adolescents.[11] However, the relationship between CRF and liver enzymes in adolescents has not been previously examined. A previous lifestyle intervention study in adults showed that CRF at baseline predicted liver fat content reduction, independently of total adiposity, body fat distribution and exercise intensity.[12] Interestingly, several studies showed that youths with excess adiposity, but also with high levels of CRF (so called fat but fit youths) had better cardiometabolic profile than those who were obese, but had low CRF (i.e. fat but unfit youths).[13] As far as we are aware, of there are no previous studies examining the associations of CRF and WC with liver enzyme levels in adolescents. Moreover, the possible protective effect of CRF on liver enzyme profile in adolescents

with increased WC is unknown. Therefore, the aims of the present study were 1) To examine whether cardiorespiratory fitness (CRF) is related to liver enzyme levels in adolescents independent of waist circumference (WC), and 2) To test whether having a high CRF is associated with an improved liver enzyme profile in adolescents with a high WC.

2. Materials and methods

The HELENA study was conducted in 10 European cities from 9 countries from 2006 to 2008 in 3528 adolescents. Details of the methods and sampling techniques have been reported elsewhere.[14] The current study comprises 811 adolescents (48.5% males) aged 12.5 to 17.5 with valid data on CRF, WC and serum liver enzyme measurement (**Supplemental figure 1**).

Before being enrolled in the study, all adolescents and their parents signed an informed written consent. The study protocol was approved by local human research review committees of each involved city.

2.1. Physical examination

Height, weight, WC were measured in triplicate using standard protocols and body mass index (BMI) was calculated. WC was used as estimate of abdominal adiposity.[15] The presence of abdominal adiposity was estimated by WC according to the age and sex-specific criteria of Katzmarzyk et al.[16] Thereafter, adolescents were categorized into two groups as having or not having high WC (high-waist *vs*. non-high waist).[16] Pubertal stage (breast, genital development and pubic hair) was assessed according to Tanner and Whitehouse[17] by a trained researcher of the same sex of the adolescent.

2.2. Liver biomarkers

Blood samples were obtained by venipuncture after an overnight fast. ALT, AST and GGT levels were measured in serum using standard protocols with the clinical chemistry system RxL (Dade Behring, Schawalbach, Germany) and the AST/ALT ratio was calculated.

2.3. Cardiorespiratory fitness assessment

CRF was measured with the multistage 20 m shuttle run test as described by Léger et al.[18] Details of the test have been published elsewhere.[18] Maximal oxygen consumption (VO_{2max}) (mL/kg/min) was estimated using the equation published by Ruiz et al.[19] This test is reliable, feasible and valid at these ages.[19] Adolescents were categorized into two groups, fit and unfit, according to the sex-specific cut points that have been associated with high cardiometabolic risk.[9] Adolescents whose CRF level was below 43.8 mL/kg/min and 34.6 mL/kg/min, for boys and girls respectively, were classified as "unfit", and those adolescents whose CRF level was above these cut points were considered as "fit".[9]

2.4. Statistical Analyses

Differences in age, pubertal stage (categories), height, weight, BMI, WC, CRF and ALT, AST, GGT and AST/ALT ratio according to CRF (unfit *vs*. fit) and WC (high waist *vs*. non-high waist) groups were calculated using t-Student tests for independent samples (continuous variables) or Chi-square tests (categorical variables).

Variables with skewed distribution were transformed in a natural logarithmic basis. Linear regression analyses were used to examine the relationships of liver enzymes (dependent variables) with CRF and WC (independent variables, separately) adjusting for sex and pubertal stage (Model 1). In model 2, analyses examining the association of liver

enzymes with CRF were further adjusted with WC, and analyses examining the relationship of liver enzymes with WC were additionally adjusted with CRF. The appropriateness of the linear regression models was checked defining the residuals and examining residual plots. In sensitivity analyses, VO_{2max} was replaced by the number of stages completed in the 20m shuttle run test as measure of CRF. The combined influence of WC and CRF groups in liver enzyme levels was examined by ANCOVA with DMS post hoc using combined groups based on categories of WC and CRF creating 4 different groups: unfit and non-high waist, fit and non-high waist, unfit and high waist and fit and high waist. Sex and pubertal stage were included into the model as covariates. All analyses were performed using the Statistical Package for Social Sciences (SPSS, version 20.0 for WINDOWS; SPSS Inc, Chicago), and the level of significance was set at α=0.05.

3. Results

The descriptive characteristics of the sample across CRF and WC categories are shown in **Table 1**. Unfit adolescents had significantly higher body weight, BMI and WC (all $p<0.001$) than fit adolescents. Moreover, AST levels and the AST/ALT ratio were lower in unfit than in fit adolescents ($p<0.001$). In addition, participants with high WC had higher levels of ALT, GGT and lower AST/ALT ratio (all $p<0.001$) than adolescents with non-high WC.

Table 1. Descriptive characteristics across fitness (fit and unfit) and abdominal adiposity (non-high and high waist circumference) categories in adolescents participating in the HELENA study.

	n	Fit	n	Unfit	p	n	Non-high waist	n	High Waist	p
Age	454	14.7 (1.2)	357	14.7 (1.2)	0.277	661	14.7 (1.2)	150	14.9 (1.3)	0.099
Tanner stage (*n* in categories 1/2/3/4/5)	435	5/28/87/174 /141	300	0/17/68/134 /81	0.143	612	5/34/139/253/18 1	123	0/11/16/55/41	0.083
Height (cm)	454	167.0 (9.6)	357	163.4 (8.8)	**<0.001**	661	164.9 (9.2)	150	167.9 (9.8)	**0.001**
Weight (kg)	454	56.4 (10.6)	357	61.4 (14.3)	**<0.001**	661	54.7 (8.8)	150	75.9 (12.3)	**<0.001**
Body mass index ($kg \cdot m^{-2}$)	454	20.1 (2.5)	357	22.8 (4.3)	**<0.001**	661	20.1 (2.3)	150	26.9 (3.4)	**<0.001**
Waist circumference (cm)	454	70.3 (6.5)	357	74.7 (10.6)	**<0.001**	661	69.0 (5.2)	150	86.7 (6.5)	**<0.001**
Cardiorespiratory fitness (mL/kg/min)	454	50.0 (8.6)	357	32.9 (3.8)	**<0.001**	661	43.5 (11.1)	150	37.8 (8.7)	**<0.001**
ALT (U/L)	454	21 (7)	357	21 (9)	0.618	661	21 (7)	150	24 (9)	**<0.001**
AST (U/L)	454	23 (7)	357	21 (7)	**<0.001**	661	22 (7)	150	22 (8)	0.847
GGT (U/L)	452	17 (5)	356	17 (8)	0.533	658	16 (5)	150	20 (11)	**<0.001**
AST/ALT ratio	454	1.13 (0.31)	357	1.05 (0.30)	**<0.001**	661	1.12 (0.30)	150	0.96 (0.28)	**<0.001**

Values are mean SD unless otherwise indicated. p values were calculated by t-test examining differences between fit and fit groups and non-high waist and high waist groups. Fit and unfit categories and non-high and high waist. Adolescents were considered as "fit" when fitness was above 43.8 mL/kg/min in males and 34.6 mL/kg/min in females, respectively.[9] Abdominal adiposity was considered as high (high waist) according to Katzmarzyk et al.[16] ALT: alanine aminotransferase; ALT/AST ratio; AST: aspartate aminotransferase; GGT: gamma-glutamyltransferase. Boldfaced values: $p<0.05$.

Table 2 shows the associations of CRF and WC with liver enzyme levels in adolescents. It was observed that CRF was negatively associated with ALT ($p<0.05$) and GGT ($p<0.001$) levels and positively with AST ($p<0.05$) and AST/ALT ratio ($p<0.001$) after controlling for pubertal stage and sex (Model 1, Table 2). However, these relationships became non-significant when WC was entered into the model (Model 2, Table 2). WC was significantly associated with ALT ($p<0.001$) and GGT ($p<0.001$) levels and negatively associated with AST/ALT ratio ($p<0.001$) regardless sex and pubertal stage ($p<0.001$, Model 1, Table 2) and CRF levels (all $p<0.001$, Model 2, Table 2). In sensitivity analyses, VO_{2max} was replaced by the number of stages completed in the 20m shuttle run test as measure of CRF, and it was observed that the associations of CRF with liver enzymes were slightly attenuated, while the relationships between WC and liver enzymes did not substantially change.

Table 2. Standardized (β) and non-standardized (B) regression coefficients of the associations of cardiorespiratory fitness and waist circumference with alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT) and aspartate aminotransferase to alanine aminotransferase (AST/ALT) ratio.

	Cardiorespiratory fitness								Waist circumference							
	Model 1				Model 2				Model 1				Model 2			
	R^2	B	β	p	R^2	B	β	p	R^2	B	β	p	R^2	B	β	p
ALT (U/L)	0.066	-0.133	-0.106	**0.049**	0.096	0.113	0.093	0.127	0.145	0.656	0.239	**<0.001**	0.156	0.756	0.285	**<0.001**
AST (U/L)	0.137	0.120	0.108	**0.036**	0.141	0.085	0.077	0.192	0.140	-0.180	-0.073	0.054	0.141	-0.149	-0.061	0.188
GGT (U/L)	0.142	-0.260	-0.225	**<0.001**	0.150	-0.095	-0.083	0.157	0.169	0.515	0.200	**<0.001**	0.186	0.544	0.213	**<0.001**
AST/ALT	0.036	0.253	0.234	**<0.001**	0.121	-0.029	-0.027	0.656	0.124	-0.836	-0.361	**<0.001**	0.129	-0.905	-0.395	**<0.001**

ALT: alanine aminotransferase; ALT/AST: aspartate aminotransferase to alanine aminotransferase ratio; AST: aspartate aminotransferase; GGT: gamma-glutamyltransferase. Model 1: adjusted for sex and pubertal stage; Model 2: In analyses examining the influence of cardiorespiratory fitness, model 1 was further adjusted with waist circumference; in analyses examining the influence waist circumference Model 1 was additionally with cardiorespiratory fitness. Boldfaced values: p<0.05.

In the high WC group, the results showed that unfit adolescents had significantly higher ALT (25±1 U/L *vs*. 22±1 U/L, unfit and fit respectively, pubertal stage and sex adjusted p=0.028) and lower AST/ALT ratio (0.93±0.03 *vs*. 1.04±0.05, unfit and fit respectively, pubertal stage and sex adjusted p=0.014) than fit adolescents (Panels *a* and *d*, respectively, **Fig. 1**). There were not statistically significant differences between fit and unfit adolescents in GGT (21±1 U/L *vs*. 18±1 U/L, in unfit and fit respectively, adjusted p=0.124) and AST levels (22±1 U/L in both unfit and fit, adjusted p=0.592).

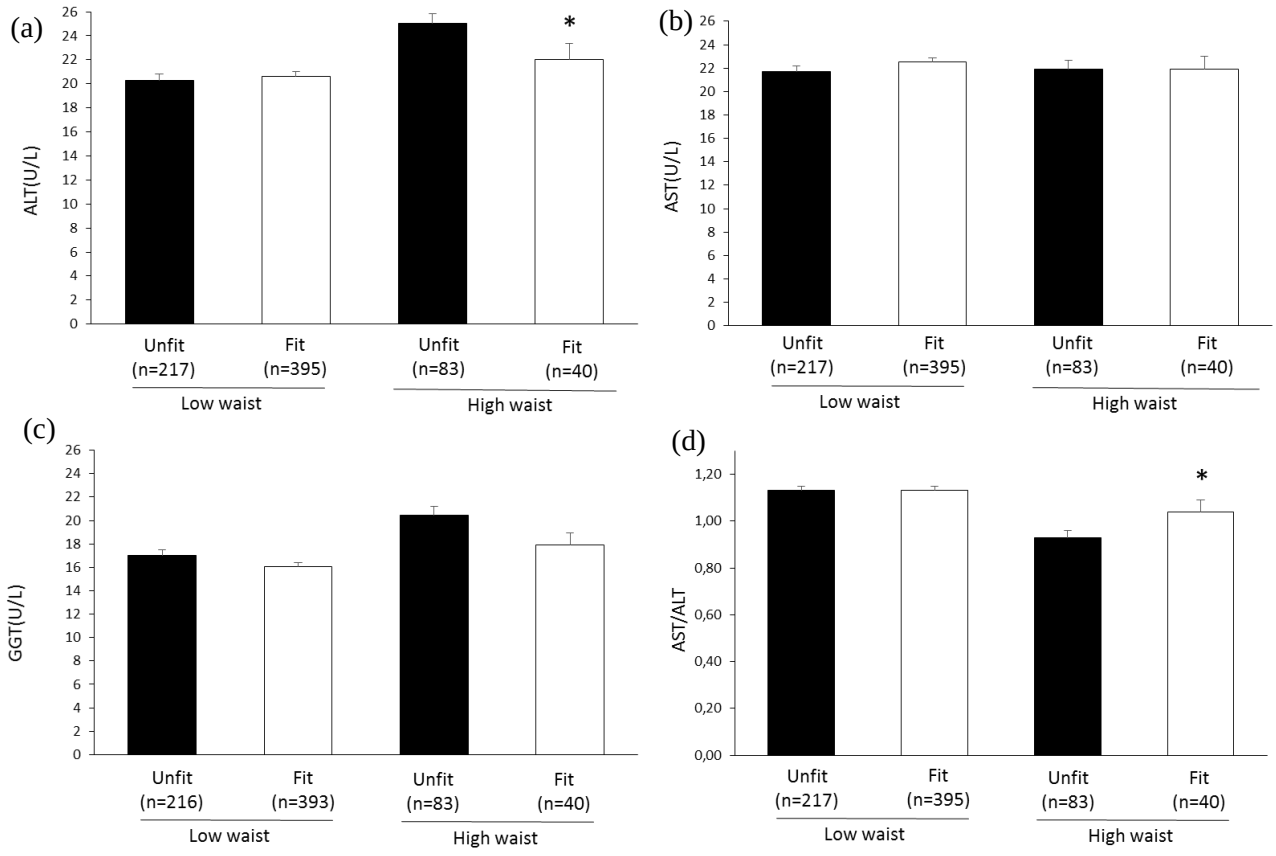


Figure 1. Levels of alanine aminotransferase (ALT) (a), aspartate aminotransferase (AST) (b), gamma-glutamyltransferase (GGT) (c) and aspartate aminotransferase to alanine aminotransferase ratio (AST/ALT) (d) according to waist circumference (non-high and high waist)[16] and CRF (unfit and fit) categories.[9] Adolescents were considered as "fit" when CRF was above 43.8 mL/kg/min in males and 34.6 mL/kg/min in females, respectively.[9] Adolescents were categorized as having high waist circumference according to Katzmarzyk et al., criteria.[16] Analyses were performed with logarithmic transformed values, but non-transformed data are shown for easier interpretation. Analyses were adjusted for sex and pubertal stage. *: p<0.05.

4. Discussion

The findings of the present study show that WC is a stronger predictor of liver enzyme levels than CRF in adolescents. The results also showed that having high CRF levels may attenuate the deleterious consequences associated with WC on liver enzymes in adolescents. We observed that those adolescents with high WC, but with high levels of CRF had lower levels of ALT and higher AST/ALT ratio than those adolescents who had high WC but who were unfit.

Several studies observed that children and adolescents classified as "fit" presented significantly lower total and abdominal adiposity than their "unfit" counterparts,[20] which concurs with our findings. Elevated levels of ALT and GGT, as well as lower AST/ALT ratio, likely reflect excessive lipid deposition in the liver.[7] Previous studies has been proposed that a healthier metabolic phenotype of overweight or obese individuals could be partially explained by higher CRF levels.[21] In contrast, other studies did not find any significant difference in CRF levels between metabolically healthy and unhealthy overweight/obese youths.[22] Interestingly, lower hepatic fat content and/or liver enzyme levels have been independently associated with the metabolically healthy phenotype in overweight/obese youths.[22]

In the current study, it was observed that fit adolescents had higher AST/ALT ratio than their unfit counterparts. Furthermore, our results showed that high CRF levels were associated with a healthier liver enzyme profile (i.e., lower ALT and GGT levels and higher AST and AST/ALT ratio) and that these associations were attenuated by WC, in agreement with previous reports in adults.[23] In this line, Church et al. in a sample of adults observed an inverse relationship between NAFLD (measured by tomography scan) and CRF that was diminished after adjustment for WC.[23] Few studies have examined the

influence of CRF on fatty liver surrogate markers in youths.[10, 24, 25] In a large sample of adolescents participating in the National Health and Nutrition Examination Survey (N=2844), non-significant associations were observed between low CRF and ALT below the 80th percentile of ALT, while the relationships became significant above this cut-off and regardless of age, race and WC.[25] In a small sample of 12-18 years old adolescents (N=95), CRF was also inversely correlated with ALT in both non-overweight and overweight youth, independently of WC.[24] As far as we are aware, no studies examined the relationship between CRF and GGT or AST/ALT in young people which hamper comparisons.

The most novel finding of the current study is that adolescents with high WC, but who were fit, had a better profile of ALT and AST/ALT ratio. Thus, it was observed that adolescents with high WC and high CRF levels had lower ALT levels and higher AST/ALT, than their peers with high WC, but low CRF levels. However, in the regression analyses performed in the whole sample of adolescents, after adjustment for WC, the relationship between CRF and ALT and AST/ALT were no longer significant. A specific protective effect of high CRF on fatty liver markers in adolescents with high WC may partially explain these apparently contradictory results. High ALT levels and low AST/ALT ratio[26] are considered surrogate markers of liver steatosis, while GGT and AST are less specific. GGT levels are increased in obesity and metabolic syndrome,[27] whereas AST is present in the liver, but in considerable amounts also in other tissues including the muscles. Liver steatosis is strongly associated with abdominal fat accumulation,[2] and individuals with excess total and abdominal adiposity are more likely to have abnormal ALT levels and AST/ALT ratios. Thus, it can be interpreted that high CRF levels improves fatty liver surrogate markers in adolescents at a higher risk of developing hepatic steatosis. Martins et al.10, in a small sample (n=79) of overweight/obese young

adolescents (11-13 years old) with abnormal ALT serum concentrations found that fit participants were less likely to have abnormal ALT values, which is in line with our results. These results suggest that adolescents with abdominal obesity may have specific health benefits in the liver from high intensity physical activity or exercise training to increase CRF levels. Previous studies reported strong correlations between and abdominal adiposity and NAFLD in children.[2] Body mass loss and WC reduction are able to reduce hepatic fat content and ALT levels.[28] However, energy restriction programs may lead to undesirable lean mass loss and could compromise healthy growth and development in youths. In contrast, intervention studies in adolescents reported that exercise training decreased hepatic fat content and improved other metabolic syndrome features even without body mass loss.[29] Moreover, Senechal et al. observed that the increase in CRF was the single best predictor of the reduction of hepatic triglyceride content in response to exercise in adolescents.[30]

Strengths of the present study include the use of fitness tests which are known to be reliable, valid and related with health in children and adolescents[19] and the standardization of the methods and collection of data throughout countries involved in the HELENA study.[14] The most important limitation of our study is that we are not able to exclude that liver enzyme concentrations could have been affected by viral hepatitis or drug-or alcohol-induced liver injury. However, youths were examined by medical doctors and all of them had a negative history of liver disease. Second, our study lacks direct assessment of liver fat content and because of our cross-sectional design causality cannot be assumed. Finally, after adjusting the analyses for multiple comparisons the majority of the results became non-significant.

5. Conclusion

In summary, the results of the current study showed that WC is a stronger predictor of liver enzyme levels than CRF in adolescent. Our findings also suggest that higher levels of CRF may attenuate the deleterious consequences associated with high WC on liver enzymes in adolescents. Exercise programs focused on increasing CRF and WC could be a good alternative in the treatment and prevention of obesity related non-alcoholic fatty liver disease in adolescents.

Practical implications

- High cardiorespiratory fitness levels may attenuate the deleterious consequences associated with a high waist circumference on liver enzymes in adolescents.
- Adolescents with a high waist circumference, but with high levels of cardiorespiratory fitness had better hepatic health profile than those adolescents who had an elevated waist circumference but who were unfit.
- Exercise programs focused on increasing CRF and decreasing abdominal adiposity could be a good alternative in the treatment and prevention of obesity related non-alcoholic fatty liver disease in adolescents.

Funding sources

The HELENA study was carried out with the financial support of the European Community Sixth RTD Framework Program (Con- tract FOODCT-2005-007034). This work was also supported by grants from Spanish Ministry of Economy and Competitiveness (RYC-2010-05957; RYC-2011-09011), Spanish Ministry of Education, Culture and Sports (FPU14/03329), Spanish Ministry of Health: Maternal, Child Health and Development Network (no. RD08/0072) and the University of the Basque Country (GIU14/21). The writing group takes sole responsibility for the content of this article.

Acknowledgements

We gratefully acknowledge all participating children and adolescents, and their parents and teachers for their collaboration. We also acknowledge all the members involved in field work for their efforts (see Online appendix file for complete list of the HELENA study members). In addition, the authors gratefully acknowledge to Anke Carstensen and Petra Pickert for their contribution to laboratory work.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jsams.2017.04. 006.

References

1. Schwimmer JB, Pardee PE, Lavine JE et al. Cardiovascular risk factors and the metabolic syndrome in pediatric nonalcoholic fatty liver disease. *Circulation* 2008; 118(3):277–283.

2. Jung JH, Jung MK, Kim KE et al. Ultrasound measurement of pediatric visceral fat thickness: correlations with metabolic and liver profiles. *Ann Pediatr Endocrinol Metab* 2016; 21(2):75–80.

3. Boppidi H, Daram SR. Nonalcoholic fatty liver disease: hepatic manifestation of obesity and the metabolic syndrome. *Postgrad Med* 2008; 120(2):E01–07.

4. Patel DA, Srinivasan SR, Xu JH et al. Persistent elevation of liver function enzymes within the reference range is associated with increased cardiovascular risk in young adults: the Bogalusa Heart Study. *Metabolism* 2007; 56(6):792–798.

5. Koehler EM, Sanna D, Hansen B et al. Serum liver enzymes are associated with all–cause mortality in an elderly population. *Liver Int* 2014; 34(2):296–304.

6. Anderson EL, Howe LD, Jones HE et al. The prevalence of non–alcoholic fatty liver disease in children and adolescents: a systematic review and meta–analysis. *PLoS One* 2015; 10(10):e0140908.

7. Labayen I, Ruiz JR, Ortega FB, Davis CL, Rodríguez G, González–Gross M, Breidenassel C, Dallongeville J, Marcos A, Widhalm K, Kafatos A, Molnar D, DeHenauw F, Moreno LA. Liver enzymes and clustering cardiometabolic risk factors in European adolescents: the HELENA study. *Pediatr Obes* 2015; 10(5):361–370.

8. Rizzo NS, Ruiz JR, Hurtig–Wennlof A et al. Relationship of physical activity, fitness, and fatness with clustered metabolic risk in children and adolescents: the European youth heart study. *J Pediatr* 2007; 150(4):388–394.

9. Ruiz JR, Huybrechts I, Cuenca–Garcia M et al. Cardiorespiratory fitness and ideal cardiovascular health in European adolescents. *Heart* 2015; 101(10):766–773.

10. Martins C, Freitas IJ, Pizarro A et al. Cardiorespiratory fitness, but not central obesity or C–reactive protein, is related to liver function in obese children. *Pediatr Exerc Sc* 2013; 25:3–11 (1543–2920 (Electronic)).

11. Ruiz JR, Labayen I, Ortega FB et al. Physical activity, sedentary time, and liver enzymes in adolescents: the HELENA study. *Pediatr Res* 2014; 75(6):798–802.

12. Kantartzis K, Thamer C, Peter A et al. High cardiorespiratory fitness is an independent predictor of the reduction in liver fat during a lifestyle intervention in non–alcoholic fatty liver disease. *Gut* 2009; 58(9):1281–1288.

13. Brouwer SI, Stolk RP, Liem ET et al. The role of fitness in the association between fatness and cardiometabolic risk from childhood to adolescence. *Pediatr Diabetes* 2013; 14(1):57–65.

14. Moreno LA, De Henauw S, Gonzalez–Gross M et al. Design and implementation of the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross–Sectional Study. *Int J Obes* 2008; 32(Suppl. 5):S4–S11.

15. Barreira TV, Broyles ST, Gupta AK et al. Relationship of anthropometric indices to abdominal and total body fat in youth: sex and race differences. *Obesity* 2014; 22(5):1345–1350.

16. Katzmarzyk PT, Srinivasan SR, Chen W et al. Body mass index, waist circumference, and clustering of cardiovascular disease risk factors in a biracial sample of children and adolescents. *Pediatrics* 2004; 114(2):e198–e205.

17. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child* 1976; 51(3):170–179.

18. Léger LA, D M, Gadoury C et al. The multistage 20 metre shuttle run test for aerobic fitness. *J Sports Sci* 1988; 6(2):93–101 (0264–0414 (Print).

19. Ruiz JR, Ramirez–Lechuga J, Ortega FB et al. Artificial neural network–based equation for estimating VO_{2max} from the 20 m shuttle run test in adolescents. *Artif Intell Med* 2008; 44(3):233–245.

20. Ostojic JM, Stojanovic MD, Stojanovic V et al. Correlation between fitness and fatness in 6–14 years old. *J Health Popul Nutr* 2011; 29(1):53–60.

21. Ortega FB, Cadenas–Sanchez C, Sui X et al. Role of fitness in the metabolically healthy but obese phenotype: a review and update. *Prog Cardiovasc Dis* 2015; 58(1):76–86.

22. Senechal M, Wicklow B, Wittmeier K et al. Cardiorespiratory fitness and adiposity in metabolically healthy overweight and obese youth. *Pediatrics* 2013; 132(1):e85–e92.

23. Church TS, Kuk JL, Ross R et al. Association of cardiorespiratory fitness, body mass index, and waist circumference to nonalcoholic fatty liver disease. *Gastroenterology* 2006; 130(7):2023–2030.

24. Kelishadi R, Cook SR, Amra B et al. Factors associated with insulin resistance and non–alcoholic fatty liver disease among youths. *Atherosclerosis* 2009; 204(2):538–543.

25. Trilk JL, Ortaglia A, Blair SN et al. Cardiorespiratory fitness, waist circumference, and alanine aminotransferase in youth. *Med Sci Sports Exerc* 2013; 45(4):722–727.

26. Sorbi D, J B, Lindor KD. The ratio of aspartate aminotransferase to alanine aminotransferase: potential value in differentiating nonalcoholic steatohepatitis from alcoholic liver disease. *Am J Gastroenterol* 1999; 94(4):1018–1022.

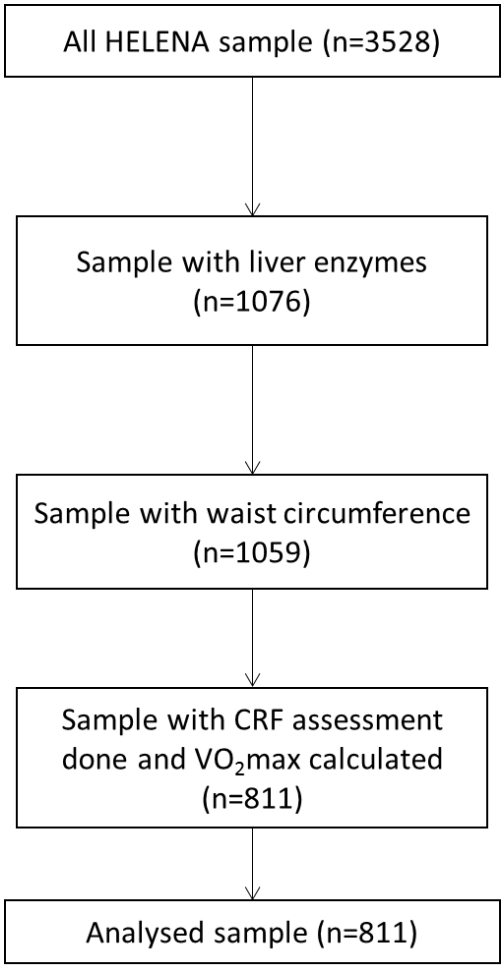
27. Sakamoto A, Ishizaka Y, Yamakado M et al. Comparison of the impact of changes in waist circumference and body mass index in relation to changes in serum gamma–glutamyltransferase levels. *J Atheroscler Thromb* 2013; 20(2):142–151.

28. Dixon JB, Bhathal PS, Hughes NR et al. Nonalcoholic fatty liver disease: Improvement in liver histological analysis with weight loss. *Hepatology* 2004; 39(6):1647–1654.

29. Lee S, Bacha F, Hannon T et al. Effects of aerobic versus resistance exercise without caloric restriction on abdominal fat, intrahepatic lipid, and insulin sensitivity in obese adolescent boys: a randomized, controlled trial. *Diabetes* 2012; 61(11):2787–2795.

30. Senechal M, Rempel M, Duhamel TA et al. Fitness is a determinant of the metabolic response to endurance training in adolescents at risk of type 2 diabetes mellitus. *Obesity* 2015; 23(4):823–832.

Supplementary data



Supplemental Figure 1. Flow diagram of participants. CRF: cardiorespiratory fitness; VO_{2max}: maximum oxygen consumption.

3.2. Study II

Status: submitted.

Associations of physical activity and fitness with hepatic steatosis, liver enzymes and insulin resistance in children with overweight/obesity

María Medrano[a], Lide Arenaza[a], Jairo Hidalgo Migueles[b], Beatriz Rodríguez-Vigil[c], Jonatan R. Ruiz[b,d], Idoia Labayen[a]

[a]Institute for Innovation & Sustainable Development in Food Chain (IS-FOOD), Public University of Navarra, Campus de Arrosadía, Pamplona, Spain.

[b]PROFITH ''PROmoting FITness and Health through physical activity'' Research Group, Department of Physical Education and Sport, Faculty of Sport Sciences, University of Granada, Granada, Spain.

[c]Osatek, Departament of Magnetic Resonance, University Hospital of Alava, Vitoria, Spain.

[d]Department of Biosciences and Nutrition at NOVUM, Karolinska Institutet, Huddinge, Sweden.

Contact Author: María Medrano, Department of Health Sciences, Public University of Navarra, Avenida Barañaín s/n, 31007 Pamplona, Spain. Phone: +34 629558766. e-mail: maria.medrano@unavarra.es

Abstract

This study analyzed the associations of physical fitness and physical activity (PA) with percentage hepatic fat and liver enzymes in children with overweight/obesity. A total of 115 children (10.6±1.1 years; 54% girls) with overweight/obesity of the EFIGRO study (ClinicalTrials.gov: NCT02258126) were included in the analyses. Cardiorespiratory fitness (CRF), musculoskeletal fitness and speed-agility were measured by the Alpha-fitness tests, and PA by wGT3X-BT accelerometers. Percentage hepatic fat was assessed by magnetic resonance imaging. Alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), insulin, glucose, triglycerides (TG) and high-density lipoprotein (HDL) levels were obtained from fasting blood samples. The homeostasis model assessment (HOMA-IR) and AST/ALT and TG/HDL ratios were calculated. Higher CRF was associated with lower percentage hepatic fat (β=-0.263, P=.01) and GGT (β=-0.315, P=.01), and higher AST/ALT ratio (β=0.304, P=.01). CRF-fit children have lower GGT levels (15±1 *vs*. 17±1 U/L, CRF-fit *vs*. CRF-unfit children, P=.02), HOMA-IR (2.2±0.1 *vs*. 2.9±0.1, P<.01) and TG/HDL ratio (1.4±0.1 *vs*. 1.9±0.1, P=.01) and higher AST/ALT ratio (1.3±0.0 *vs*. 1.2±0.0, P=.03), than CRF-unfit children. These findings emphasize the importance of considering the improvement of CRF as a target of programs for preventing hepatic steatosis, type 2 diabetes and cardiovascular diseases in children with overweight.

Keywords: percentage hepatic fat, NAFLD, cardiometabolic risk, HOMA, TG/HDL, pediatric obesity.

Introduction

The global pandemic of overweight and obesity in children has contributed to a rise in associated cardiovascular and metabolic complications, such as insulin resistance and type 2 diabetes, already in childhood and earlier in adult life[1]. Fat accumulation in hepatocytes leads to an excess of percentage hepatic fat, associated with the development of non-alcoholic fatty liver disease (NAFLD) in children[2]. NAFLD is the most common liver disease in childhood[3] affecting nearly 8% of pediatric general population, and 34% of children with obesity[4]. Moreover, it is strongly associated with insulin resistance and cardiovascular risk factors in children with overweight/obesity[5]. High levels of liver enzymes are considered surrogate measures of NAFLD and are also associated with insulin resistance and cardiometabolic health in youth[6]. Liver enzymes alone are inadequate for identification of hepatic steatosis[7], whereas magnetic resonance imaging (MRI) is considered as an accurate method for detecting hepatic steatosis in children[8]. The AST/ALT ratio is a biomarker of hepatic liver function, and it was also associated with cardiovascular abnormalities in adolescents[6]. The triglyceride-to-high density lipoprotein (TG/HDL) ratio, which has been proposed as the best estimate of cardiometabolic risk in youths[9], is also associated with insulin resistance and hepatic steatosis in children and adolescents[10].

There is considerable evidence supporting the associations of high levels of physical fitness and physical activity (PA) with insulin resistance and cardiometabolic risk factors in children and adolescents[11–14]. However, evidence supporting that higher levels of fitness and PA are associated with lower percentage hepatic fat[15,16], liver enzymes (including the AST/ALT ratio)[17,18] or TG/HDL ratio[19], is scarce in youths, particularly in preadolescent children. Therefore, the aims of the present study were: i) to explore the associations of physical fitness and PA with percentage hepatic fat and liver enzymes in children with overweight/obesity and ii) to test the relationships of physical fitness and PA with insulin resistance and cardiometabolic risk in preadolescent children with overweight/obesity.

Material and methods

Design

The present cross-sectional study was based on data derived from the EFIGRO study (ClinicalTrials.gov ID: NCT02258126) whose overall aim was to investigate the effect of exercise on percentage hepatic fat in children with overweight/obesity participating in a 22-week family-based program that included healthy lifestyle and psychological education. This study was conducted from September 2014 to June 2017 in Vitoria-Gasteiz, Spain[20]. Details of sample calculation, randomization, characteristics of participants, design, methods and measurements have been published elsewhere[20]; however, the most relevant information is briefly described below.

Participants

The planned sample size for the EFIGRO study was 160 children with overweight or obesity. A total of 134 children were assessed for eligibility, and 121 meeting all the inclusion criteria were included in the study. For the current purpose, 115 children with overweight/obesity (10.6±1.1 years of age, 54% girls) with complete baseline data on percentage hepatic fat were included in the present study. Before being enrolled in the study, parents or legal guardians signed an informed written consent. The study protocol was approved by the Ethics Committee of Clinical Investigation of Euskadi (PI2014045) and complied with the revised ethical guidelines of the Declaration of Helsinki (revision of 2013).

Anthropometry and physical fitness

Body mass (SECA 760; SECA, Hamburg, Germany) and height (SECA 220) were measured in duplicate using standard protocols. Body mass index (BMI) was calculated as body mass (kg)/height (m^2).

The three main health-related physical fitness components (cardiorespiratory fitness (CRF), musculoskeletal fitness (MF), and speed-agility) were evaluated using the Alpha-fitness test battery, whose reliability and validity for measuring health-related fitness components in children of this age have been previously reported[21]. The number of laps obtained in the 20m shuttle run test (20mSRT) was selected as CRF estimate. Upper and lower MF were assessed by the handgrip (HG) (kg) and the standing broad jump tests (SBJ) (cm), respectively[21]. Finally, speed-agility was measured using the 4 × 10m (seconds). Thereafter, children were classified as fit or unfit for each fitness test and according to the sex- and age-specific 20th centiles created by Tomkinson et al.[22] Children were considered as CRF-fit or CRF-unfit, HG-fit or HG-unfit, and SBJ-fit or SBJ-unfit when their results were above (fit) or below (unfit) the sex- and age-specific 20th centile[22] for the corresponding test (laps in the 20mSRT, HG and SBJ, respectively). These categories are independent, in such a way that a same participant could be classified as CRF-fit, but HG- or SBJ-unfit, and *vice versa*.

Physical activity

PA was measured by accelerometry (wGT3X-BT monitors; Actigraph, Pensacola, FL, USA). Children wore an accelerometer (sampling frequency of 30 Hz) on the non-dominant wrist for seven consecutive days, except during water-based activities. Children, helped by her parents/caregivers, also reported non-wear time, bedtime and

wake time in a daily log. A researcher member instructed them how to report this information.

Accelerometer data was downloaded in comma-separated values format using the Actilife software (ActiGraph, Pensacola, FL, USA) and then processed and analyzed in ENMO metric using raw acceleration (mg, being 1g=9.81 m/sg) in the R software (v. 3.1.2, (http://www.cran.r-project.org) using the GGIR package (v. 1.5-12, https://cran.r-project.org/web/packages/GGIR/). Waking hours were classified as sedentary time, light (LPA), moderate (MPA), vigorous (VPA) and moderate-to-vigorous PA (MVPA) according to the Hildebrand et al.[23] cut-points.

Mean daily PA intensity levels were then calculated as: (mean of available weekdays*5 + mean of available weekend days*2)/7. The participants were excluded from the analyses if they recorded less than 4 valid days (i.e., ≥ 16 wearing hours), including at least 1 weekend day as recommended[24,25]. Children were categorized as active (≥ 60 min/day of MVPA) or inactive (< 60 min/day of MVPA) according to international PA recommendations[26].

Percentage hepatic fat

Percentage hepatic fat was measured by magnetic resonance imaging (Magnetom Avanto, Siemens Healthcare, Erlangen, Germany), using a multiecho gradient-echo sequence with Dixon reconstruction[20]. Children were then categorized into two groups according to the absence or presence of hepatic steatosis (percentage hepatic fat <5% or ≥5%, respectively)[27].

Biochemical variables

Blood samples were obtained after an overnight fast. Blood extraction and collection details have been published elsewhere[20]. Serum concentrations of liver

enzymes (alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT) and aspartate aminotransferase (AST)), insulin, glucose, triglycerides (TG) and high-density lipoprotein (HDL) were measured using standard protocols. Thereafter, the homeostasis model assessment (HOMA-IR) (insulin (mU/L) x glucose (mmol/L)/22.5), and AST/ALT and TG/HDL ratios were calculated.

Statistical analyses

Chi-square and Student´s t-tests were used to calculate differences in categorical (prevalence of children with overweight/obesity, hepatic steatosis, high HOMA-IR, high TG/HDL, and inactive children) or continuous (remainder of the variables) variables between boys and girls. Linear regression analysis was performed to evaluate the associations of physical fitness (continuous variables: number of laps, HG, SBJ and speed-agility) and PA (LPA, MPA, VPA, MVPA and total PA) (independent variables) with percentage hepatic fat, liver enzymes (ALT, GGT and AST/ALT ratio), insulin resistance (HOMA-IR) and TG/HDL ratio (continuous dependent variables). Differences in dependent variables between CRF-fit and CRF-unfit, HG-fit and HG-unfit, SBJ-fit and SBJ-unfit, or active and inactive children (fixed factors) were examined by ANCOVA. Analyses were controlled for age and sex in Model 1. In Model 2, the analyses were further adjusted for BMI. PA variables were additionally adjusted for waking time in both models.

Data were analyzed to detect extreme values and winsorized to limit their influence on the analyses (i.e. ALT ≥35 U/L in N=6), AST ≥34 U/L in N=3, GGT ≥29 U/ in N=2, Insulin ≥25.5 microU/mL in N=5, TG ≥199 mg/dL in N=2). Transformation to their natural logarithms was used for variables non-normally distributed (BMI, CRF, speed-agility, VPA, percentage hepatic fat, ALT, GGT, HOMA-IR and TG/HDL ratio).

However, non-transformed data are presented in tables and figures. All analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 20.0 for WINDOWS; SPSS Inc., Chicago), and the level of significance was set at α=.05.

Results

The descriptive characteristics of the children by sex are shown in **Table 1**. MRI analysis revealed that 44.4% of children had hepatic steatosis. Also, LPA was significantly higher and VPA significantly lower in girls than in boys (P<.05). No significant differences were observed in the remainder of the variables between boys and girls. A total of 49, 104 and 12 children were classified as CRF-fit, HG-fit and SBJ-fit, respectively.

Table 1. Descriptive characteristics of boys and girls participating in the study.

	Boys		Girls		P for sex
	N		N		
Age (years)	53	10.6 (1.1)	62	10.5 (1.1)	.57
Body mass (kg)	53	55.1 (9.7)	62	54.1 (11.2)	.62
Height (m)	53	1.47 (0.08)	62	1.46 (0.08)	.54
Body mass index (kg/m^2)	53	25.5 (3.1)	62	25.4 (3.4)	.73
Children with overweight, obesity (N)	53	21, 32	62	28, 34	.75
Alpha-fitness tests					
20-meters shuttle run test (laps)	50	22 (13)	60	20 (11)	.46
Handgrip (kg)	52	18 (4)	62	18 (4)	.50
Standing broad jump (cm)	51	101 (19)	62	99 (16)	.61
Speed-agility (seconds)	50	13.9 (1.5)	62	13.7 (1.1)	.48
Physical activity					
Light PA (min/day)	49	277 (46)	59	303 (42)	**<.01**
Moderate PA (min/day)	49	52 (18)	59	47 (17)	.19
Vigorous PA (min/day)	49	10 (6)	59	6 (5)	**.02**
MVPA (min/day)	49	61 (22)	59	54 (20)	.07
Total PA (m*g*)	49	66 (17)	59	63 (15)	.29
Inactive children (n, %)		25, 51		40, 68	.06
Percentage hepatic fat (%)	53	6.2 (5.0)	62	5.1 (2.8)	.17
Children with hepatic steatosis (N, %)		24, 45		27, 44	.50
Biochemical variables					
HOMA-IR	52	2.5 (1.0)	61	2.7 (1.2)	.36
High HOMA-IR (N, %)		20, 39		29, 47	.27
Triglycerides (mg/dL)	53	80 (40)	62	85 (39)	.39
HDL (mg/dL)	53	53 (12)	62	49 (10)	.09
TG/HDL ratio	53	1.7 (1.0)	62	1.8 (1.1)	.19
High TG/HDL (N, %)		16, 31		20, 32	.51
AST (U/L)	52	25 (5)	62	23 (3)	.81
ALT (U/L)	52	22 (11)	62	19 (5)	.06
GGT (U/L)	51	17 (5)	61	16 (5)	.51
AST/ALT ratio	52	1.2 (0.4)	62	1.3 (0.3)	.81

Abbreviations: ALT: alanine aminotransferase; AST/ALT: aspartate aminotransferase to alanine aminotransferase ratio; GGT: gamma-glutamyl transferase; HOMA-IR: insulin resistance homeostasis model assessment; MVPA: moderate to vigorous physical activity; PA: physical activity; TG/HDL: triglycerides to high density lipoprotein ratio. Values are means (standard deviation) unless otherwise indicated. Non-transformed data are presented in the table, but statistical analysis was performed on logarithmically transformed data in those variables not normally distributed. Boldfaced values: P<.05.

Missing data: 20-meters shuttle run test (N=5), handgrip (N=1), standing broad jump (N=2), speed-agility (N=3), PA (N=7), HOMA-IR (N=2), AST (N=1), ALT (N=1) and GGT (N=2). Reasons: it was not possible to perform the test (equipment problems, injuries), families did not attend the tests, or there were not enough valid days in accelerometer data collection or analysis.

Associations of physical fitness and PA with percentage hepatic fat and liver enzymes

Higher CRF was significantly associated with lower percentage hepatic fat (Ps<.01) (Model 1, **Table 2**). After further adjustment for BMI, the association between CRF and percentage hepatic fat remained statistically significant (Model 2, Table 2). Neither upper, lower MF, nor speed-agility were significantly associated with percentage hepatic fat (Table 2). No differences were found in percentage hepatic fat between CRF-fit and CRF-unfit children (Panel *a* in **Figure 1)** or between HG-fit and HG-unfit, or SBJ-fit and SBJ-unfit children (data not shown). There were no significant relationships between PA levels and percentage hepatic fat (Table 2), and we did not find any significant difference in percentage hepatic fat between active and inactive overweight/obese children (**Supplemental Table 1**).

CRF was negatively associated with GGT levels (Ps<.04) and positively with the AST/ALT ratio (Ps<.02) (Table 2). Similarly, CRF-fit children had significantly lower levels of GGT (15±1 U/L *vs*. 17±1 U/L, in CRF-fit and CRF-unfit children, respectively, P<.02) and a higher AST/ALT ratio (1.3±0.0 U/L *vs*. 1.2±0.0 U/L in CRF-fit and CRF-unfit children, respectively, P<.03) than their CRF-unfit peers (Figure 1, panel *c* for GGT, and *d* for AST/ALT ratio). In contrast, no significant associations were observed between fitness components and ALT (Table 2). Likewise, there were no differences in ALT levels between fit and unfit children in any of the fit/unfit categories (Panel *b* in Figure 1 for CRF-fit and CRF-unfit categories) (data not shown for HG and SBJ fit/unfit categories). PA was not associated with liver enzyme levels (Table 2). Similarly, there were no significant differences in liver enzyme levels between active or inactive children (Supplemental Table 1).

Table 2. Associations of physical fitness and physical activity with percentage hepatic fat, liver enzymes, insulin resistance and TG/HDL ratio in children with overweight/obesity.

	Percentage hepatic fat (%)				ALT (U/L)			
	Model 1		Model 2		Model 1		Model 2	
	β	P	β	P	β	P	β	P
Alpha-fitness tests								
20 meters shuttle run test (laps)	-0.263	**.01**	-0.236	**.04**	-0.155	.13	-0.129	.27
Handgrip (kg)	0.102	.35	0.044	.69	-0.174	.11	0.135	.23
Standing broad jump (cm)	-0.007	.94	0.054	.60	0.036	.71	0.090	.39
Speed-agility (seconds)	0.099	.33	0.055	.60	-0.092	.37	-0.141	.18
Physical Activity								
Light PA (min/day)	-0.056	.58	-0.009	.93	-0.084	.42	-0.037	.73
Moderate PA (min/day)	-0.049	.61	-0.008	.93	-0.054	.58	-0.012	.90
Vigorous PA (min/day)	-0.062	.54	-0.001	.99	-0.007	.94	0.063	.55
MVPA (min/day)	-0.059	.54	-0.016	.88	-0.020	.84	0.028	.78
Total PA (m*g*)	-0.077	.43	-0.026	.80	0.004	.97	0.064	.53

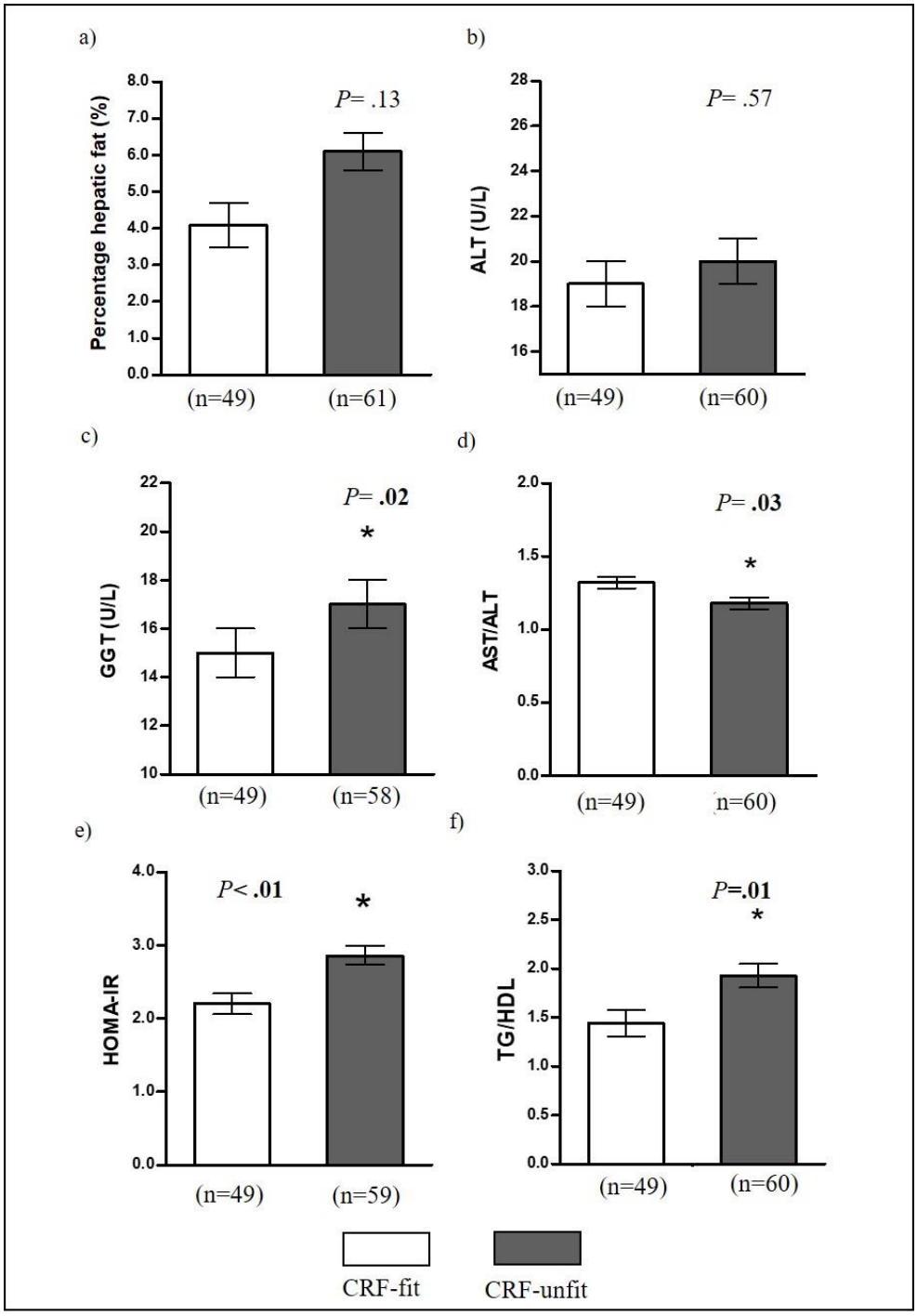
	GGT (U/L)				AST/ALT ratio			
	Model 1		Model 2		Model 1		Model 2	
	β	P	β	P	β	P	β	P
Alpha-fitness tests								
20 meters shuttle run test (laps)	-0.315	**<.01**	-0.250	**.03**	0.304	**<.01**	0.288	**.01**
Handgrip (kg)	0.199	.07	0.123	.26	-0.154	.16	-0.095	.39
Standing broad jump (cm)	-0.143	.12	-0.060	.56	0.008	.93	-0.060	.56
Speed-agility (seconds)	0.146	.16	0.074	.49	-0.121	.23	-0.074	.49
Physical Activity								
Light PA (min/day)	-0.180	.09	-0.091	.38	0.024	.82	-0.050	.63
Moderate PA (min/day)	-0.107	.28	-0.028	.78	0.119	.22	0.061	.53
Vigorous PA (min/day)	-0.095	.35	0.023	.82	0.105	.30	0.017	.87
MVPA (min/day)	-0.096	.34	-0.011	.91	0.107	.28	0.044	.66
Total PA (m*g*)	-0.110	.27	-0.010	.92	0.092	.36	0.015	.89

	HOMA-IR				TG/HDL ratio			
	Model 1		Model 2		Model 1		Model 2	
	β	P	β	P	β	P	β	P
Alpha-fitness tests								
20 meters shuttle run test (laps)	-0.370	**<.01**	-0.197	.06	-0.327	**<.01**	-0.350	**<.01**
Handgrip (kg)	0.116	.28	-0.001	.99	-0.095	.39	-0.144	.20
Standing broad jump (cm)	-0.218	**.02**	-0.072	.44	-0.116	.23	-0.090	.39
Speed-agility (seconds)	0.237	**.02**	0.114	.23	0.251	**.01**	0.238	**.02**
Physical Activity								
Light PA (min/day)	-0.163	.12	-0.042	.67	0.085	.42	0.134	.21
Moderate PA (min/day)	-0.125	.20	-0.023	.80	-0.078	.43	-0.046	.65
Vigorous PA (min/day)	-0.188	.06	-0.042	.67	-0.214	**.04**	-0.226	.09
MVPA (min/day)	-0.138	.16	-0.029	.76	-0.119	.23	-0.086	.40
Total PA (m*g*)	-0.171	.08	-0.043	.65	-0.105	.29	-0.066	.53

Model 1: adjusted for age and sex. Model 2: Model 1 further adjusted for body mass index. Physical activity variables further adjusted for accelerometer waking time in both models.

Abbreviations: ALT: alanine aminotransferase; AST/ALT: aspartate aminotransferase to alanine aminotransferase ratio; CRF: cardiorespiratory fitness; GGT: gamma-glutamyl transferase; HOMA-IR: insulin resistance homeostasis model assessment; MVPA: moderate to vigorous physical activity; PA: physical activity; TG/HDL: triglycerides to high density lipoprotein ratio. Boldfaced values: P<.05.

Figure 1. Differences in percentage hepatic fat, liver enzymes, insulin resistance and TG/HDL ratio between CRF-fit and CRF-unfit children, adjusted for age and sex.



CRF-fit and CRF-unfit categories were classified according to the number of laps in the 20-meter shuttle run test, categorizing children as being above (CRF-fit) or below (CRF-unfit) the sex- and age-specific 20th centile published by Tomkinson et al.25. Non-transformed data are presented in the figure, but ANCOVA was performed on log-transformed data in the case of variables not normally distributed. *Abbreviations*: ALT: alanine aminotransferase; AST/ALT: aspartate aminotransferase to alanine aminotransferase ratio; CRF: cardiorespiratory fitness; GGT: gamma-glutamyl transferase; HOMA-IR: insulin resistance homeostasis model assessment; min: minutes; TG/HDL: triglycerides to high density lipoprotein ratio. Boldfaced values: P<.05.

Associations of physical fitness and PA with insulin resistance and TG/HDL ratio

Results showed that better performance in all fitness components was significantly associated with lower HOMA-IR, except in the HG test (Model 1, Table 2). However, after further adjustment for BMI, the relationship between physical fitness components and HOMA-IR became non-significant (Model 2, Table 2). Moreover, children categorized as CRF-fit had a lower HOMA-IR value than CRF-unfit children (2.2±0.1 *vs*. 2.9±0.1 in CRF-fit and CRF-unfit children, respectively, Ps<.01) (panel *e*, Figure 1). By contrast, there were no significant differences in HOMA-IR between HG-fit and HG-unfit or between SBJ-fit and SBJ-unfit children (data not shown). In addition, none of the PA estimates was associated with HOMA-IR in either of the models (Table 2). Similarly, there were no significant differences between active and inactive children in HOMA-IR values (Supplemental Table 1).

CRF and speed agility were significantly associated with the TG/HDL ratio irrespective of age, sex and BMI (Model 2, Table 2). Likewise, CRF-fit children had a lower TG/HDL ratio than CRF-unfit children (1.4±0.1 *vs*. 1.9±0.1, for CRF-fit and CRF-unfit, respectively; P<.01) (panel *f*, Figure 1). With regards to PA, higher VPA was significantly associated with a lower TG/HDL ratio (P<.05, Model 1, Table 2). After further controlling for BMI, the association of VPA and TG/HDL ratio was diminished and became non-significant (Model 2, Table 2). Neither LPA, MPA, MVPA, nor total PA was significantly associated with TG/HDL ratio. Active children did not have a significant lower TG/HDL ratio than inactive children (Supplemental Table 1).

Discussion

The present study examined the association of physical fitness and PA with percentage hepatic fat, liver enzymes, insulin resistance and cardiometabolic risk in preadolescent children with overweight/obesity. The main finding of this study is that higher CRF is associated with lower percentage hepatic fat and a healthier profile of liver enzymes in preadolescent children with overweight/obesity. Moreover, our results confirm that CRF-fit children with overweight/obesity have lower liver enzymes, insulin resistance and TG/HDL ratio than their CRF-unfit peers.

In our study, 44.4% of children with overweight/obesity had hepatic steatosis, which was slightly higher than the prevalence reported by other studies in which percentage hepatic fat was measured by MRI in youths with obesity[28,29]. In samples comprising both children and adolescents with obesity, Kim et al.[28] and Radetti et al.[29] estimated that 38% and 31.8% of youth, respectively, had hepatic steatosis. However, these authors established the cut-off point for hepatic steatosis in a hepatic fat fraction (HFF) ≥5.5% or ≥9%. In a meta-analysis based on various diagnostic methods (ALT, ultrasonography (US) and MRI), Anderson et al.[4] estimated a NAFLD prevalence of 34.2% in a large sample of children and adolescents from clinical obese population studies around the world (N=23892, age range from 2 to 14 years). The observed prevalence of hepatic steatosis in children with overweight/obesity is clinically relevant and a major public health concern because it is still under-diagnosed, and it increases the risk of liver disease, diabetes, cardiovascular disease and mortality[2].

To our knowledge, the present study is the first to examine the association of the main health-related physical fitness components and PA intensities with percentage hepatic fat and liver enzymes in children. We observed that CRF, but not MF or speed-agility, was negatively associated with percentage hepatic fat, GGT and AST/ALT. Few

studies have analyzed the association between physical fitness and percentage hepatic fat, GGT or AST/ALT in youth. In agreement with our results, previous studies showed significant associations between CRF and percentage hepatic fat (determined by (1)H-magnetic resonance spectroscopy)[15] and with ALT, GGT and AST/ALT[18] in adolescents.

In our study, CRF-fit children had lower GGT and AST/ALT, but not statistically significant lower percentage hepatic fat or ALT than their unfit counterparts. In agreement with our results, other studies observed that youth classified in the higher categories of CRF (assessed by the 20mSRT and classified according to their calculated VO_2peak) had lower levels of liver enzymes than those in the lower CRF categories[18,30]. We did not find any study where differences in percentage hepatic fat (specifically measured by MRI) between fit and unfit children were analyzed. In a previous study with obese male adults, those participants categorized as fit had lower levels of intrahepatocellular lipids (MRI measurement) than their unfit counterparts[31]. A plausible mechanism that might explain the observed association between CRF and percentage hepatic fat could be that intrinsic aerobic capacity is linked with hepatic mitochondrial oxidative capacity and susceptibility to hepatic steatosis[32]. An experiment performed in animal models showed that rats with low aerobic fitness had a reduced hepatic mitochondrial oxidative capacity and content. This difference was associated with a greater liver triglycerides content, a higher percentage of hepatocytes with lipid droplets and lipid peroxidation in low CRF rats, which was associated with hepatic steatosis. Additionally, rats with low CRF had significantly higher hepatic fibrosis and apoptosis[32].

Regarding PA, we did not find any significant association between PA levels and percentage hepatic fat or liver enzyme levels in children with overweight/obesity. To the best of our knowledge, this is the first study analyzing the relationship between objectively measured PA and percentage hepatic fat (measured by MRI) in preadolescent

children. Nevertheless, other studies have reported significant associations with other PA (questionnaires) and hepatic steatosis (ultrasound[16,33], liver enzymes[17,33]) measurements in youths. A longitudinal analysis reported that objectively measured total PA and MVPA at 12 years of age was inversely associated with the risk of liver fat (ultrasound), and abnormal GGT levels, but not with ALT levels at 17.8 years of age (N=1292)[33]. Another study, performed exclusively in adolescents, reported significant associations of total PA and MVPA (measured by accelerometry) with AST/ALT, but not with ALT or GGT[17]. The authors also observed that active adolescents had a lower AST/ALT ratio than their inactive peers[17]. It should be noted that this study included both normal weight and overweight adolescents. In summary, direct comparisons among studies are difficult because of differences in the age, or BMI categories of participants, as well as the different methodology used to assess physical fitness, PA or percentage hepatic fat. A possible explanation for the lack of association between PA and percentage hepatic fat in our study could be that our sample is exclusively composed by prepubertal children with overweight/obesity with a narrow range of PA levels.

We observed that the inverse associations between physical fitness (CRF, lower body MF and speed-agility) and insulin resistance (HOMA-IR) in children with overweight/obesity disappeared after controlling for BMI. In line with our results, some studies showed that associations of physical fitness with HOMA-IR disappeared after adjusting for BMI or other adiposity estimates[14,34]. However, in other studies, the associations between physical fitness components and HOMA-IR were irrespective of BMI[13]. Finally, in a study performed in European adolescents (N=1053), the strength of the associations between MF and HOMA-IR varied depending on the adiposity variable used for adjustment (BMI, waist circumference or skinfold thickness) and sex of participants (boys or girls)[35]. For example, the association between SBJ and HOMA-IR

was statistically significant after controlling for waist circumference, but not after controlling for BMI or skinfold thickness in boys[35]. Thereby, the potential influence of physical fitness on insulin resistance in youth, independently of BMI or adiposity, remains unclear. The non-significant association between physical fitness and HOMA-IR after controlling for BMI could be explained both by the influence of BMI on insulin resistance and by the association of physical fitness levels and BMI in children[36]. Differences in the results among studies could be related to the various adiposity variables used for adjustment, physical fitness tests used and children's characteristics.

In addition, we found that HOMA-IR levels were lower in CRF-fit than in CRF-unfit children. In line with our results, Llorente-Cantarero et al.[37] observed that Spanish prepubertal children (N=141) categorized in the higher CRF group had lower levels of HOMA-IR than those categorized in the lower CRF group (according to other reference values). When children were classified as HG-fit or HG-unfit and SBJ-fit or SBJ-unfit, we did not find differences in HOMA-IR levels. By contrast, other authors found differences in HOMA-IR across HG[35,38] or SBJ[35] fit/unfit categories. Maturational state (adolescents[35] or both children and adolescents[38]), as well as ethnic and or regional differences[38] could be underlying discrepancies among studies' findings.

Regarding PA, we did not observe any significant associations of PA with insulin resistance in children with overweight/obesity. Nevertheless, other studies in youth reported significant associations between different PA intensities measured by questionnaires and HOMA-IR[39,40]. Moreover, we did not find differences in HOMA-IR levels between active and inactive children with overweight/obesity. In agreement with our findings, no significant differences in HOMA-IR levels were found between active and inactive (>60 min/day or <60 min/day MVPA, respectively) children and adolescents

(N=745) in week days PA (measured by accelerometry) in the study conducted by Janssen et al.[41].

Several studies have examined the association between physical fitness or/and PA and cardiometabolic risk factors, included as individual risk factors[42,43] or in clustered scores[44] in children. However, we only found one study examining the relationship between physical fitness or PA and TG/HDL ratio[19]. In line with our results, this study observed inverse correlations between CRF (VO_2peak calculated from a progressive cycle ergometer test) and TG/HDL ratio in a similar sample size (N=155) of school children (10–14 years old) in all BMI categories. They also found that CRF-fit children had a lower TG/HDL ratio than CRF-unfit children from 10 to 14 years of age[19]. In contrast to our findings, this study did not observe any significant association between VPA and TG/HDL in the sample of both children and adolescents[19]. Moreover and according to our results, these authors did not find significant differences in TG/HDL levels across other PA categories. In the study of Bailey et al.[19], different model of accelerometers ((RT3® triaxial accelerometers) and different cut-offs for defining PA intensities were employed which could have a large impact in the outcomes[24].

The cross-sectional design of this study limits the ability to determine any causality in the results. Lack of data about dietary intake could be considered as a limitation of the current study. Nevertheless, the current study has strengths. The use of MRI for percentage hepatic fat measurement is an important strength in the present study. Moreover, the reliability and validity of Alpha-fitness tests for measuring fitness in this population[21], the methodology used to objectively measure PA, as well as the accelerometry analyses using raw data (R software and packages) are strengths of the present study. Longitudinal and intervention programs performed in larger samples are

needed to confirm the influence of physical fitness and PA on hepatic steatosis in children with overweight/obesity.

In summary, the present study shows that higher CRF is associated with lower percentage hepatic fat, liver enzyme levels, insulin resistance and cardiometabolic risk in preadolescent children with overweight/obesity. Likewise, those preadolescent children with overweight/obesity, but classified as CRF-fit, had lower liver enzymes, insulin resistance, and cardiometabolic risk than their CRF-unfit peers. In addition, higher VPA levels are inversely associated with cardiometabolic risk in this population. Overall, these findings suggest that improving cardiorespiratory fitness through physical activity interventions should be a target in prevention strategies aiming to reduce the risk of developing NAFLD, type 2 diabetes, and cardiovascular diseases in children with overweight or obesity.

Disclosure Statement

The authors report no conflict of interest.

Acknowledgements

Funding details: this work was supported by the Spanish Ministry of Health, "Fondo de Investigación Sanitaria del Instituto de Salud Carlos III", "Fondos de la Unión Europea (FEDER), una manera de hacer Europa" (PI13/01335) and by the University of the Basque Country (GIU14/21). MM is supported by grant from the Spanish Ministry of Education, Culture and Sport (FPU14/03329 and EST17/00210). LA is supported by a grant from Education, Linguistic Policy and Culture Department of the Government of the Basque Country (PRE_2016_1_0057). JHM was supported by a Grant from the Spanish Ministry of Education, Culture and Sport (FPU15/02645). JRR was supported by

the University of Granada, Plan Propio de Investigación 2016, Excellence actions: Units of Excellence; Unit of Excellence on Exercise and Health (UCEES).

The authors thank the families, children and parents, for their participation and collaboration in the study. We also acknowledge the pediatricians and all the members involved in the recruitment and assessments for their efforts and to the pre- and post-graduate students for their collaboration in the evaluations and healthy lifestyle education and exercise training sessions.

References

1. Chung ST, Onuzuruike AU, Magge SN. Cardiometabolic risk in obese children. *Ann N Y Acad Sci*. 2018;1411(1):166-183.

2. Vos MB, Abrams SH, Barlow SE, et al. NASPGHAN Clinical Practice Guideline for the Diagnosis and Treatment of Nonalcoholic Fatty Liver Disease in Children: Recommendations from the Expert Committee on NAFLD (ECON) and the North American Society of Paediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN). *J Pediatr Gastroenterol Nutr*. 2017;64(2):319-334.

3. Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics*. 2006;118(4):1388-1393.

4. Anderson EL, Howe LD, Jones HE, Higgins JPT, Lawlor DA, Fraser A. The Prevalence of Non-Alcoholic Fatty Liver Disease in Children and Adolescents: A Systematic Review and Meta-Analysis. *PLos One*. 2015;10(10):e0140908.

5. Schwimmer JB, Pardee PE, Lavine JE, Blumkin AK, Cook S. Cardiovascular risk factors and the metabolic syndrome in pediatric nonalcoholic fatty liver disease. *Circulation*. 2008;118(3):277-283.

6. Labayen I, Ruiz JR, Ortega FB, et al. Liver enzymes and clustering cardiometabolic risk factors in European adolescents: The HELENA study. *Pediatr Obes*. 2015;10(5):361-370.

7. Vajro P, Lenta S, Socha P, et al. Diagnosis of nonalcoholic fatty liver disease in children and adolescents: Position paper of the ESPGHAN hepatology committee. *J Pediatr Gastroenterol Nutr*. 2012;54(5):700-713.

8. Zand KA, Shah A, Heba E, et al. Accuracy of multiecho magnitude-based MRI (M-MRI) for estimation of hepatic proton density fat fraction (PDFF) in children. *J Pediatr Gastroenterol Nutr*. 2015;42(5):1223-1232.

9. Di Bonito P, Moio N, Scilla C, et al. Usefulness of the high triglyceride-to-HDL cholesterol ratio to identify cardiometabolic risk factors and preclinical signs of organ damage in outpatient children. *Diabetes Care*. 2012;35(1):158-162.

10. Pacifico L, Bonci E, Andreoli G, et al. Association of serum triglyceride-to-HDL cholesterol ratio with carotid artery intima-media thickness, insulin resistance and nonalcoholic fatty liver disease in children and adolescents. *Nutr Metab Cardiovasc Dis*. 2014;24(7):737-743.

11. Ortega FB, Ruiz JR, Castillo MJ, Sjöström M. Physical fitness in childhood and adolescence: a powerful marker of health. *Int J Obes (Lond)*. 2008;32(1):1-11.

12. Nyström CD, Henriksson P, Martínez-Vizcaíno V, et al. Does Cardiorespiratory Fitness Attenuate the Adverse Effects of Severe/Morbid Obesity on Cardiometabolic Risk and Insulin Resistance in Children? A Pooled Analysis. *Diabetes Care*. 2017;40(11):1580-1587.

13. Li S, Zhang R, Pan G, Zheng L, Li C. Handgrip strength is associated with insulin resistance and glucose metabolism in adolescents: Evidence from National Health and Nutrition Examination Survey 2011 to 2014. *Pediatr Diabetes*. 2018;19(3):375-380.

14. Krekoukia M, Nassis GP, Psarra G, Skenderi K, Chrousos GP, Sidossis LS. Elevated total and central adiposity and low physical activity are associated with insulin resistance in children. *Metabolism*. 2007;56(2):206-213.

15. Wittmeier KDM, Wicklow BA, MacIntosh AC, et al. Hepatic Steatosis and Low Cardiorespiratory Fitness in Youth With Type 2 Diabetes. *Obesity (Silver Spring).* 2012;20(5):1034-1040.

16. Nobili V, Liccardo D, Bedogni G, et al. Influence of dietary pattern, physical activity, and I148M PNPLA3 on steatosis severity in at-risk adolescents. *Genes Nutr*. 2014;9(3):392.

17. Ruiz JR, Labayen I, Ortega FB, et al. Physical activity, sedentary time, and liver enzymes in adolescents: the HELENA study. *Pediatr Res*. 2014;75(6):798-802.

18. Medrano M, Labayen I, Ruiz JR, et al. Cardiorespiratory fitness, waist circumference and liver enzyme levels in European adolescents: The HELENA cross-sectional study. *J Sci Med Sport*. 2017;20(10):932-936.

19. Bailey DP, Savory LA, Denton SJ, Davies BR, Kerr CJ. The triglyceride to high-density lipoprotein ratio identifies children who may be at risk of developing cardiometabolic disease. *Acta Paediatr*. 2014;103(8):e349-e353.

20. Medrano M, Maiz E, Maldonado-Martín S, et al. The effect of a multidisciplinary intervention program on hepatic adiposity in overweight-obese children: Protocol of the EFIGRO study. *Contemp Clin Trials*. 2015;45(Pt B):346-355.

21. Ruiz JR, Castro-Pinero J, Espana-Romero V, et al. Field-based fitness assessment in young people: the ALPHA health-related fitness test battery for children and adolescents. *Br J Sports Med*. 2011;45(6):518-524.

22. Tomkinson GR, Carver KD, Atkinson F, et al. European normative values for physical fitness in children and adolescents aged 9–17 years: results from 2 779 165 Eurofit performances representing 30 countries. *Br J Sports Med*. 2017;52(22):1445-1456.

23. Hildebrand M, VAN Hees VT, Hansen BH, Ekelund U. Age group comparability of raw accelerometer output from wrist- and hip-worn monitors. *Med Sci Sports Exerc*. 2014;46(9):1816-1824.

24. Migueles JH, Cadenas-Sanchez C, Ekelund U, et al. Accelerometer Data Collection and Processing Criteria to Assess Physical Activity and Other Outcomes: A Systematic Review and Practical Considerations. *Sports Med*. 2017;47(9):1821-1845.

25. Hildebrand M, Hansen BH, van Hees VT, Ekelund U. Evaluation of raw acceleration sedentary thresholds in children and adults. *Scand J Med Sci Sports*. 2017;27(12):1814-1823.

26. WHO - World Health Organization. Recommended levels of physical activity for children aged 5 - 17 years. Global recommendations on physical activity for health. http://www.who.int/dietphysicalactivity/factsheet_young_people/en/. Published 2010. Accessed May 25, 2017.

27. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: Practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Am J Gastroenterol*. 2012;107(6):811-826.

28. Kim G, Giannini C, Pierpont B, et al. Longitudinal effects of MRI-measured hepatic steatosis on biomarkers of glucose homeostasis and hepatic apoptosis in obese youth. *Diabetes Care*. 2013;36(1):130-136.

29. Radetti G, Kleon W, Stuefer J, Pittschieler K. Non-alcoholic fatty liver disease in obese children evaluated by magnetic resonance imaging. *Acta Paediatr*. 2006;95(7):833-837.

30. Trilk JL, Ortaglia A, Blair SN, Bottai M, Church TS, Pate RR. Cardiorespiratory Fitness, Waist Circumference and Aminotransferase in Youth. *Med Sci Sports Exerc*. 2014;45(4):722-727.

31. O'Donovan G, Thomas EL, McCarthy JP, et al. Fat distribution in men of different waist girth, fitness level and exercise habit. *Int J Obes (Lond).* 2009;33(12):1356-1362.

32. Thyfault JP, Rector RS, Uptergrove GM, et al. Rats selectively bred for low aerobic capacity have reduced hepatic mitochondrial oxidative capacity and susceptibility to hepatic steatosis and injury. *J Physiol.* 2009;587(Pt 8):1805-1816.

33. Anderson EL, Fraser A, Howe LD, et al. Physical Activity Is Prospectively Associated With Adolescent Nonalcoholic Fatty Liver Disease. *J Pediatr Gastroenterol Nutr*. 2016;62(1):110-117.

34. Slinger JD, van Breda E, Keizer H, Rump P, Hornstra G, Kuipers H. Insulin resistance, physical fitness, body composition and leptin concentration in 7-8 year-old children. *J Sci Med Sport*. 2008;11(2):132-138.

35. Jimenez-Pavon D, Ortega FB, Valtuena J, et al. Muscular strength and markers of insulin resistance in European adolescents: the HELENA Study. *Eur J Appl Physiol*. 2012;112(7):2455-2465.

36. Huang Y-C, Malina RM. BMI and health-related physical fitness in Taiwanese youth 9-18 years. *Med Sci Sports Exerc*. 2007;39(4):701-708.

37. Llorente-Cantarero FJ, Perez-Navero JL, de Dios Benitez-Sillero J, Munoz-Villanueva MC, Guillen-del Castillo M, Gil-Campos M. Non-traditional markers of metabolic risk in prepubertal children with different levels of cardiorespiratory fitness. *Public Health Nutr*. 2012;15(10):1827-1834.

38. Cohen DD, Gomez-Arbelaez D, Camacho PA, et al. Low muscle strength is associated with metabolic risk factors in Colombian children: the ACFIES study. *PLos One*. 2014;9(4):e93150.

39. Rizzo NS, Ruiz JR, Oja L, Veidebaum T, Sjostrom M. Associations between physical activity, body fat, and insulin resistance (homeostasis model assessment) in adolescents: the European Youth Heart Study. *Am J Clin Nutr*. 2008;87(3):586-592.

40. Owen CG, Nightingale CM, Rudnicka AR, et al. Physical activity, obesity and cardiometabolic risk factors in 9- to 10-year-old UK children of white European, South Asian and black African-Caribbean origin: the Child Heart And health Study in England (CHASE). *Diabetologia*. 2010;53(8):1620-1630.

41. Janssen I, Wong SL, Colley R, Tremblay MS. The fractionalization of physical activity throughout the week is associated with the cardiometabolic health of children and youth. *BMC Public Health*. 2013;13:554.

42. Puder JJ, Schindler C, Zahner L, Kriemler S. Adiposity, fitness and metabolic risk in children: a cross-sectional and longitudinal study. *Int J Pediatr Obes*. 2011;6(2-2):e297-306.

43. Chaput J-P, Saunders TJ, Mathieu M-E, et al. Combined associations between moderate to vigorous physical activity and sedentary behaviour with cardiometabolic risk factors in children. *Appl Physiol Nutr Metab*. 2013;38(5):477-483.

44. Andersen LB, Sardinha LB, Froberg K, Riddoch CJ, Page AS, Anderssen SA. Fitness, fatness and clustering of cardiovascular risk factors in children from Denmark, Estonia and Portugal: the European Youth Heart Study. *Int J Pediatr Obes*. 2008;3 Suppl 1:58-66.

Supplemental Material

Supplemental table 1. Differences in percentage hepatic fat, liver enzymes, insulin resistance, and TG/HDL ratio between children that followed or not the ≥60 min/day MVPA recommendations, adjusted for age, sex and accelerometer waking time.

		Active children (MVPA ≥60 min/day)		Inactive children (MVPA <60 min/day)	*P*
	N		N		
Percentage hepatic fat (%)	43	5.8 (0.6)	65	5.5 (0.5)	.76
ALT (U/L)	43	20 (1)	65	20 (1)	.84
GGT (U/L)	41	16 (1)	65	16 (1)	.62
AST/ALT ratio	43	1.3 (0.1)	65	1.2 (0.0)	.38
HOMA-IR	42	2.5 (0.2)	65	2.7 (0.1)	.50
TG/HDL ratio	43	1.7 (0.2)	65	1.9 (0.1)	.24

Abbreviations: ALT: alanine aminotransferase; AST/ALT: aspartate aminotransferase to alanine aminotransferase ratio; GGT: gamma-glutamyl transferase; HOMA-IR: insulin resistance homeostasis model assessment; min: minutes; MVPA: moderate-to-vigorous physical activity; TG/HDL: triglycerides to high density lipoprotein ratio. Non-transformed data are presented in the table, but ANCOVA was performed on log-transformed data in the case of variables not normally distributed.

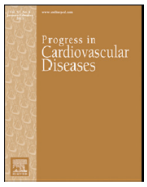
3.3. Study III

Published in: Progress in Cardiovascular Diseases. 2018;61(2);222-231.

Contents lists available at ScienceDirect

Progress in Cardiovascular Diseases

journal homepage: www.onlinepcd.com



Evidence-Based Exercise Recommendations to Reduce Hepatic Fat Content in Youth- a Systematic Review and Meta-Analysis

María Medrano[a,*], Cristina Cadenas-Sanchez[b], Celia Álvarez-Bueno[c], Iván Cavero-Redondo[c], Jonatan R. Ruiz[b,d], Francisco B. Ortega[b,d], Idoia Labayen[a]

[a]Department of Health Sciences, Public University of Navarra, Pamplona, Spain

[b]Department of Physical Education and Sport, School of Sport Sciences, University of Granada, Granada, Spain

[c]Universidad de Castilla-La Mancha: Health and Social Research Center, Castilla-La Mancha, Cuenca, Spain

[d]Department of Biosciences and Nutrition at NOVUM, Karolinska Institutet, Huddinge, Sweden

*Corresponding author: María Medrano, MSc, Department of Health Sciences, Public University of Navarra, Avenida Barañain s/n, 31008, Pamplona, Spain. E-mail address: maria.medrano.echeverria@gmail.com (M. Medrano).

Abstract

The main purposes of this study were to elucidate the effects of supervised-exercise training (ET) interventions on hepatic fat content and on non-alcoholic fatty liver disease (NAFLD) prevalence in children and adolescents and to provide information about the optimal ET prescription (type, intensity, volume, and frequency) needed to reduce hepatic fat content in youths. Supervised-ET interventions performed in children and adolescents (6–19 years) that provided results of exercise effects on hepatic fat content or NAFLD prevalence were included. Supervised-exercise significantly reduced hepatic fat content compared to the control groups. Lifestyle interventions that included supervised-ET significantly reduced the prevalence of NAFLD. This systematic review and meta-analysis shows that supervised-ET could be an effective strategy in the management and prevention of NAFLD in children and adolescents. Both aerobic and resistance ET, at vigorous or moderate-to-vigorous intensities, with a volume ≥60 min/session and a frequency ≥3 sessions/week, aiming to improve cardiorespiratory fitness and muscular strength, had benefits on hepatic fat content reduction in youth. These data concur with the international recommendations of physical activity for health promotion in youth and may be useful when designing ET programs to improve and prevent hepatic steatosis in the pediatric population.

Key words: evidence based review, exercise, liver fat, children, adolescent.

Abbreviations:

%HRmax, Percentage of maximum heart rate

%HRR, Percentage of heart rate reserve

1RM, One-repetition maximum

AeT, Aerobic training group

C, Control group

CI, Confidence Intervals

CRF, Cardiorespiratory fitness

CVD, Cardiovascular disease

EPHPP, Quality Assessment Tool for Quantitative Studies of the Effective Public Health Practice Project

ES, Effect size

ET, Exercise training

ITT, Intention-to-treat

MI, Moderate Intensity

MusS, Muscular strength

NAFLD, Non-alcoholic fatty liver disease

OR, Odd ratio

PA, Physical activity

PICOS, Participants, intervention, comparison, outcome and study designs

PRISMA, Preferred reporting Items for Systematic Reviews and Meta-analysis

PROSPERO, International Prospective Register of Systematic Reviews

RCT, Randomized controlled trial

RT, Resistance training group

T2D, Type 2 diabetes

VI, Vigorous intensity

VO_2max, Maximum oxygen consumption

Introduction

The global pandemic of obesity in children has led to a concomitant rise and early incidence of many health complications and metabolic abnormalities that used to be exclusive to adulthood[1]. Excess adiposity is associated with an accumulation of fat in multiple organs and tissues, such as skeletal muscle, heart and liver, among others[2]. Likewise, the excessive accumulation of triglycerides in hepatocytes leads to hepatic steatosis, the earliest manifestation of non-alcoholic fatty liver disease (NAFLD)[3]. Pediatric NAFLD is a major public health concern because of its elevated prevalence[4] and its association with insulin resistance, metabolic syndrome components and elevated risk to develop type 2 diabetes (T2D) and cardiovascular disease (CVD)[5–7]. Strategies to reduce or prevent NAFLD in overweight/obese children have the potential to improve overall cardiometabolic risk and reduce the risk to develop CVD, T2D and liver dysfunction in the future[3,8]. In adults, body mass loss is able to reduce hepatic fat content or even reverse the course of NAFLD[9]. Therefore, lifestyle interventions including hypocaloric diets and physical activity (PA) aiming to reduce body mass remain the cornerstone of NAFLD treatment for adults[10]. In children, however, energy restriction programs lead to undesirable lean mass loss and could compromise healthy growth and development. PA and Exercise training (ET) are effective in the reduction of cardiometabolic risk[11,12], also in overweight/obese youths[10,13]. Nevertheless, most children and adolescents do not meet the guidelines of PA recommendations in the United States and over the world[14]. Guidelines of the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology recommend ET as a primary form of treatment of NAFLD[15]. However, evidence supporting the effect of ET on the reduction of hepatic fat content and NAFLD prevalence is limited in the pediatric population. A meta-analysis on overweight and

obese children and adolescents showed that supervised-ET programs significantly reduced the intrahepatic fat percentage[16]. However, the effects of exercise on NAFLD prevalence or optimal ET prescription describing type (i.e. aerobic, resistance or combined ET), intensity (i.e. moderate or vigorous), volume and frequency of exercise needed to reduce hepatic fat content in children and adolescents is unclear. Cardiorespiratory fitness (CRF) is associated with CVD[11,17,18] in all weight status[19] and is a predictor of all cause and disease mortality in varied populations[20]. More specifically, CRF and muscular strength (MusS) are associated with CVD risk factors[17,21], abdominal obesity[21], as well as with other health components in children and adolescents[17,21]. Similarly, CRF is associated with liver enzyme levels and liver function in this population[22,23]. However, little is known about the relationship between both CRF and MusS with hepatic fat content in children and adolescents. Therefore, the aims of the current systematic review and meta-analysis are: i) to systematically examine the effects of supervised-ET interventions on hepatic fat content and NAFLD prevalence in children and adolescents ii) to examine the specific effect of the type of ET (aerobic *vs*. resistance), intensity, volume and frequency on hepatic fat content in children and adolescents, and iii) to explore whether exists an association of improvements in CRF and MusS with the reduction of hepatic fat in youth.

Methods

Protocol and registration

The systematic review and meta-analysis were conducted following the Preferred reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement[24]. The review protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO reference number CRD42017055834) and is available from https://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42017055834.

Eligibility criteria

The participants, intervention, comparison, outcome, and study designs (PICOS) framework[25] was followed to define research question and study eligibility criteria. Studies were considered eligible for their inclusion if: 1) the age of participants was from 6 to 19 years, 2) the duration of the supervised-ET intervention was at least 8 weeks in order to be able to detect effects on the outcome[26], 3) they provided results that allowed outcome comparison between the intervention and control group and/or pre and post intervention results within groups, 4) they provided results on hepatic fat content quantified by proton magnetic resonance, magnetic resonance imaging, or prevalence of NAFLD measured by ultrasonography, and 5) they were either controlled trials or experimental studies without a control group.

Studies that involved other lifestyle activities (nutritional, motivational, psychological support, *etc.*) apart from supervised-ET were also included in the systematic-review and meta-analysis. In the case of controlled trial studies, they were only included when these lifestyle activities were performed by children in ET and control groups allowing to discriminate the independent effect of ET.

Studies that were not written in English or were grey literature, as well as reviews, editorials, opinions, letters, and meeting abstracts were excluded.

Data sources and search strategies

We conducted a systematic literature search in PubMed, Web of Science, EMBASE, and SportDiscuss databases for studies that showed results of the effect of supervised-ET on hepatic fat content or NAFLD prevalence in children and/or adolescents published until the 11th January 2017.

The keywords used in search strategy were related to the following topics: 1) Participants: children and adolescents, 2) Intervention: exercise, 3) Outcome: hepatic fat and 4) Design: trial. The four search topics were combined using the Boolean searching with *and* and *or* operators. Keywords search terms and search strategies used were different for each database (**Supplemental material 1**). As an example, the search strategy in PubMed database was: ("children" OR "adolescent" OR "youth" OR "teenager" OR "boy" OR "girl") AND ("obesity AND hepatic fat" OR "obesity AND non-alcoholic fatty liver disease" OR "obesity AND non-alcoholic steatosis" OR "non-alcoholic steatosis" OR "non-alcoholic fatty liver disease" OR "fatty liver" OR "hepatic fat" OR "metabolic risk" OR "cardiometabolic risk" OR "type 2 diabetes") AND ("exercise" OR "physical activity" OR "exercise intervention" OR "training") AND ("intervention" OR "program" OR "trial" OR "treatment" OR "pre-post study"). Additionally, the reference lists of retrieved studies were examined to identify other articles.

Once the search strategies were executed in the different databases, they were imported into EndNote software (version ×7, Thomson Reuters, USA). Duplicate files were removed, firstly automatically by the software and secondly by visual checking.

Study selection process

Two reviewers (MM and CC-S) independently performed the study selection process according to a form established *a priori*. The title and abstract of each retrieved article were examined to identify those that were likely to report the effects of supervised-exercise on hepatic fat and/or NAFLD prevalence. Studies appearing eligible based on their abstract or those that could not be excluded based on their title and abstract were read full-text against the inclusion criteria for their final inclusion or exclusion in the systematic review. Attempts to contact authors were made when articles did not contain

all the information necessary for their selection. Disagreements about study selection were resolved by reaching consensus among reviewers.

Among the articles included in the systematic review, those studies that provided pre and post intervention values of hepatic fat content or NAFLD prevalence were included in the meta-analysis. **Supplemental Fig 1** outlines the flow diagram of the study selection process.

Data collection process and data items

Reviewers created a study specific database in Excel (Microsoft Corp., USA) for data collection. Data were extracted from the studies included in the database by one reviewer, and checked for accuracy by a second reviewer.

The extracted data were the following: 1) author's name, study name, and year of publication of the article, 2) number, age, gender, and weight status of the participants, 3) type, intensity, volume, frequency, and duration of the exercise intervention, and activities (nutritional and/or physiological) of the lifestyle intervention, 4) study design (*i.e.* type of trial and presence of a control group), 5) method of measurement of hepatic fat content or NAFLD prevalence and their results, 6) covariates used in meta-regressions (*i.e.* cardiorespiratory fitness, muscular strength, *etc*.), if any. When the studies presented both intention-to-treat (ITT) and per protocol values, only the results from the ITT data were extracted.

We contacted the corresponding authors in those cases in which the full-text did not provide all the relevant information for the data extraction and/or meta-analysis.

Study quality and risk of bias assessment

Study quality was assessed by two independent reviewers (MM and CC-S). Critical methodological components were assessed and disagreements were solved in a consensus meeting. Concordance among the reviewers' scores in the risk of bias assessment was measured using the Kappa (*k*) statistic.

All the included randomized controlled trials (RCT) were assessed using the Cochrane risk of bias tool[27]. Each study was assessed for selection bias (random sequence generations and allocation concealment), performance bias (blinding of participants and research staff), detection bias (blinding of the outcome assessment), attrition bias (incomplete outcome data), reporting bias (selective reporting), and other sources of bias.

The methodological quality of non-RCT and studies without a control group were assessed using the Quality Assessment Tool for Quantitative Studies of the Effective Public Health Practice Project (EPHPP)[28,29]. This tool is used to evaluate a variety of intervention study designs, such as non-RCT or pre-post studies[30]. EPHPP assesses study quality in six domains: selection bias, study design, confounders, blinding, data collection method, and withdrawals/dropouts.

Data synthesis and analysis

Data synthesis was narrative and descriptive, and is presented in text and in detailed tabular summaries (**Table 1**).

Meta-analysis for hepatic fat content was performed in 1) intervention *vs.* control groups, and 2) pre *vs.* post intervention values for each intervention group, and in those studies without control group. In the analysis of studies comparing intervention vs. control group, mean difference (post *minus* pre intervention values) and standardized mean difference were calculated for each intervention group. We used Campbell Effect-

Size Calculator[31] to calculate effect size (d-Cohen) (ES) and 95% confidence intervals (CI). ES was used to standardize changes of hepatic fat. For the analysis of pre *vs*. post analysis, ES and CI were calculated using post minus pre mean values and standardized differences. Pooled ES of the effect of ET on hepatic fat was obtained using random-effects models, due to heterogeneity detected among the analyzed studies. Heterogeneity was quantified using the I^2 statistic (the percentage of total variability attributed to between-study heterogeneity). A large I^2 value indicates more heterogeneity. Values of 25%, 50% and 75% denoted cut-off points for low, moderate, and high degrees of heterogeneity, respectively[32]. Potential sources of heterogeneity were assessed with meta-regression. In addition, we performed meta-analysis among subgroups by ET type (aerobic and resistance), intensity (moderate and moderate-to-vigorous and vigorous), volume (<60 or ≥60 min/session), or frequency (<3or ≥3 sessions/week) with the aim of knowing which of the subgroups had a greater influence on the reduction of liver fat content and NAFLD prevalence. Subgroup analyses were performed when there were enough studies to compare.

As most of the studies examining the effect of exercise on NAFLD prevalence did not include a control group, a meta-analysis was performed using post minus pre percentages of prevalence. Odds ratio (OR) for each intervention group and pooled OR estimates were obtained.

Furthermore, we performed meta-regressions to examine the relationship between the ES estimates for hepatic fat content and the following covariates: 1) changes in CRF [measured as maximum oxygen consumption (VO_{2max}, mL/kg/min)] and 2) changes in MusS [measured as MusS Index (sum of one-repetition maximum scores per kilogram of body weight)]. In meta-regressions, standardized β and P values were obtained.

For assessing the risk of potential publication bias, we used visual inspection of funnel plots and we calculated the P value of Egger's intercept. We followed the Duval and Tweedie “trim and fill” procedure as a method of adjustment for suspected publication bias. In this procedure, pooled ES was recalculated incorporating hypothetical missing studies as if they existed.

The influence of each independent study in the pooled estimates of ES was detected using sensitivity analysis. The leave-one-out analysis helped to examine the influence of *various* exclusions on the combined ES by omitting one study in turn.

P values of <0.05 were accepted to indicate statistical significance. Statistical analyses were performed with the Comprehensive Meta-Analysis software version 3 (Englewood, NJ: Biostat. USA).

Results

Study selection

A total of 4153 studies were identified through databases search. Duplicate references were identified and removed (*N*=933). After examining titles and abstracts, 72 full-texts were further screened and 16 studies were identified as meeting inclusion criteria[33–48] and included in the systematic review. The reasons for exclusion at this full-text level (*N*=56) are listed in Supplemental Fig 1. Of the 16 articles included in the systematic review, 14 provided pre and post intervention values of hepatic fat content[33,34,36,41,42] or NAFLD prevalence[38–40,43–48], and were thus included in the meta-analysis. The flow diagram summarizing the study selection process of the systematic review and meta- analysis is shown in Supplemental Fig 1.

Study characteristics

The characteristics of the 16 studies included in the systematic review sorted by study design are available in Table 1. Of the 16 included studies, five were RCTs[33–37], two were non-RCTs[43,44], and nine were intervention studies without control group (pre-post studies)[38–42,45–48]. All together, the selected studies comprised 1201 participants. Sample size ranged from 12 to 180 children; from 24% to 59% boys, except in three studies in which only girls[34,37] or boys[36] participated. The majority of the analyzed studies (*N*=11) were performed in adolescents[33–38,41,42,46–48] and the remaining five studies included both children and adolescents[39,40,43–45]. None of the studies included only children (≤12 years). Two studies included youths with overweight and obesity[33,37], one only with overweight[44], and 11 studies exclusively youths with obesity[34–36,38–40,42,43,46–48]. Two interventions[41,45] included lean and obese children, although one of them[41] only reported outcome data for obese children.

Seven studies quantified hepatic fat content by proton magnetic resonance[33,34,36] or magnetic resonance imaging[35,37,41,42], whereas nine reported NAFLD prevalence measured by ultrasonography[38–40,43–48]. Hepatic steatosis was defined as a peak triglyceride to water ratio higher than 5.5%[33,49] or 5.6%[41,42,50]. In ultrasonography measurements, the definition of steatosis or the quantification of fatty liver was based on Sabir[38,46,51], Saadeh[38,46–48,52], Mazhar[39,53], or Saverymuttu[40,44,45,54] criteria.

All the studies conducted supervised-ET sessions; however, the type, intensity, volume, frequency, and duration of ET varied across studies. According to the type of ET, the design of three studies included exclusively aerobic[33,41,46], two of them only resistance[35,42], two combined both aerobic and resistance ET[37,38], while two studies compared the effects of aerobic *vs*. resistance[34,36]. The remaining studies did not provide details of the type of ET and described the design as general exercise (ball games, jogging,

etc.)[43–45], supervised activities (bicycling, walking, running, *etc*.)[39], or recreational sports, activities, gymnastics, and walking[47], or did not report any detail about the type of exercise[40,48]. ET intensity was based on percentage of heart rate reserve (% HRR)[33], percentage of maximum heart rate (% HRmax)[37], percentage of VO_{2max}[34,36,41], percentage of one-repetition maximum (1RM)[34–36], or 3RM[42]. Two studies established the intensity at a workload corresponding to the first ventilatory threshold (50–70% of oxygen consumption test)[38,46]. The rest of studies described the intensity of ET as "moderate PA"[47], "moderate strenuous"[39] or did not specify at all[40,43–45,48].Three studies[33,34,36] measured energy expenditure in the sessions and one of them[33] adjusted ET volume according to this variable. Due to the differences in the intensity measurements across studies, we classified the ET intensity according to the American College of Sport Medicine classification[55] (as an example, considering the %HRmax, vigorous intensity ranges from 76% to 96%). The volume (min/session) of exercise was 60 min in 10 of the studies[34–36,38–40,42,46–48], 50 min/session in one study[41], two of the studies used a progression in session time from 30 min to 45 min[33] or from 60 min to 90 min[37], and three of them did not report the session volume data[43–45]. Most of the studies had a volume ≥60 min/session[34–40,42,46–48] and two had a volume <60 min/session[33,41]. Frequency of exercise sessions varied from 2 to 7 sessions per week, being a frequency of 3 sessions per week the most com- mon (N=5)[33,34,36,38,40,46]. Nine of the studies had a frequency <3sessions/week[35,37,41–45,47,48] and seven studies ≥3 sessions/week[33,34,36,38–40,46]. The duration of the intervention ranged from 10 to 52 weeks. Seven studies reported changes in CRF (estimated by changes in VO_{2max}[33,34,36,37,41]) and/or in MusS (estimated as MusS index[34,36], chest and press weight in kilograms or pounds[35–37,42] or sets *x* repetitions[42]) after the intervention. In addition to ET, the design of several studies also included nutritional[35,39], physiological[37] or both nutritional and physiological[38,40,43–48] activities in their interventions.

Table 1- Study design, participants, outcome, exercise and lifestyle intervention characteristics of included studies (*N*=16) in the systematic review.

	Study design	Participant characteristics			Outcome	Exercise characteristics					Lifestyle intervention
Study reference	**Study design**	***N* (analysed in the original study)**	**Age (yr) (range or mean)**	**Sex (%male)**	**Liver fat content or NAFLD measurement**	**Exercise type intervention groups (*N* analysed per group)**	**Intensity**	**Volume (minutes/ session)**	**Frequency (session/ week)**	**Duration (weeks)**	**Other Lifestyle interventions**
Hay et al. 2016[24]	RCT	106	13-19	24%	Proton magnetic resonance	AeT moderate intensity and AeT vigorous intensity (*N*=32 and 38)	Moderate or Vigorous	30-45	3	24	
Lee et al. 2013[25]	RCT	44	12-18	0%	Proton magnetic resonance	AeT and RT (*N*=16 and 16)	Moderate to Vigorous	60	3	12	
Hasson et al. 2012[26]	RCT	100	14-18	41%	MRI assessment	RT (*N*=31)	Moderate to Vigorous	60	2	16	Dietary and motivational interviewing
Lee et al. 2012[27]	RCT	45	12-18	100%	Proton Magnetic Resonance	AeT and RT (*N*=15 and 16)	Moderate to Vigorous	60	3	12	
Davis et al. 2011[28]	RCT	38	14-18	0%	MRI assessment	Circuit training (AeT+RT) (**N**=14)	Moderate to Vigorous	60-90	2	16	Motivational interviews
Dâmaso et al. 2013[29]	Study without control group	77	15-19	42%	US assessment	AeT+RT	Moderate	60	3	52	Nutritional and psychological
Gronbaek et al. 2012[30]	Study without control group	117	12.1 ±1.3	44%	US assessment	Supervised activities: bicycling, walking, running, *etc*.	Moderate to Vigorous	60	7	10	Nutritional (Weight loss camp)

Koot et al. 2011[31]	Study without control group	144	14.1 ±2.3	38%	Obesity	US assessment	Exercise n.s.	n.s.	60	3	24	Nutritional and behaviour modification therapy
Van der Heijden et al. 2010[32]	Study without control group	29	15.6 ±0.4	59%	Obesity (*N*=15) Normoweight (N=14)	MRI assessment (just in obese children)	AeT	Vigorous	50	4 (2 supervised)	12	
Van der Heijden et al. 2010[33]	Study without control group	12	15.5 ±0.5	50%	Obesity	MRI assessment	RT	Moderate to Vigorous	60	2	12	
Reinehr et al. 2009[34]	Control Clinical Trial	152	6-16	M & F n.s.	Obesity	US assessment prevalence	General Exercise (Ball games, jogging, *etc*.) (N=109)	n.s.	n.s.	1	52	Nutrition education and behavioural therapy
Reinehr et al. 2009[35]	Control Clinical Trial	180	10.7 ± 2.5	48%	Overweight	US assessment prevalence	General Exercise (Ball games, jogging, *etc*.) (N=130)	n.s.	n.s.	1	52	Nutrition education and behavioural therapy
Reinehr et al.2008[36]	Study without control group	36	6-14	45%	Normoweight (*N*=14) and Obesity (*N*=36)	US assessment prevalence	General Exercise (Ball games, jogging, *etc*.)	n.s.	n.s.	1	52	Nutrition education and behavioural therapy
de Piano et al. 2007[37]	Study without control group	34	15-19	44%	Obesity	US assessment	AeT	Moderate	60	3	12	Dietary and Psychological
Damaso et al. 2006[38]	Study without control group	28	15-19	43%	Obesity	US assessment	Recreational sports activities, gymnastics and walking	Moderate	60	2	12	Nutritional and Psychological
Tock et al. 2006[39]	Study without control group	73	15-19	33%	Obesity	US assessment (hepatocytes affected)	Exercise n.s.	n.s.	60	2	12	Dietary, clinical Psychological support

Abbreviations: AeT: Aerobic Training; MRI: magnetic resonance imaging; NAFLD: non-alcoholic fatty liver disease; n.s.: not specified; RCT: randomized control trials; s.e.: standard error; RT: Resistance Training; US: ultrasonography; yr: years.

Risk of bias within studies

The quality of the included studies was generally suboptimal. Methodological quality assessment is shown in **Supplemental Fig 2A** for RCTs and in **Supplemental Fig 2B** for non-RCTs and studies without a control group.

Details of randomization[35,37], allocation concealment[35,37], and study blinding[34–37] were inadequately reported or rated as an "unclear risk" (categorized when the information of those domains of bias was not specified in the article) for most RCTs.

None of the non-RCTs or the studies without a control group reported an adequate selection bias. Double-blind design (participants and researchers) was not applied due to the nature of the interventions. Retention rates ranged from 79% to 100%. Seven of the included studies did not explicitly report dropout rate or number of participants that finished the intervention. Only 3 studies used ITT analysis[33,34,36].

There was strong agreement among the reviewers in allocating risk of bias scores in both Cochrane risk of bias tool and EPHPP quality assessment tool (k=0.828 and 0.884, respectively).

Effects of exercise on hepatic fat content comparing ET intervention vs. control groups

Meta-analysis of the ET vs. control effects showed a significant pooled ES for the efficacy of the exercise on hepatic fat content reduction (ES=−2.10, 95% CI:−3.25 to−0.95; P< 0.001, I^2=92%, 181 participants) (**Fig 1**). A high heterogeneity (>75%) was observed among studies. Differences in ET intensity explained partially the heterogeneity among studies (P<0.001; I^2=59%), whereas the number of sessions per week did not seem to affect it (P>0.05).

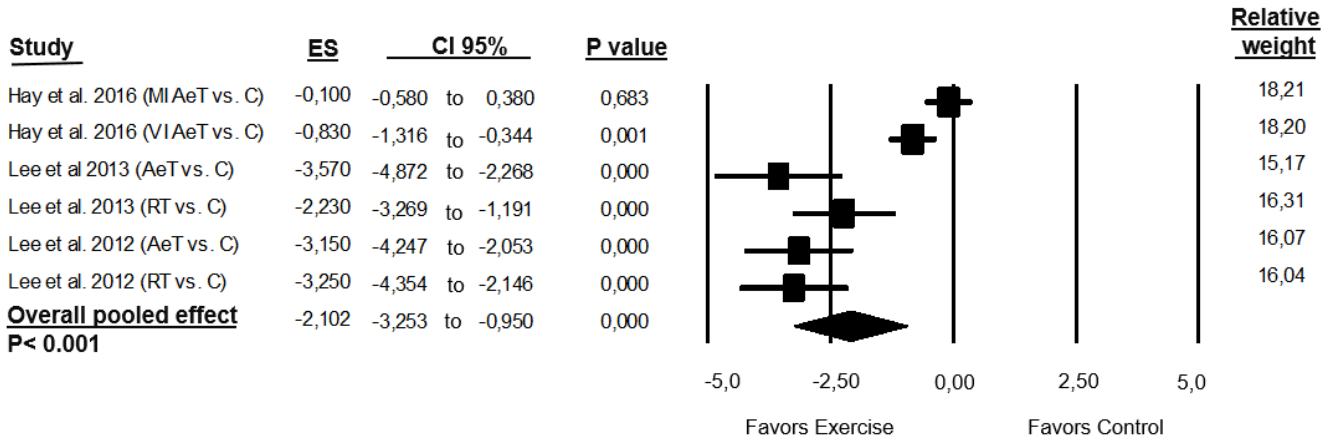


Fig 1. Meta-analysis of the pooled effect size (ES) and confidence intervals (CI) (95%) of ET intervention *vs*. control group on hepatic fat content measured by resonance magnetic imaging. The size of the square represents the weight of each study. *Abbreviations*: AeT: aerobic training group; C: control group; MI: moderate intensity; RT: resistance training group; VI: vigorous intensity. If nothing is specified about intensity of ET, it corresponds to a moderate-to-vigorous ET intensity.

There was a statistically significant publication bias according to Egger's test (P=0.003) or a visual inspection of the funnel plot for hepatic fat outcomes (**Supplemental Fig 3A**). However, after having incorporated the imputed studies (N=3) using "trim and fill" procedure, the ES estimate was −0.63 (95% CI:−0.90 to −0.37). Thus, correction for potential publication bias did not alter the significant association.

Subgroup analysis was conducted by ET type (aerobic and resistance), intensity (moderate or moderate-to-vigorous and vigorous), and volume (<60 or ≥60 min/session) (**Supplemental Fig 4A, B** and **C** respectively). Both aerobic (ES=−1.79; 95% CI:−3.13 to −0.45; P=0. 009, I^2=93%, 101 participants) and resistance ET interventions (ES=−2.72, 95% CI:−3.72 to −1.72; P<0.001, I^2=42%, 32 participants) were associated with a significant reduction of hepatic fat content. When the analysis was performed removing the moderate aerobic intensity group, it was observed that the pooled ES of the aerobic sub- group increased (ES=−2.45 95% CI:−4.39 to −0.51; P=0.013, I^2=92%, 69 participants) (Supplemental Fig 4A). Only vigorous and moderate-to-vigorous intensities (ES=−2.55, 95% CI:−3.80 to−1.29; P<0.001, I^2=89%, 101 participants) and a volume

≥60min/session (ES=−2.99, 95% CI:−3.56 to −2.42; *P*<0.001, I^2=4%, 63 participants) were associated with a significant reduction of hepatic fat content (Supplemental Fig 4B and C, respectively).

Meta-regression analysis showed a significant correlation between changes in CRF (VO_{2max}, mL/kg/min) and ES of hepatic fat content (β=−0.43, 95% CI −0.54 to −0.33; *P*<0.001) (**Fig 2A**). In other words, those youth participating in ET interventions that improved more their CRF levels as compared to children participating in the control groups, had higher reductions in hepatic fat content. A non-significant correlation between changes in MusS (MusS index) and ES of hepatic fat content (β=−4.08, 95% CI 8.42 to 0.27; *P*=0.066) was also observed (**Fig 2B**).

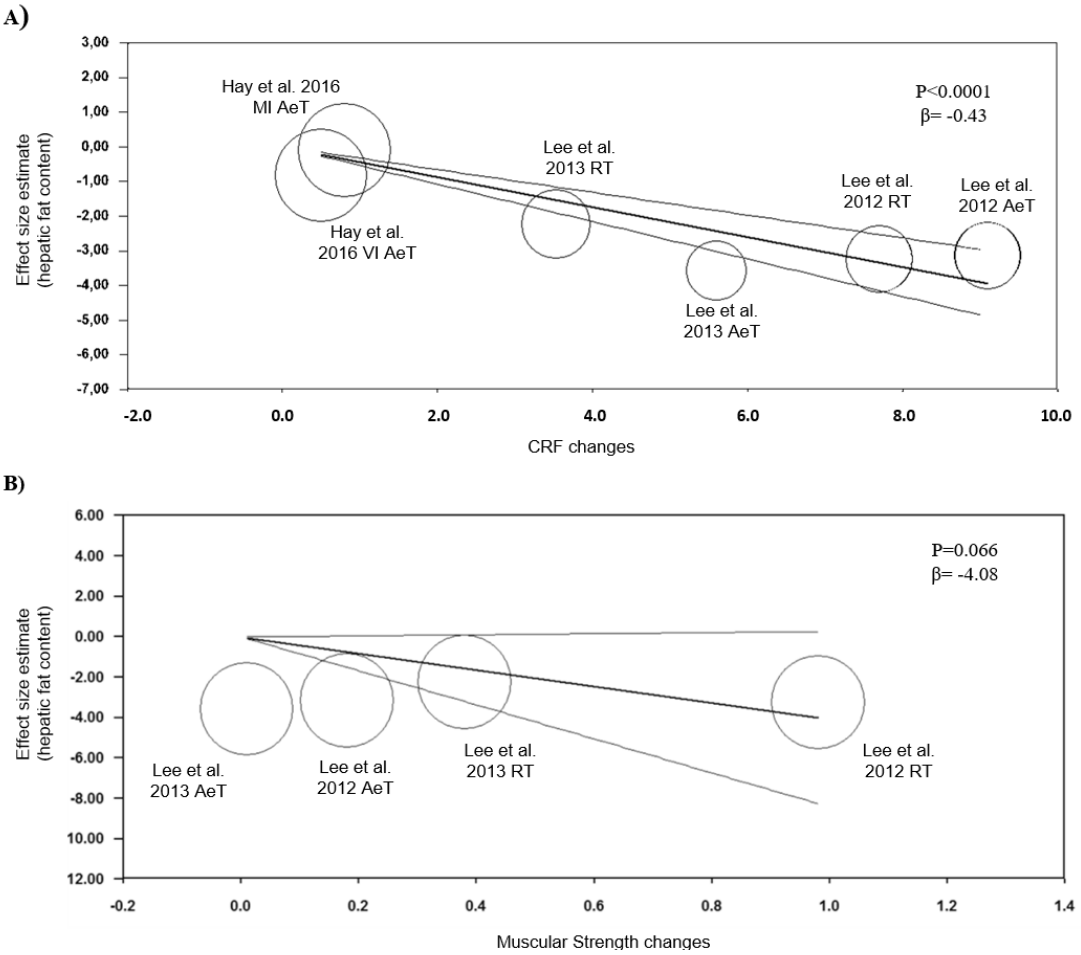


Fig 2. Meta-regression analysis (standardized *β* coefficient and *P* values in exercise *vs*. control analysis) of changes in CRF (VO_{2max}, mL/kg/min) (A) or changes in MusS (MusS Index) (B) (X axis) against the effect size on hepatic fat content (Y axis). The more negative the effect size is, the more effective is the ET intervention group compared to control group reducing hepatic fat content. For studies without control group see supplementary material. The size of the circle represents the weight of each study. *Abbreviations*: AeT: aerobic training group; C: control group; CRF: cardiorespiratory fitness; MI: moderate intensity; RT: resistance training group; VI: vigorous intensity. If nothing is specified about intensity of ET, it corresponds to a moderate to vigorous ET intensity.

Effects of ET on hepatic fat content comparing post vs. pre intervention values

The meta-analysis of ET effects (post *vs*. pre) on hepatic fat content showed a non-significant pooled ES (ES=−0.32, 95% CI:–0.79 to 0.16; *P*=0.189, I^2=77%, 222 participants) (**Fig 3A**). However, in the leave-one-out analysis by omitting one study in turn, the pooled ES be- came significant (ES=−0.50, 95% CI:−0.79 to −0.20; *P*=0.001) after having removed the moderate intensity ET intervention group from the analysis (**Fig 3B**).

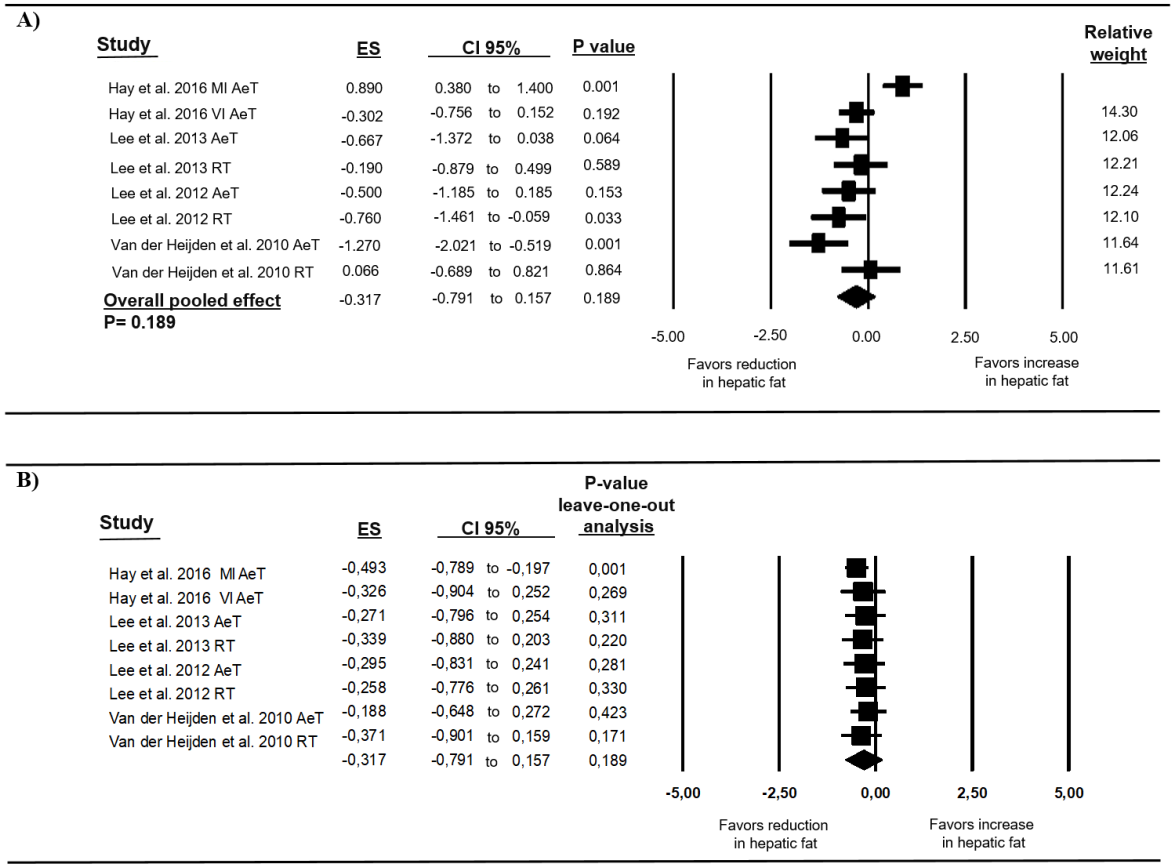


Fig 3. Meta-analysis of the pooled effect size (ES) and confidence intervals (CI) (95%) (post *vs*. pre values) of ET on liver fat content measured by resonance magnetic imaging (A) and sensitivity analysis by leave-one-out analysis, in which the pooled effect size (point) and significance (*p*-value) are shown if each study were eliminated (B). The size of the square represents the weight of each study. *Abbreviations*: AeT: aerobic training group; C: control group; MI: moderate intensity; RT: resistance training group; VI: vigorous intensity. If nothing is specified about intensity of exercise, it corresponds to a moderate to vigorous ET intensity.

There was not risk of publication bias according to either the Egger's test (*P*=0.19) or a visual asymmetry of the funnel plot (**Supplemental Fig 3B**).

Type of ET: It was observed that aerobic ET performed at least at moderate-to-vigorous intensity (ES=−0.62, 95% CI:−1.02 to −0.22; *P*=0.002, I^2=37%, 81 participants), but not resistance ET (ES=−0.31, 95% CI:−0.78 to 0.17; *P*=0.205, I^2=25, 44 participants), was effective in the reduction of hepatic fat content (**Supplemental Fig 5A**).

Intensity: The moderate intensity subgroup showed a significant increase in the hepatic fat content (ES=0.89, 95% CI:0.38 to 1.40; *P*=0.001, I^2=0%, 32 participants), whereas the moderate-to-vigorous and vigorous subgroup significantly reduced hepatic fat (ES=−0.49, 95% CI:−0.79 to −0.20; *P*=0.001, I^2=29%, 125 participants) (**Supplemental Fig 5B**).

Volume and frequency: Significant reductions of hepatic fat content were observed with at least ≥60min of ET/session (ES=−0.42, 95% CI:–0.74 to −0.11; *P*=0.009, I^2= 0%, 82 participants) and with a minimum frequency of 3 sessions/week at moderate-to-vigorous or vigorous intensity (ES=−0.44, 95% CI:–0.72 to −0.17; *P*=0.002, I^2= 0%, 101 participants) (**Supplemental Fig 5C and D**, respectively).

In the meta-regression analysis, there was a significant association between changes in CRF and hepatic fat content reduction (*β*=−0.09, 95% CI −0.18 to −0.01; *P*=0.039) (**Supplemental Fig 6A**), which was strengthened after having removed the moderate aerobic intensity group from the analysis (*β*=−0.10, 95% CI −0.16 to −0.04; *P*=0.001) (**Supplemental Fig 6B**), as well as between changes in MusS and hepatic fat content reduction (*β*=−0.80, 95% CI −1.52 to −0.08; *P*=0.030) (**Supplemental Fig 6C**).

Effects of ET on NAFLD prevalence comparing post vs. pre intervention values in interventions including other lifestyle activities

Seven studies were included in the meta-analysis of the effects of supervised-ET interventions on NAFLD prevalence. In this case, all the studies also performed multidisciplinary lifestyle interventions (ET + nutrition and/or physiological activities). Thus, the effect on NAFLD prevalence was not only because of ET, but the combined effect of all the lifestyle components. Four out of seven studies reported a significant reduction in the prevalence of NAFLD after the lifestyle intervention. The pooled OR of changes in NAFLD prevalence was 0.383 (95% CI 0.23 to 0.63; P<0.001, I^2=67%, 748 participants) (**Fig 4**). Thus, the participation in a lifestyle intervention program, including ET was associated with a significant decrease in the risk of NAFLD.

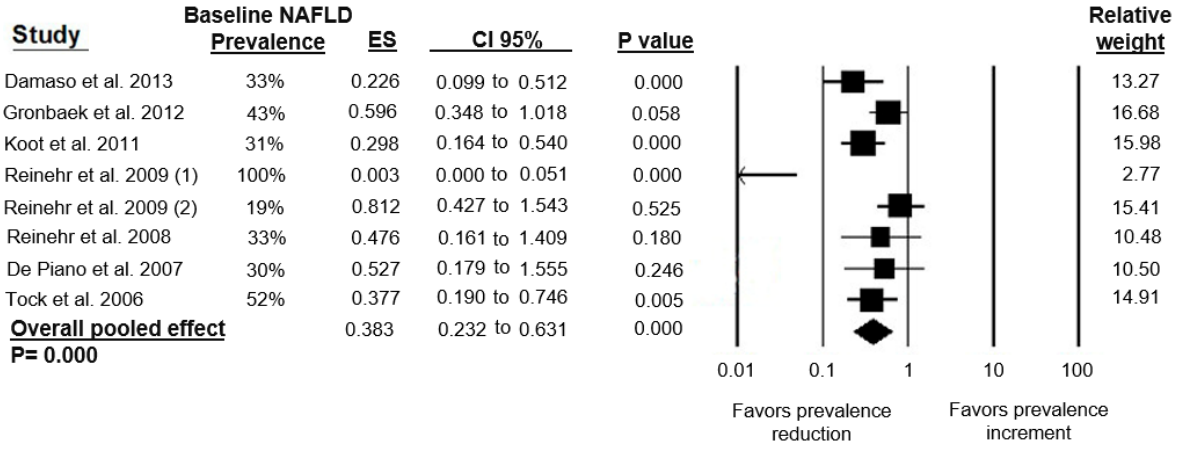


Fig 4. Meta-analysis forest plot of pooled Odd ratio of post *vs.* pre NAFLD prevalence measured by ultrasonography. All the studies included other lifestyle intervention activities apart from ET (*e.g.* nutritional advices). The size of the square represents the weight of each study. *Abbreviations*: NAFLD: non-alcoholic fatty liver disease.

A significant publication bias estimated by either the Egger's test (*P*=0.08) or by visual asymmetry of the funnel plot (**Supplemental Fig 3C**) was observed. In any case, the effect of lifestyle interventions on NAFLD was not diminished when the 'trim and fill' procedure of publication bias was applied (ES=0.45; 95% CI:0.34 to 0.59).

We did not examine differences in NAFLD prevalence according to the type of ET, intensity, or volume, because the studies did not report this information. Nevertheless, subgroup analysis by frequency showed that both frequencies (<3 and ≥3 sessions/week) were associated with significant reductions in NAFLD prevalence (**Supplemental Fig 7**).

Discussion

In this systematic review and meta-analysis, we examined the effects of supervised-ET on hepatic fat content and NAFLD prevalence in children and adolescents. The main findings of our study are the following: i) supervised-ET interventions reduced hepatic fat content and NAFLD prevalence in children and adolescents, ii) both aerobic and resistance ET at vigorous or moderate-to-vigorous intensity, with a volume of ≥60 min/session and a minimum frequency of 3 sessions/week significantly reduced hepatic fat content, and iii) higher increases in CRF and MusS levels after the supervised-ET interventions are associated with greater reductions in hepatic fat content. To our knowledge, this is the first systematic review and meta-analysis assessing the effects of supervised-ET on hepatic fat content and NAFLD prevalence in children and adolescents, and providing evidence of the efficacy of the type, intensity, volume, and frequency of ET, as well as of the role of improvements in aerobic and muscular fitness on hepatic fat content. These findings could help to provide more specific exercise recommendations for NAFLD management in pediatric population.

Our results show that ET alone has beneficial and strong effects on hepatic fat content, compared to the non-ET control group. This finding is consistent with a recently published systematic review and meta- analysis[16] which showed that exercise significantly reduced liver fat content in youth. Nevertheless, this study did not analyze the effects of ET on NAFLD prevalence or the specific effects of different type of ET, intensities, volumes, or frequencies on hepatic fat content. Neither the association of changes in CRF nor MusS in hepatic fat reduction were provided. Other narrative review[56] also suggested that ET alone may improve hepatic steatosis in children and adolescents. Keating et al.[57] in adults, and Orci et al.[58] in adults and adolescents also demonstrated benefits of ET alone on hepatic fat content and NAFLD management. The effectiveness of ET on hepatic fat content was not statistically significant in the meta-analyses performed comparing post *vs*. pre intervention values, although some of the studies showed a significant ES in favors of the post-ET intervention. The differences between the results observed in analyses examining the results of ET *vs*. control groups and post *vs*. pre analyses could be precisely due to the comparison with a control group. Adolescents who participated in the ET intervention reduced hepatic fat content, whereas participants in the control group increased it[33,34,36]. Thus, ET may counteract the increment on hepatic fat content observed in youth participating in control groups (without ET). Also, ET showed a beneficial effect on NAFLD prevalence. The majority of the studies reporting the effect of supervised-ET on NAFLD prevalence also included nutritional and/or physiological activities in the interventions. Likewise, the reduction of NAFLD prevalence was due to supervised-ET in conjunction with other lifestyle components. We did not find previous reviews specifically referring to the effects of lifestyle intervention programs, including supervised-ET on NAFLD prevalence. However, several reviews showed the benefits of lifestyle intervention programs on the management of NAFLD in adults[59,60] and also in children and adolescents[56,61]. In this regard,

lifestyle interventions involving diet and ET are considered a cornerstone of NAFLD treatment in the pediatric population[3].

The subgroup analyses revealed that both aerobic and resistance ET at vigorous and moderate-to-vigorous intensities, in sessions of ≥60min and a frequency ≥3 sessions per week are more effective reducing hepatic fat content in children and adolescents. These results are in line with the international recommendations of PA for the promotion of health in young people of the World Health Organisation[62] or the "Centers for Disease Control and Prevention"[63] which recommend 60min of aerobic and muscle strengthening ET at moderate and vigorous intensity every day or at least 3 days per week in this population. Thus, these results suggest that the design of future ET interventions for NAFLD management and prevention in children and adolescents should include both aerobic and resistance ET. It should be performed at vigorous or moderate-to-vigorous intensity, with at least a volume of 60 min/ session and a frequency of three sessions/week since they have shown greater benefits in reducing hepatic fat content and NAFLD prevalence. However, these results should be taken with caution given the reduced number of studies included in the analysis. A recent systematic-review in adults concluded that both aerobic and resistance ET similarly reduced hepatic steatosis[64], which concurs with our findings. In contrast, a meta-analysis in adults concluded that aerobic ET was more effective than resistance training in the reduction of intrahepatic lipid content[58]. Hashida et al. in their meta-analysis[64] proposed as a median effective protocol to improve hepatic steatosis in adults a volume of 40min/session, three times/week for aerobic ET or 45 min/session and three times/week for resistance exercise, which is similar to our results.

In the post *vs*. pre analyses, the effect of ET on hepatic fat content became significant when moderate ET intervention was excluded from the leave-one-out sensitivity analyses.

This result also suggests that at least part of the ET should be performed at vigorous intensity to achieve a beneficial effect on hepatic adiposity. In general, vigorous intensity ET maximize the benefits of ET on CVD risk factors and health[65]. Regarding hepatic health, several trials have reported beneficial effects of high intensity ET on hepatic fat content in adults[66,67]. However, as far as we are aware, there is only one study comparing the effects between vigorous and moderate ET intensities on hepatic fat content in youth[33]. Of note is that, although there were no significant differences on changes in hepatic fat among the control, moderate, and vigorous intensity groups, only those children participating in the vigorous intensity group reduced their hepatic adiposity. In fact, both youths of the control and moderate intensity groups increased their hepatic fat content after the intervention[33]. Balducci et al.[68] did not find differences between low-to-moderate and moderate-to-vigorous exercise groups on hepatic fat index, which was calculated from gamma-GT, body mass index, waist circumference, and triglyceride levels. To the best of our knowledge, there are no previous studies in humans comparing the effects of moderate-to-vigorous ET with just vigorous ET on hepatic fat content or NAFLD prevalence; nevertheless, a study performed in mice showed that vigorous was more effective than moderate exercise improving hepatic steatosis[69].

The meta-regression analyses showed that higher improvements in CRF and MusS are associated with greater reductions in hepatic fat content. These findings are in accordance with those reported in adults. Similarly, Kantartzis et al.[70] reported a significant correlation between changes in VO_{2max} and hepatic fat content after a lifestyle intervention that included unsupervised aerobic ET and sports. It was also observed that the association of changes in CRF levels and changes in hepatic fat content was stronger when aerobic ET at a moderate intensity was excluded from the analyses. This suggests that ET interventions aiming to improve CRF levels in the management and prevention of NAFLD should be performed at

least at a moderate-to-vigorous intensity. As it has previously been reported that vigorous intensity ET is more effective than moderate intensity increasing CRF levels, intervention programs should be focused on improving CRF and/or MusS levels, including ET programs designed to reach vigorous intensity levels[71,72].

The current review has several limitations. Firstly, there is a high degree of heterogeneity among the analyzed studies, in part due to the differences in inclusion criteria, type, intensity, volume, frequency, and duration of the ET interventions. Secondly, meta-analysis and meta-regression analyses enclosed a reduced number of studies, some of them with small simple sizes. Publication bias in some of the analysis could be another limitation. However, after performing the "trim and fill" analysis, the significant results persisted. This review is also limited by the suboptimal methodological quality of the included interventions. Therefore, results should be taken with caution; and more research on the effect of ET interventions is required to reinforce the recommendations of ET in the treatment and prevention of NAFLD in youth. Despite the limitations, several strengths need to be acknowledged in this study. For this systematic review and meta-analysis many different reference databases were used. Furthermore, we were able to include meta-analysis and meta-regressions that may help to clarify the overall effects of supervised-ET, as well as the specific effects of the different type, intensity, volume, and frequency of ET on hepatic fat content and NAFLD prevalence. These data may allow the design of exercise programs for the management and prevention of NAFLD in the pediatric population.

Conclusions and clinical health implications

This review shows that supervised-ET alone reduces hepatic fat content in children and adolescents, and that its combination with other lifestyle activities is an effective approach to reduce NAFLD prevalence in youth. Hence, ET programs can be recommended

not only in combination with other lifestyle changes, but also independently as an effective approach to improve hepatic steatosis in the pediatric population. Moreover, this study provides, for the first time, data on type, intensity, volume, and frequency of ET needed to effectively reduce hepatic fat content in the pediatric population. Of note is that these data concur with the international recommendations of PA for health promotion in youths. Likewise, both aerobic and resistance ET at vigorous or moderate-to-vigorous intensities in sessions of at least 60 min and with a minimum of 3 sessions per week, focused on increasing CRF and MusS, should be included in the design of ET programs for the prevention and management of NAFLD in children and adolescents. Although further research with more large-scale intervention studies in youths is needed, particularly in preadolescent children, current evidence is promising and reinforces the recommendations of ET therapy for NAFLD management and prevention in the pediatric population.

Statement of conflict of interest

All Authors declare that there are no conflicts of interest.

Acknowledgments

This review was conducted under the umbrella of the EFIGRO study (ClinicalTrials.gov ID: NCT02258126) which was supported by the Spanish Ministry of Health, “Fondo de Investigación Sanitaria del Instituto de Salud Carlos III”, “Fondos de la Unión Europea (FEDER), una manera de hacer Europa” (PI13/01335), the PREDIKID study, supported by the Spanish Ministry of Economy, Competition and Competitiveness, grant “Programa Estatal de Investigación, Desarrollo e Innovación Orientada a los Retos de la Sociedad” (DEP2016-78377-R), and by the University of the Basque Country (GIU14/21), and the SmarterMove (DEP2016-79512-R), supported by the Spanish Ministry of Economy, Competition and Competitiveness. MM is supported by grant from

the Spanish Ministry of Education, Culture and Sport (FPU14/03329). CC-S is supported by a grant from the Spanish Ministry of Economy and Competitiveness (BES-2014-068829). CA-B is supported by grant from the Spanish Ministry of Education, Culture and Sport FPU13/03137. IC-R is supported by a grant from the Universidad de Castilla La Mancha (Fpu13/01582). FBO is supported by the Spanish Ministry of Science and Innovation (RYC-2011-09011). Additional support was obtained from the University of Granada, Plan Propio de Investigación 2016, Excellence actions: Units of Excellence, Unit of Excellence on Exercise and Health (UCEES); the SAMID III network, RETICS, funded by the PN I+D+I 2017-2021 (Spain), ISCIII- Sub-Directorate General for Research Assessment and Promotion, the European Regional Development Fund (ERDF) (Ref. RD16/0022) and the EXERNET Research Network on Exercise and Health in Special Populations (DEP2005-00046/ACTI). This review does not have a sponsor and all the authors declare themselves independent from funders.

We thank Vicente Martínez-Vizcaíno, for his helpful advice and indispensable and valuable acquired learning about the development and understanding of systematic review and meta-analysis. We are grateful to Ms. Carmen Sainz Quinn for her assistance with the English language.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pcad.2018.01.013

References

1. Skinner AC, Perrin EM, Moss LA, et al. Cardiometabolic risks and severity of obesity in children and young adults. *N Engl J Med*. 2015;373(14):1307-1317.

2. Ayer J, Charakida M, Deanfield JE, et al. Lifetime risk: childhood obesity and cardio- vascular risk. *Eur Heart J*. 2015;36(22):1371-1376.

3. Vos MB, Abrams SH, Barlow SE, et al. NASPGHAN clinical practice guideline for the diagnosis and treatment of nonalcoholic fatty liver disease in children: recommendations from the expert committee on NAFLD (ECON) and the North American Society of Paediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr*. 2017;64(2):319-334.

4. Schwimmer JB, Deutsch R, Kahen T, et al. Prevalence of fatty liver in children and adolescents. *Pediatrics*. 2006;118(4):1388-1393.

5. Schwimmer JB, Pardee PE, Lavine JE, et al. Cardiovascular risk factors and the metabolic syndrome in pediatric nonalcoholic fatty liver disease. *Circulation*. 2008;118(3):277-283.

6. Wittmeier KDM, Wicklow BA, MacIntosh AC, et al. Hepatic steatosis and low cardiorespiratory fitness in youth with type 2 diabetes. *Obesity*. 2012;20(5):1034-1040.

7. AlKhater SA. Paediatric non-alcoholic fatty liver disease: an overview. *Obes Rev*. 2015;16(5):393-405.

8. Armstrong MJ, Newsome PN. Editorial: treatment for NASH - helping the liver or helping the heart? *Aliment Pharmacol Ther*. 2015;41(5):487.

9. Dixon JB, Bhathal PS, Hughes NR, et al. Nonalcoholic fatty liver disease: improvement in liver histological analysis with weight loss. *Hepatology*. 2004;39(6):1647-1654.

10. Garcia-Hermoso A, Sanchez-Lopez M, Martinez-Vizcaino V. Effects of aerobic plus resistance exercise on body composition related variables in pediatric obesity: a systematic review and meta-analysis of randomized controlled trials. *Pediatr Exerc Sci*. 2015;27(4):431-440.

11. WisloffU, Lavie CJ. Taking physical activity, exercise, and fitness to a higher level. *Prog Cardiovasc Dis*. 2017;60(1):1-2.

12. Zisko N, Skjerve KN, Tari AR, et al. Personal activity intelligence (PAI), sedentary behavior and cardiovascular risk factor clustering – the HUNT study. *Prog Cardiovasc Dis*. 2017;60(1):89-95.

13. Garcia-Hermoso A, Saavedra JM, Escalante Y. Effects of exercise on resting blood pressure in obese children: a meta-analysis of randomized controlled trials. *Obes Rev*. 2013;14(11):919-928.

14. Katzmarzyk PT, Lee I-M, Martin CK, et al. Epidemiology of physical activity and exercise training in the United States. *Prog Cardiovasc Dis*. 2017;60(1):3-10.

15. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology*. 2012;142(7):1592-1609.

16. Gonzalez-Ruiz K, Ramirez-Velez R, Correa-Bautista JE, et al. The effects of exercise on abdominal fat and liver enzymes in pediatric obesity: a systematic review and meta- analysis. *Child Obes*. 2017;13(4):272-282.

17. Castro-Piñero J, Perez-Bey A, Segura-Jiménez V, et al. Cardiorespiratory fitness cutoff points for early detection of present and future cardiovascular risk in children. *Mayo Clin Proc*. 2017;92:1753-1762.

18. Lavie CJ, Kokkinos P, Ortega FB. Survival of the fittest—promoting fitness throughout the life span. *Mayo Clin Proc*. 2017;92(12):1743-1745.

19. Oktay AA, Lavie CJ, Kokkinos PF, et al. The interaction of cardiorespiratory fitness with obesity and the obesity paradox in cardiovascular disease. *Prog Cardiovasc Dis*. 2017;60(1):30-44.

20. Harber MP, Kaminsky LA, Arena R, et al. Impact of cardiorespiratory fitness on all- cause and disease-specific mortality: advances since 2009. *Prog Cardiovasc Dis*. 2017;60(1):11-20.

21. Ortega FB, Ruiz JR, Castillo MJ, et al. Physical fitness in childhood and adolescence: a powerful marker of health. *Int J Obes*. 2008;32(1):1-11.

22. Trilk JL, Ortaglia A, Blair SN, et al. Cardiorespiratory fitness, waist circumference, and alanine aminotransferase in youth. *Med Sci Sports Exerc*. 2013;45(4):722-727.

23. Martins C, Freitas Jr I, Pizarro A, et al. Cardiorespiratory fitness, but not central obesity or C-reactive protein, is related to liver function in obese children. *Pediatr Exerc Sci*. 2013;25(1):3-11.

24. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ*. 2009;339, b2535.

25. Schardt C, Adams MB, Owen T, et al. Utilization of the PICO framework to improve searching PubMed for clinical questions. *BMC Med Inform Decis Mak*. 2007;15(7):16.

26. Kessler HS, Sisson SB, Short KR. The potential for high-intensity interval training to reduce cardiometabolic disease risk. *Sports Med*. 2012;42(6):489-509.

27. Higgins JPT, Green S. Cochrane handbook for systematic reviews of interventions version 5.1.0 [Updated March 2011]. The Cochrane Collaboration; 2011. Available from www.handbook.cochrane.org.

28. Armijo-Olivo S, Stiles CR, Hagen NA, et al. Assessment of study quality for systematic reviews: a comparison of the Cochrane collaboration risk of bias tool and the effective public health practice project quality assessment tool: methodological research. *J Eval Clin Pract*. 2012;18(1):12-18.

29. Effective Public Health Practice P. Quality Assessment Tool for Quantitative Studies. 2009. Available from: http://www.ephpp.ca/tools.html.

30. Thomas BH, Ciliska D, Dobbins M, et al. A process for systematically reviewing the literature: providing the research evidence for public health nursing interventions. *Worldviews Evid-Based Nurs*. 2004;1(3):176-184.

31. Wilson DB. Practical meta-analysis effect size calculator [online calculator]. (n.d.) Retrieved February 19, 2017, from https://www.campbellcollaboration.org/this-is-a-web-based-effect-size-calculator/explore/this-is-a-web-based-effect-size-calculator.

32. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med*. 2002;21(11):1539-1558.

33. Hay J, Wittmeier K, MacIntosh A, et al. Physical activity intensity and type 2 diabetes risk in overweight youth: a randomized trial. *Int J Obes*. 2016;40(4):607-614.

34. Lee S, Deldin AR, White D, et al. Aerobic exercise but not resistance exercise reduces intrahepatic lipid content and visceral fat and improves insulin sensitivity in obese adolescent girls: a randomized controlled trial. *Am J Physiol Endocrinol Metab*. 2013;305(10):E1222-9.

35. Hasson RE, Adam TC, Davis JN, et al. Randomized controlled trial to improve adiposity, inflammation, and insulin resistance in obese African-American and Latino youth. *Obesity*. 2012;20(4):811-818.

36. Lee S, Bacha F, Hannon T, et al. Effects of aerobic versus resistance exercise without caloric restriction on abdominal fat, intrahepatic lipid, and insulin sensitivity in obese adolescent boys a randomized, controlled trial. *Diabetes*. 2012;61(11):2787-2795.

37. Davis JN, Gyllenhammer LE, Vanni AA, et al. Startup circuit training program reduces metabolic risk in Latino adolescents. *Med Sci Sports Exerc*. 2011;43(11):2195-2203.

38. Dâmaso AR, de Piano A, Campos RM, et al. Multidisciplinary approach to the treatment of obese adolescents: effects on cardiovascular risk factors, inflammatory

profile, and neuroendocrine regulation of energy balance. *Int J Endocrinol*. 2013;12(5): 237-241.

39. Gronbaek H, Lange A, Birkebaek NH, et al. Effect of a 10-week weight loss camp on fatty liver disease and insulin sensitivity in obese Danish children. *J Pediatr Gastroenterol Nutr*. 2012;54(2):223-228.

40. Koot BGP, van der Baan-Slootweg OH, Tamminga-Smeulders CLJ, et al. Lifestyle intervention for non-alcoholic fatty liver disease: prospective cohort study of its efficacy and factors related to improvement. *Arch Dis Child*. 2011;96(7):669-674.

41. Van der Heijden G-J, Wang ZJ, Chu ZD, et al. A 12-week aerobic exercise program reduces hepatic fat accumulation and insulin resistance in obese, Hispanic adolescents. *Obesity*. 2010;18(2):384-390.

42. Van Der Heijden G-J, Wang ZJ, Chu Z, et al. Strength exercise improves muscle mass and hepatic insulin sensitivity in obese youth. *Med Sci Sports Exerc*. 2010;42(11):1973-1980.

43. Reinehr T, Schmidt C, Toschke AM, et al. Lifestyle intervention in obese children with non-alcoholic fatty liver disease: 2-year follow-up study. *Arch Dis Child*. 2009;94(6):437-442.

44. Reinehr T, Schmidt C, de Sousa G, et al. Association between leptin and transaminases: 1-year follow-up study in 180 overweight children. *Metabolism*. 2009;58(4):497-503.

45. Reinehr T, Roth CL. Fetuin-A and its relation to metabolic syndrome and fatty liver disease in obese children before and after weight loss. *J Clin Endocrinol Metab*. 2008;93(11):4479-4485.

46. de Piano A, Prado WL, Caranti DA, et al. Metabolic and nutritional profile of obese adolescents with nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr*. 2007;44(4):446-452.

47. Dâmaso R, Tock L, Tufik S, et al. Multidisciplinary treatment reduces visceral adiposity tissue, leptin, ghrelin and the prevalence of non-alcoholic fat liver disease (NAFLD) in obese adolescents. *Rev Bras Med Esporte*. 2006;12(5):263-267.

48. Tock L, Prado WL, Caranti DA, et al. Nonalcoholic fatty liver disease decrease in obese adolescents after multidisciplinary therapy. *Eur J Gastroenterol Hepatol*. 2006;18(12):1241-1245.

49. Wicklow BA, Wittmeier KDM, MacIntosh AC, et al. Metabolic consequences of hepatic steatosis in overweight and obese adolescents. *Diabetes Care*. 2012;35(4):905-910.

50. Szczepaniak LS, Nurenberg P, Leonard D, et al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab*. 2005;288(2):E462-8.

51. Sabir N, Sermez Y, Kazil S, et al. Correlation of abdominal fat accumulation and liver steatosis: importance of ultrasonographic and anthropometric measurements. *Eur J Ultrasound*. 2001;14(2–3):121-128.

52. Saadeh S, Younossi ZM, Remer EM, et al. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology*. 2002;123(3):745-750.

53. Mazhar SM, Shiehmorteza M, Sirlin CB. Noninvasive assessment of hepatic steatosis. *Clin Gastroenterol Hepatol*. 2009;7(2):135-140.

54. Saverymuttu SH, Joseph AE, Maxwell JD. Ultrasound scanning in the detection of hepatic fibrosis and steatosis. *Br Med J (Clin Res Ed).* 1986;292(6512):13-15.

55. Pescatello LS, Arena R, Riebe D, Thompson PD. *ACSM's Guidelines for Exercise Testing and Prescription9th edition. . 2013:165*. [*Chapter 7, Table 7.1*].

56. Africa JA, Newton KP, Schwimmer JB. Lifestyle interventions including nutrition, exercise, and supplements for nonalcoholic fatty liver disease in children. *Dig Dis Sci*. 2016;61(5):1-12.

57. Keating SE, Hackett DA, George J, et al. Exercise and non-alcoholic fatty liver disease: a systematic review and meta-analysis. *J Hepatol*. 2012;57(1):157-166.

58. Orci LA, Gariani K, Oldani G, et al. Exercise-based interventions for nonalcoholic fatty liver disease: a meta-analysis and meta-regression. *Clin Gastroenterol Hepatol*. 2016;14(10):1398-1411.

59. Nseir W, Hellou E, Assy N. Role of diet and lifestyle changes in nonalcoholic fatty liver disease. *World J Gastroenterol*. 2014;20(28):9338-9344.

60. Hannah Jr WN, Harrison SA. Lifestyle and dietary interventions in the Management of Nonalcoholic Fatty Liver Disease. *Dig Dis Sci*. 2016;61(5):1365-1374.

61. Mitchel EB, Lavine JE. Review article: the management of paediatric nonalcoholic fatty liver disease. *Aliment Pharmacol Ther*. 2014;40(10):1155-1170.

62. WHO - World Health Organization. Recommended levels of physical activity for children aged 5–17 years. Global recommendations on physical activity for health. http://www.who.int/dietphysicalactivity/factsheet_young_people/en/. Accessed May 25, 2017. [Published 2010].

63. Centers for Disease Control and Prevention. How much physical activity do children need? Physical activity guidelines.

https://www.cdc.gov/physicalactivity/basics/ children/index.htm. Accessed May 25, 2017. [Published 2015].

64. Hashida R, Kawaguchi T, Bekki M, et al. Aerobic *vs*. resistance exercise in non-alcoholic fatty liver disease: a systematic review. *J Hepatol*. 2017;66(1):142-152.

65. Karlsen T, Aamot I-L, Haykowsky M, et al. High intensity interval training for maxi- mizing health outcomes. *Prog Cardiovasc Dis*. 2017;60(1):67-77.

66. Cassidy S, Thoma C, Hallsworth K, et al. High intensity intermittent exercise improves cardiac structure and function and reduces liver fat in patients with type 2 diabetes: a randomised controlled trial. *Diabetologia*. 2016;59(1):56-66.

67. Hallsworth K, Thoma C, Hollingsworth KG, et al. Modified high-intensity interval training reduces liver fat and improves cardiac function in non-alcoholic fatty liver disease: a randomized controlled trial. *Clin Sci (Lond)*. 2015;129(2):1097-1105.

68. Balducci S, Cardelli P, Pugliese L, et al. Volume-dependent effect of supervised exercise training on fatty liver and visceral adiposity index in subjects with type 2 diabetes The Italian Diabetes Exercise Study (IDES). *Diabetes Res Clin Pract*. 2015;109(2):355-363.

69. Cho J, Kim S, Lee S, et al. Effect of training intensity on nonalcoholic fatty liver disease. *Med Sci Sports Exerc*. 2015;47(8):1624-1634.

70. Kantartzis K, Thamer C, Peter A, et al. High cardiorespiratory fitness is an independent predictor of the reduction in liver fat during a lifestyle intervention in non-alcoholic fatty liver disease. *Gut*. 2009;58(9):1281-1288.

71. Gutin B, Barbeau P, Owens S, et al. Effects of exercise intensity on cardiovascular fitness, total body composition, and visceral adiposity of obese adolescents. *Am J Clin Nutr*. 2002;75(5):818-826.

72. Swain DP, Franklin BA. VO(2) reserve and the minimal intensity for improving cardiorespiratory fitness. *Med Sci Sports Exerc*. 2002;34(1):152-157.

Appendix A. Supplementary data

Supplemental material 1. Search terms combination used in each database and number of studies found in each database.

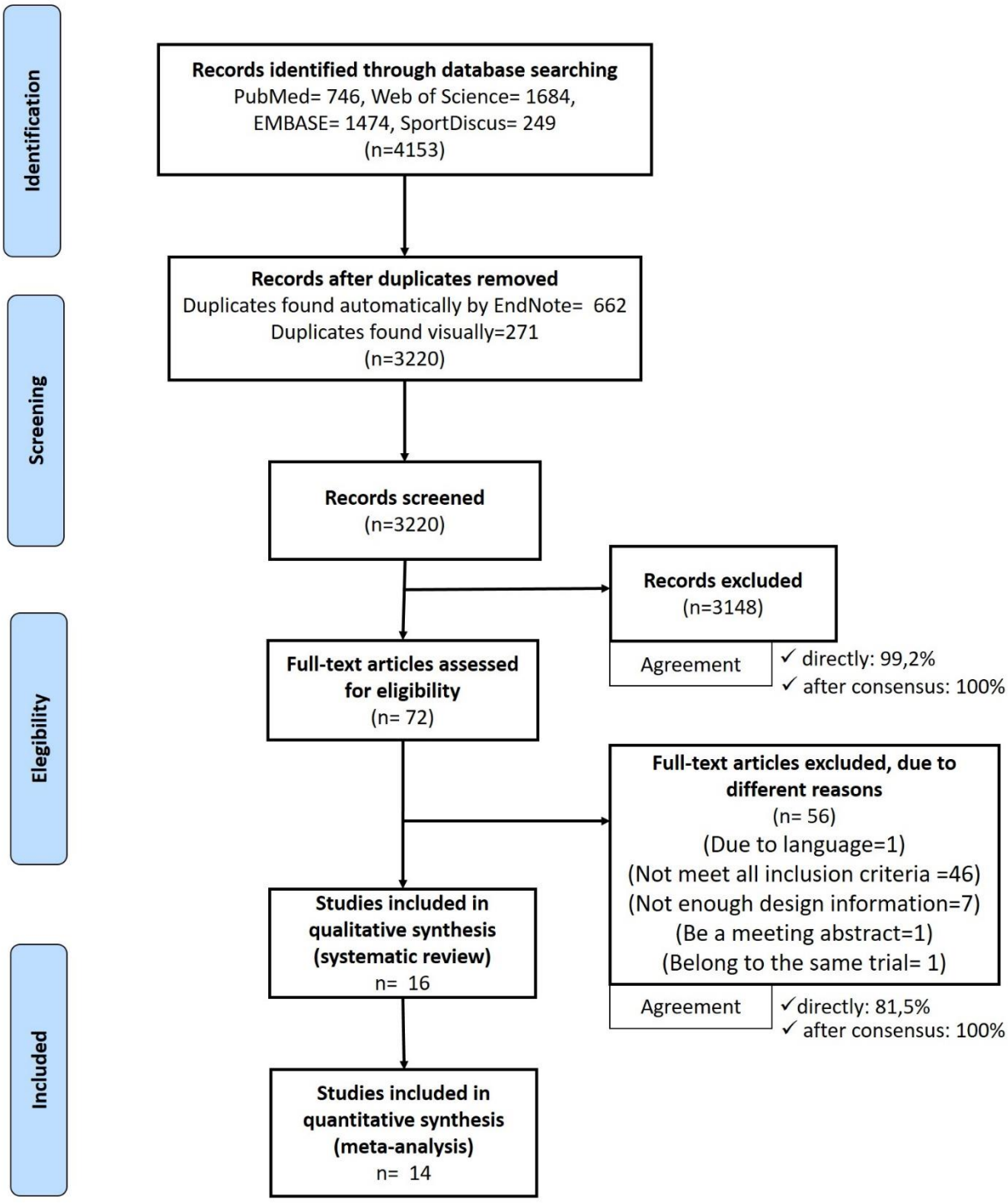
Medline search (N=746): ("children" OR "adolescent" OR "youth" OR “teenager” OR “boy” OR “girl”) AND (“obesity AND liver fat” OR “obesity AND non-alcoholic fatty liver disease” OR “obesity AND non-alcoholic steatosis” OR "non-alcoholic steatosis" OR "non-alcoholic fatty liver disease" OR "fatty liver" OR "hepatic fat" OR "metabolic risk" OR "cardiometabolic risk" OR "type 2 diabetes") AND ("exercise" OR "physical activity" OR "exercise intervention" OR "training") AND ("intervention" OR "program" OR "trial" OR "treatment" OR “pre-post study”)

Web of Science Search (N=1684): ("child*" OR "adolesc*" OR "youth" OR “teen*” OR “boy*” OR “girl*”) AND (“obesity AND liver fat” OR “obesity AND non-alcoholic fatty liver disease” OR “obesity AND non-alcoholic steatosis” OR "non-alcoholic steatosis" OR "non-alcoholic fatty liver disease" OR "fatty liver" OR "hepatic fat" OR "metabolic risk" OR "cardiometabolic risk" OR "type 2 diabetes") AND ("exercise" OR "physical activity" OR "exercise intervention" OR "training") AND ("intervention*" OR "program*" OR "trial*" OR "treatment*" OR “pre-post stud*”)

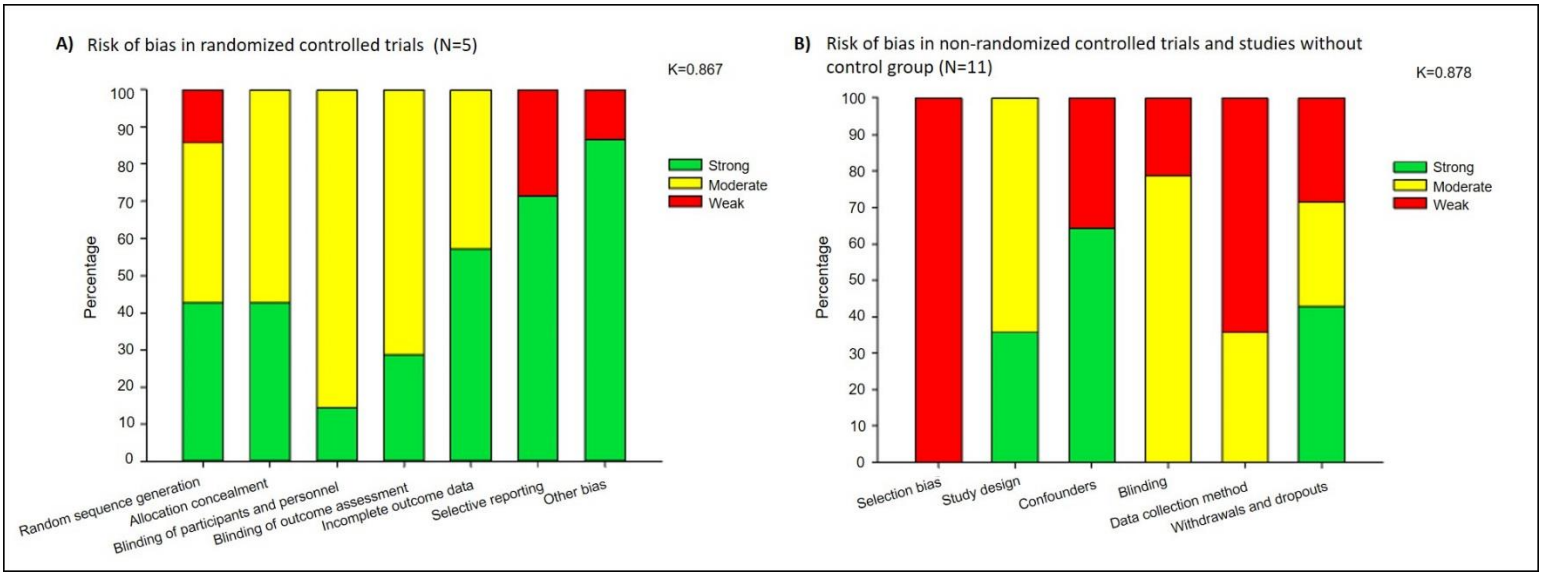
Scopus Search (N=1474): TITLE-ABS-KEY (children OR adolescent OR youth OR teenager) AND ({obesity liver fat} OR {obesity non-alcoholic fatty liver disease} OR {obesity non-alcoholic steatosis} OR {non-alcoholic steatosis} OR {non-alcoholic fatty liver disease} OR {fatty liver}) AND (exercise OR {physical activity} OR {exercise intervention} OR training) AND (intervention OR program OR trial OR treatment)

SportDiscus Search (N=249): ("child" OR "adolesc*" OR "youth" OR “teen*” OR “boy*” OR “girl*”) AND (((“liver fat” OR “non-alcoholic fatty liver disease” OR “non-

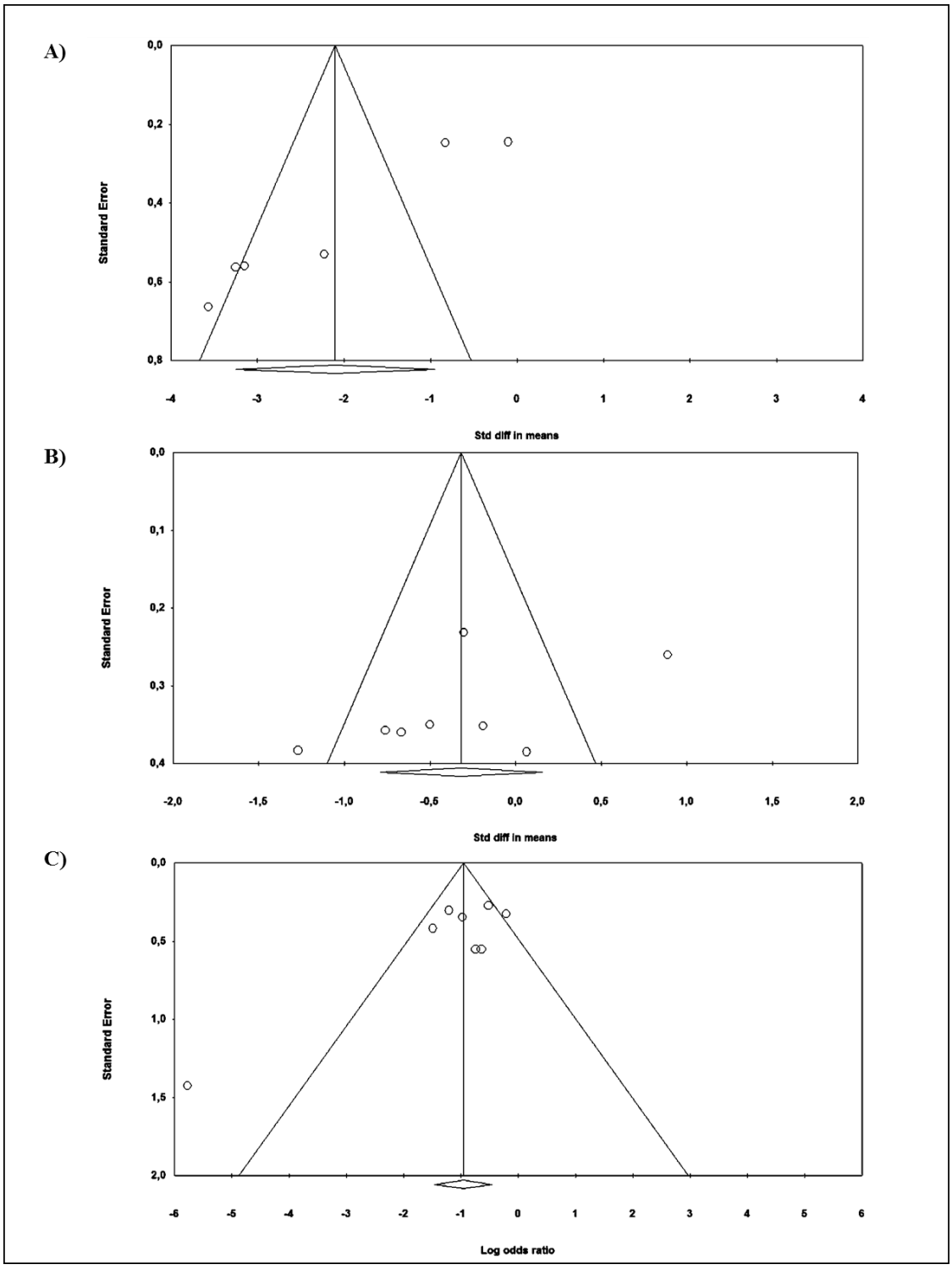
alcoholic steatosis”) AND “obesity”) OR "non-alcoholic steatosis" OR "non-alcoholic fatty liver disease" OR "fatty liver" OR "hepatic fat") AND ("exercise" OR "physical activity" OR "exercise intervention" OR "training") AND ("intervention*" OR "program*" OR "trial*" OR "treatment*" OR “pre-post stud*”)



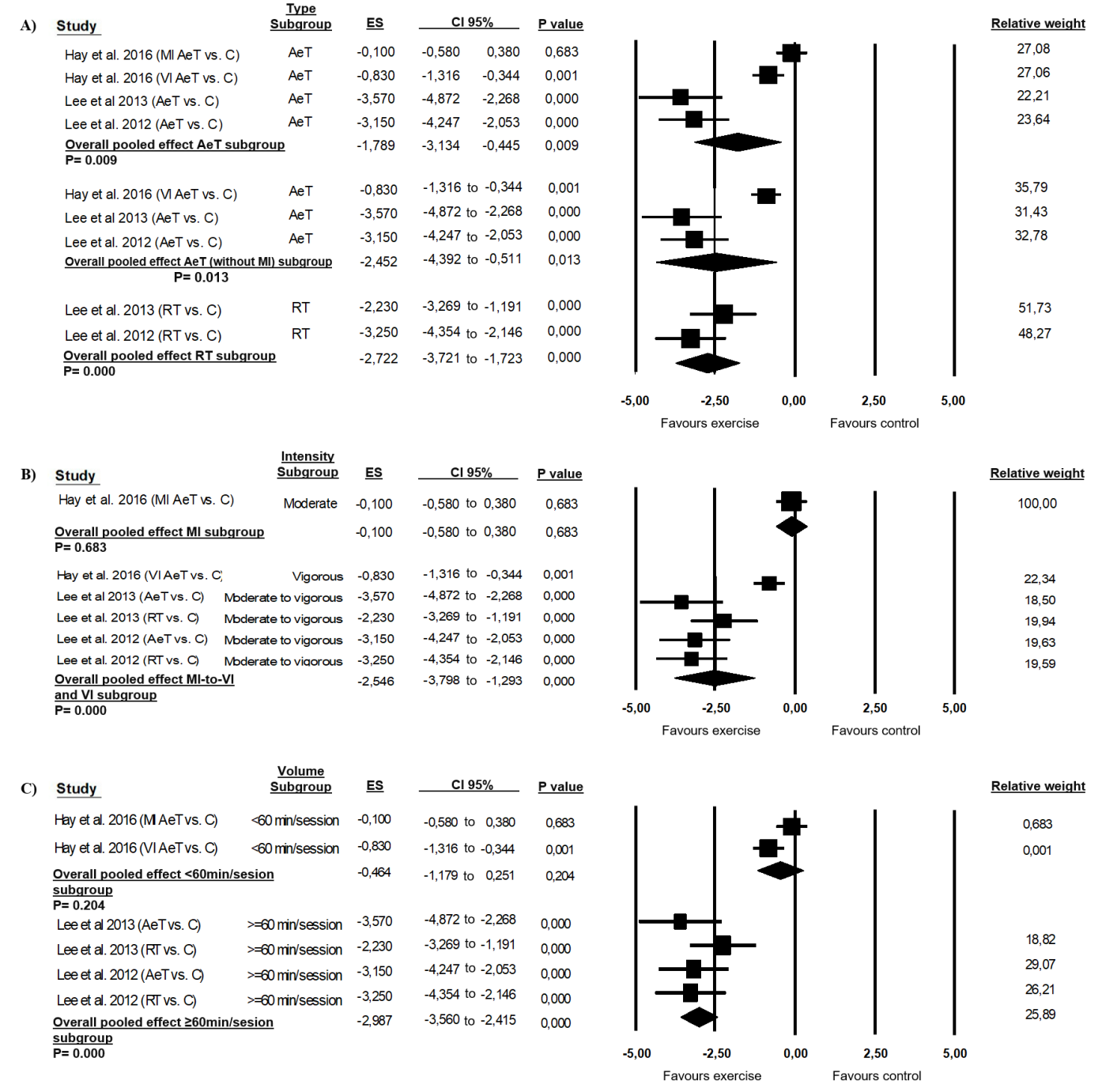
Supplemental Fig 1- Flow diagram for the selection of studies included in the systematic review and meta-analysis of the effect of supervised-exercise interventions on liver fat content and non-alcoholic fatty liver disease in children and adolescents, according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[17].



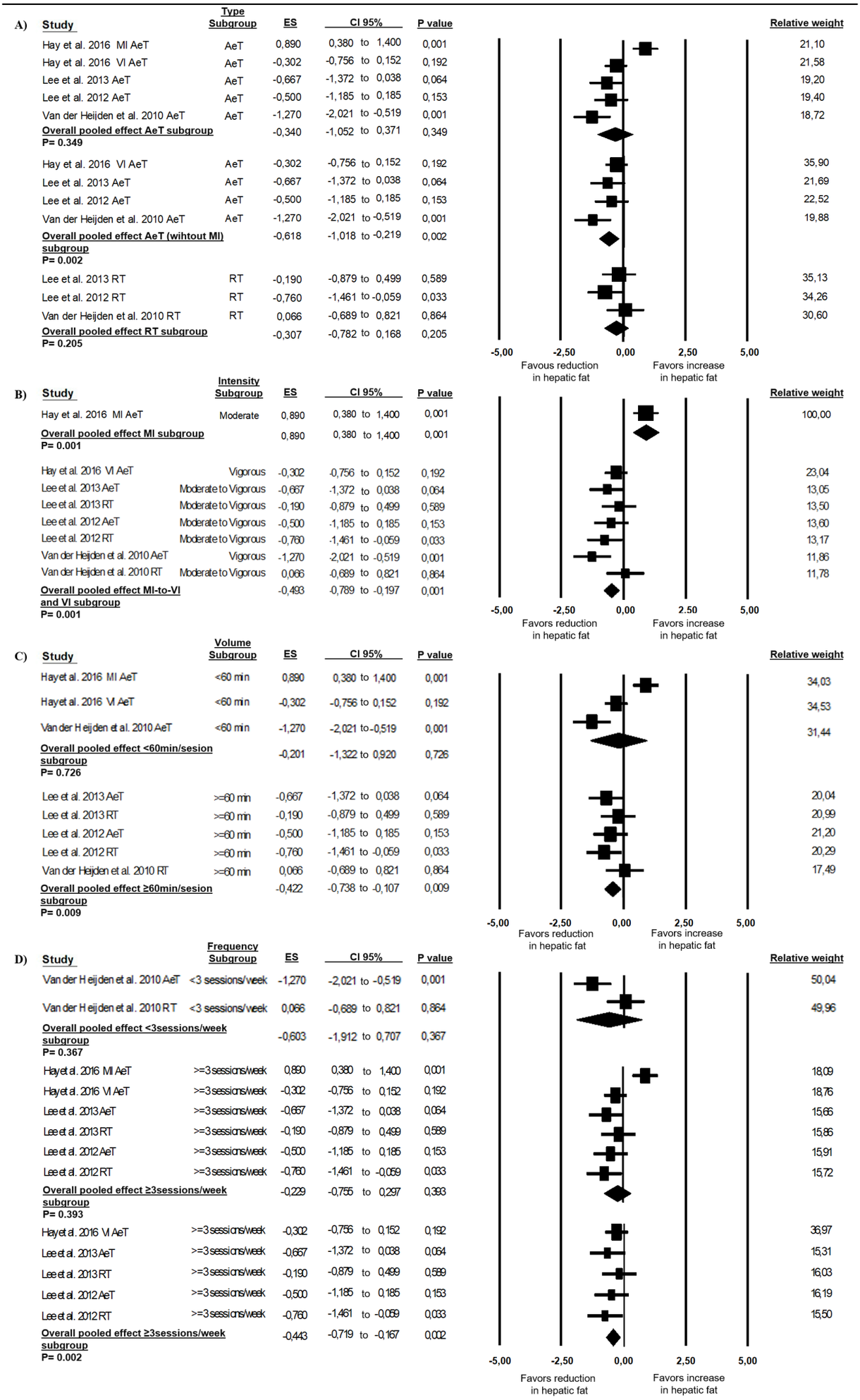
Supplemental Fig 2- Methodological quality of the included studies. The methodological quality of randomized controlled trials (N=5) was assessed using the Cochrane risk of bias tool (7 evaluation critical methodological components)[20] (A). The methodological quality of non-randomized controlled trials and studies without control group (N=11) was assessed using the Quality Assessment Tool for Quantitative Studies of the Effective Public Health Practice Project (EPHPP) (6 evaluation critical methodological components)[21,22] (B). K= concordance inter-reviewers in risk of bias scores.



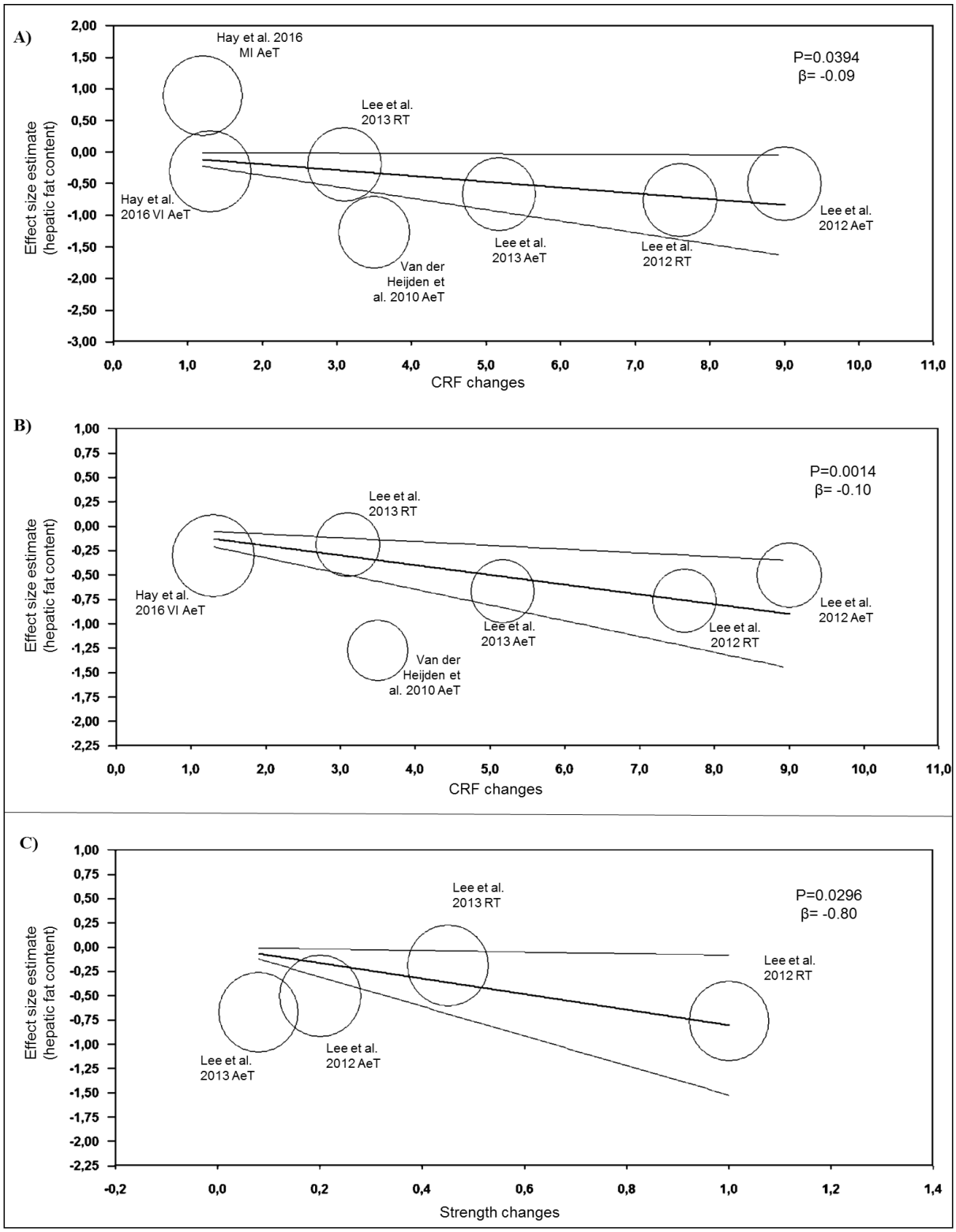
Supplemental Fig 3- Funnel plots to assess publication bias in effects of exercise on liver fat content in exercise *vs.* control analysis (A), exercise post *vs.* pre analysis (B) and prevalence of NAFLD (C) included in the meta-analysis. Each white point represents a meta-analyzed group. Diagonal lines represent pseudo-95% confidence intervals. In reference of Y axis, studies located at the lower part of the graph have a higher standard error (a lower weight in the pooled analysis). The vertical line represents the calculated estimate effect of liver fat content.



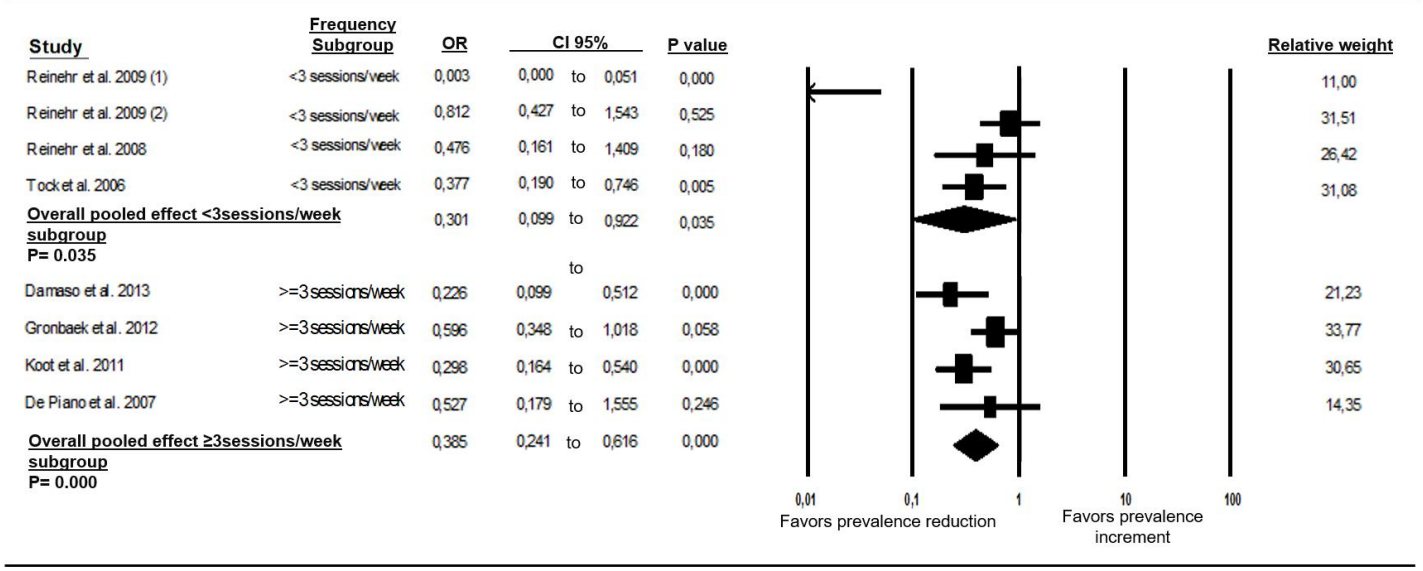
Supplemental Fig 4- Meta-analysis (pooled effect size and 95% confidence intervals) among subgroup by type (aerobic *vs.* resistance exercise) (A), intensity (moderate *vs.* moderate-to-vigorous and vigorous) (B), volume (<60 *vs.* ≥60 min/session) (C), and frequency in intervention *vs.* control analysis. The size of the square represents the weight of each study. *Abbreviations*: AeT: aerobic training group; C: control group; CI: confidence intervals; ES: effect size; MI: moderate intensity; RT: resistance training group; VI: vigorous intensity.



Supplemental Fig 5- Meta-analysis (pooled effect size and 95% confidence intervals) among subgroup by type (aerobic *vs.* resistance exercise) (A), intensity (moderate *vs.* moderate-to-vigorous and vigorous) (B), volume (<60 *vs.* ≥60 min/session) (C), and frequency (<3 *vs.* ≥3 sessions/week) (D) in post *vs.* pre analysis. The size of the square represents the weight of each study. *Abbreviations*: AeT: aerobic training group; CI: confidence intervals; ES: effect size; MI: moderate intensity; RT: resistance training group; VI: vigorous intensity.



Supplemental Fig 6- Meta-regression analysis (standardized β coefficient and P values in post *vs.* pre analysis) of changes in cardiorespiratory fitness (VO_{2max} in ml/kg/min) (A and B) or in strength (muscular strength index) (C), in X axis, against the effect size of the liver fat content, in Y axis. The more negative the effect size is, the more effective is the exercise at reducing liver fat content after the intervention. Figure Supplementary 4B shows the CRF changes analyses but removing the group where exercise intensity was moderate (Hay et al. 2010[26] Moderate Intensity training group). The size of the circle represents the weight of each study. *Abbreviations*: AeT: aerobic training group; C: control group; MI: moderate intensity; RT: resistance training group; VI: vigorous intensity. If nothing is specified about intensity of exercise, it corresponds to a moderate to vigorous exercise intensity.



Supplemental Fig 7- Meta-analysis (pooled effect size and 95% confidence intervals) among subgroup by frequency (<3 *vs.* ≥3 sessions/week) (A) in NAFLD analysis. The size of the square represents the weight of each study. *Abbreviations*: AeT: aerobic training group; C: control group; MI: moderate intensity; RT: resistance training group; VI: vigorous intensity. If nothing is specified about intensity of exercise, it corresponds to a moderate to vigorous exercise intensity.

3.4. Study IV

American Diabetes Association. Diabetes Care

Effects of exercise in addition to a family-based lifestyle intervention program on hepatic fat in children with overweight

Idoia Labayen[1], María Medrano[1], Lide Arenaza[1], Edurne Maíz[2], Maddi Osés[1], Vicente Martínez-Vizcaíno[3,4], Jonatan R. Ruiz[5], Francisco B. Ortega[5]

[1]Institute for Innovation & Sustainable Development in Food Chain (IS-FOOD), Public University of Navarra, Pamplona, Spain.

[2]University of the Basque Country, Donostia, Spain

[3]Universidad de Castilla-La Mancha, Health and Social Research Center, Spain

[4]Universidad Autónoma de Chile, Chile

[5]PROFITH ''PROmoting FITness and Health through physical activity'' Research Group, Department of Physical Education and Sport, Faculty of Sport Sciences, University of Granada, Granada, Spain.

Corresponding author: Idoia Labayen, idoia.labayen@unavarra.es

Abstract

Objective: Pediatric hepatic steatosis is highly prevalent and closely related to type 2 diabetes. To determine whether the addition of supervised exercise to a family-based lifestyle and psycho-educational intervention results in greater reduction of percentage of hepatic fat (HF), adiposity, and cardiometabolic risk factors in children with overweight/obesity.

Research Design and Methods: The study subjects of this nonrandomized, two-arm, parallel design clinical trial were 116 overweight/obese children (10.6±1.1 years of age, 53.4% girls) living in Vitoria-Gasteiz (Spain). For 22 weeks, they followed either a lifestyle- and psycho-education program (control intervention [CInt], *N*=57), consisting of two family-based education sessions/month, or the same plus supervised exercise (intensive intervention [II], *N*=59) focused mainly on high-intensity aerobic workouts (3 sessions/week, 90 min/session). The primary outcome was the change in percentage of HF (as measured by MRI) between baseline and the end of the intervention period. Secondary outcomes included changes in BMI, fat mass index (FMI), abdominal fat (measured by DEXA), blood pressure, triglycerides, HDL, LDL, γ-glutamyl-transferase, glucose, and insulin concentrations.

Results: A total of 102 children completed the trial (*N*=53 and *N*=49 in the CInt and II groups, respectively). Percentage of HF decreased only in the II group (-1.20±0.31% *vs.* 0.04±0.30%, II and CInt groups, respectively), regardless of baseline value and any change in adiposity ($P<0.01$). BMI, FMI, abdominal fat ($P\leq0.001$), and insulin ($P<0.05$) were reduced in both groups.

Conclusions: Multicomponent intervention programs that include exercise training may help to reduce adiposity, insulin resistance, and hepatic steatosis in overweight/obese children.

Keywords: hepatic steatosis, childhood, physical activity, nutrition, psychology, obesity

ClinicalTrials.gov ID: NCT02258126

Introduction

Children with overweight/obesity are at greater risk of the development of cardiovascular diseases (CVDs), type 2 diabetes, and nonalcoholic fatty liver disease (NAFLD) in adulthood (1,2) and even in childhood (3). Pediatric NAFLD is affecting nearly 34% of children with obesity (4). It is estimated that by 2025, 38 million children will have hepatic steatosis, the earliest manifestation of NAFLD (5).

Pediatric hepatic steatosis is an independent risk factor for type 2 diabetes (6,7). Excessive body mass gains during childhood may eventually induce the histological features of adult NAFLD (8). The treatment of overweight/obesity before puberty, however, can reduce the risk of the development of type 2 diabetes in adulthood (9). The early treatment of childhood overweight/obesity and its comorbidities is therefore vital.

Currently, there are no pharmacological options for NAFLD in children (10). Lifestyle modification is therefore the primary option (10). A systematic review of lifestyle interventions for the treatment of overweight/obesity in children 6–11 years of age observed that multicomponent behavior-changing programs achieved small, short-term reductions in BMI (11). However, no studies have examined the effect of a nonhypocaloric diet–based lifestyle intervention program on the percentage of hepatic fat (HF) in children.

Exercise training seems to be effective in reducing HF in adolescents (12), and in reducing the risk of the development of CVD and type 2 diabetes (13) in children. In a systematic review (12), it was reported that supervised exercise, designed following the international recommendations of physical activity, reduced HF in youths. However, there has been no such study exclusively involving children under 12 years of age, and certainly none that has used imaging methods to assess HF.

Given the above, lifestyle and behavioral modifications plus exercise training might be expected to have greater effects on HF as well as on children's health and psychological well-being) than interventions based on lifestyle modifications alone. The aim of the present work was to determine whether a multicomponent intervention program designed according to current evidence and guidelines, and including a family-based lifestyle and psycho-education program plus a supervised exercise intervention, is more effective at reducing the percentage of HF than the lifestyle program alone, in children with overweight/obesity who were 8–12 years of age. The effects of these interventions on fat mass, CVD, and type 2 diabetes risk factors, physical fitness, dietary habits, physical activity, and psychological well-being are also discussed.

Research Design and Methods

Study design

The EFIGRO project is a nonrandomized two-arm, parallel-design controlled trial (Clinical trial reg. no. NCT02258126, clinicaltrials.gov) (14). The study was conducted at the University of the Basque Country in Vitoria-Gasteiz (northern Spain), from September 2014 to June 2017. The Euskadi Clinical Research Ethics Committee approved the study protocol (PI2014045), which complies with the ethical guidelines of the Declaration of Helsinki (2013 revision). All parents/legal guardians gave their informed, written consent for their children to be included in the study; the children also gave their assent before enrolment.

The EFIGRO project compared difference in changes in the percentage of HF, and cardiometabolic and diabetes risk factors between two groups: one group received a family-based lifestyle and psycho-educational program hereafter referred as the control

intervention (CInt), and another group received the same intervention plus an exercise program hereafter referred as the intensive intervention (II).

Participants

Children and their families were recruited at the Pediatric Endocrinology Unit of the University Hospital of Araba, and at Primary Care Clinics in the city of Vitoria-Gasteiz. The main entry criterion was having overweight/obesity, as defined by the cutoffs for sex and age (by month) established by the World Obesity Federation (15). All children had to be 8–12 years of age. Subjects whose medical condition or medication limited their physical activity, or might affect the results obtained, were excluded. None of the children had diabetes or any other endocrine disorder, and all were nonsmokers.

Assignment to intervention arms and blinding

Children were allocated to the CInt or II groups after baseline measurements. The design of the current project was conceived as a randomized controlled trial; however, this assignment was not completely random. A number of children/families (N=11) did not have time to attend the exercise sessions and were thus allocated to the CInt group. This decision was made since the children/families had been encouraged to participate in the study by their pediatricians, citing probable health benefits.

Neither the staff delivering the intervention nor the study subjects were blinded to the arm assignment. However, the researcher in charge of analyzing the outcomes was thus blinded.

Intervention protocols

Lifestyle and psycho-educational program

Both the CInt and the II groups participated in the lifestyle and psychoeducational

program. Parents/caregivers and children separately attended the lifestyle (45 min) and psycho-educational (45 min) programs once every 2 weeks (i.e., 11 sessions in total), as has been explained in detail previously (14). The focus of the program was to increase 1) the parents' and children's knowledge about healthier dietary habits, 2) their physical activity level, and 3) to promote sleep hygiene. The aim of the psychoeducational program was to provide skills to the parents/caregivers in order to optimize the family environment for making positive lifestyle changes and to learn assertive communication skills. The program also provided skills to children for managing their emotions and feelings, and for improving their self-esteem and psychological well-being.

Design of the supervised exercise program

Only the subjects in the II group took part in the exercise program. The full design of the program is available elsewhere (14). Children attended three sessions per week. Briefly, sessions were designed and supervised by exercise specialists and consisted of 5 min of instruction time, 10 min of warm-up, 60 min of game-based cardiovascular endurance, 10 min of muscle strength exercises, and 5 min of cooling down and stretching exercises (overall, 90 min/session). Subjects were encouraged through motivation and game strategies to spend as much time as possible during the session in vigorous, high-intensity physical activity (16). The maximum heart rate was defined as the highest value obtained during the cardiopulmonary exercise test in the laboratory, or in the 20 m shuttle run test (msrt). Children wore a Polar RS300X HR monitor during the sessions for recording exercise intensity (16).

Participant retention and addressing compliance and adherence

The attendance of both the children and their parents/caregivers at the lifestyle and psycho-educational sessions was recorded. In the exercise program, children were

marked “absent” if they did not attend a session or refused to participate in the proposed games or activities. Regardless of their adherence record, all subjects were encouraged to return for postintervention data collection.

Measurements

Primary and secondary outcomes were assessed at baseline and after 22 weeks of intervention by the same trained researchers.

Primary outcome measure: percentage of HF

The percentage of HF was measured by MRI using a MAGNETOM Avanto 1.5-T System (Siemens Healthcare, Erlangen, Germany) equipped with a phased-array surface coil and a spine array coil, and running Siemens Medical System software v.syngo.MR B17A, following the manufacturer instructions (17). Children with ≥5.5% HF were deemed to have hepatic steatosis (18).

Secondary outcomes measurements

Physical examination and body composition

Body mass and height were measured in duplicate. The cut points established by the World Obesity Federation (http://www.worldobesity.org/) by sex and for each month of age were used to define overweight and obesity (15). Pubertal stage was recorded by a pediatrician (19). Systolic and diastolic blood pressure were measured following recommendations for children (20). Participants were deemed hypertensive when their systolic or diastolic blood pressure was >90th age-, sex-, and height-specific percentile (20).

Total and abdominal fat, as well as lean mass, were measured by DEXA using a HOLOGIC QDR 4500 W device. The fat mass index (FMI) was then determined as (FMI: fat mass [kg]/stature^2 [m^2]) and lean mass index (LMI: lean mass [kg]/ stature^2 [m^2]).

Physical fitness

Cardiorespiratory fitness (CRF) and muscle strength were determined following validated protocols for children (21). CRF was assessed by two tests: 1) an incremental CRF treadmill protocol with respiratory gas analysis until exhaustion, and 2) the 20msrt. Upper and lower body muscular strength were assessed by the handgrip and the standing broad jump tests, respectively.

Biochemical measurements

Plasma triglycerides (TGs), HDL cholesterol (HDLc), LDL cholesterol (LDLc), insulin, glucose, and γ-glutamyl transferase (GGT) concentrations were determined as reported previously (14). Insulin resistance-HOMA and the TG/HDLc ratio were then calculated. Insulin resistance was defined according to the age- and sex-specific cut points for HOMA values (22). Subjects with TG/HDLc ratio values of ≥2.0 were deemed to be at increased cardiometabolic risk (23).

Physical activity, sedentary time, sleep and dietary habits assessment

Physical activity was determined by accelerometry (wGT3X-BT; Actigraph, Pensacola, FL), and dietary habits were assessed using two nonconsecutive 24-h recall records and food frequency questionnaires, as detailed previously (14).

Psychological assessment

Anxiety was assessed using the State-Trait Anxiety Inventory for Children; stress was determined using the Children's Daily Stressors Inventory; self-concept was assessed

using the Self-Concept Form-5 Questionnaire; and depression was examined using the translated version of the Children's Depression Inventory (14).

Statistical analyses

Since no information on the effect of exercise on the percentage of HF in children was available, the required sample size was calculated using information available for closely associated secondary outcome variables. The sample size actually obtained was N=116. This figure was expected to provide at least 80% power for detecting an effect size (Cohen's d) between the two experimental groups of 0.7 for insulin resistance and total body fat (minimum number required in each group=34 for 80% power and α=0.05).

The baseline characteristics of the subjects' in the two intervention groups were compared using either the Student t test (for continuous variables) or the χ^2 test (for categorical variables). Differences between the intervention groups in terms of postintervention values for the primary and secondary outcomes were also examined using the Student t test. Within group differences (pre vs. post values) in primary and secondary outcomes were examined using the Student paired t test following per protocol and intention-to-treat principles. For the intention-to-treat analyses, missing values at follow-up were obtained by multiple imputation using the following predictor variables: preintervention values, postintervention values, age, sex, and intervention group. Imputations were performed as four blocks: body composition variables (HF, body mass, BMI, FMI, LMI, abdominal fat), blood pressure variables (systolic and diastolic blood pressure), cardiometabolic risk variables (TG, HDLc, LDLc, TG/HDLc ratio, insulin, glucose, HOMA, GGT), and fitness variables (VO_2 peak, end-time treadmill test, 20msrt result, handgrip strength, and standing broad jump).

In both the per protocol and the intention-to-treat analyses, differences between the CInt and II groups in terms of changes in primary and secondary outcomes were examined by ANCOVA adjusting for baseline values. Cohen's d was used to estimate the effect size and 95% CI. Differences between the CInt and II groups in terms of changes in the percentage of HF were examined in extended models: Model 1, unadjusted; Model 2, adjusted by the corresponding baseline value and changes in height; Model 3, adjusted by baseline values and changes in the FMI; and Model 4, adjusted by baseline values and changes in abdominal fat.

Finally, the relationship between the changes (preintervention to postintervention values) in physical fitness variables and in the percentage of HF in each intervention group (CInt and II) were examined by regression analysis 1) using an unadjusted model, and 2) adjusting for baseline physical fitness and percentage of HF.

Significance was set at $P<0.05$. All calculations were made using the Statistical Package for Social Sciences version 24.0 for Windows (SPSS Inc, Chicago, IL).

Results

Figure 1 shows the flow chart for the trial, plus subject inclusions and exclusions.

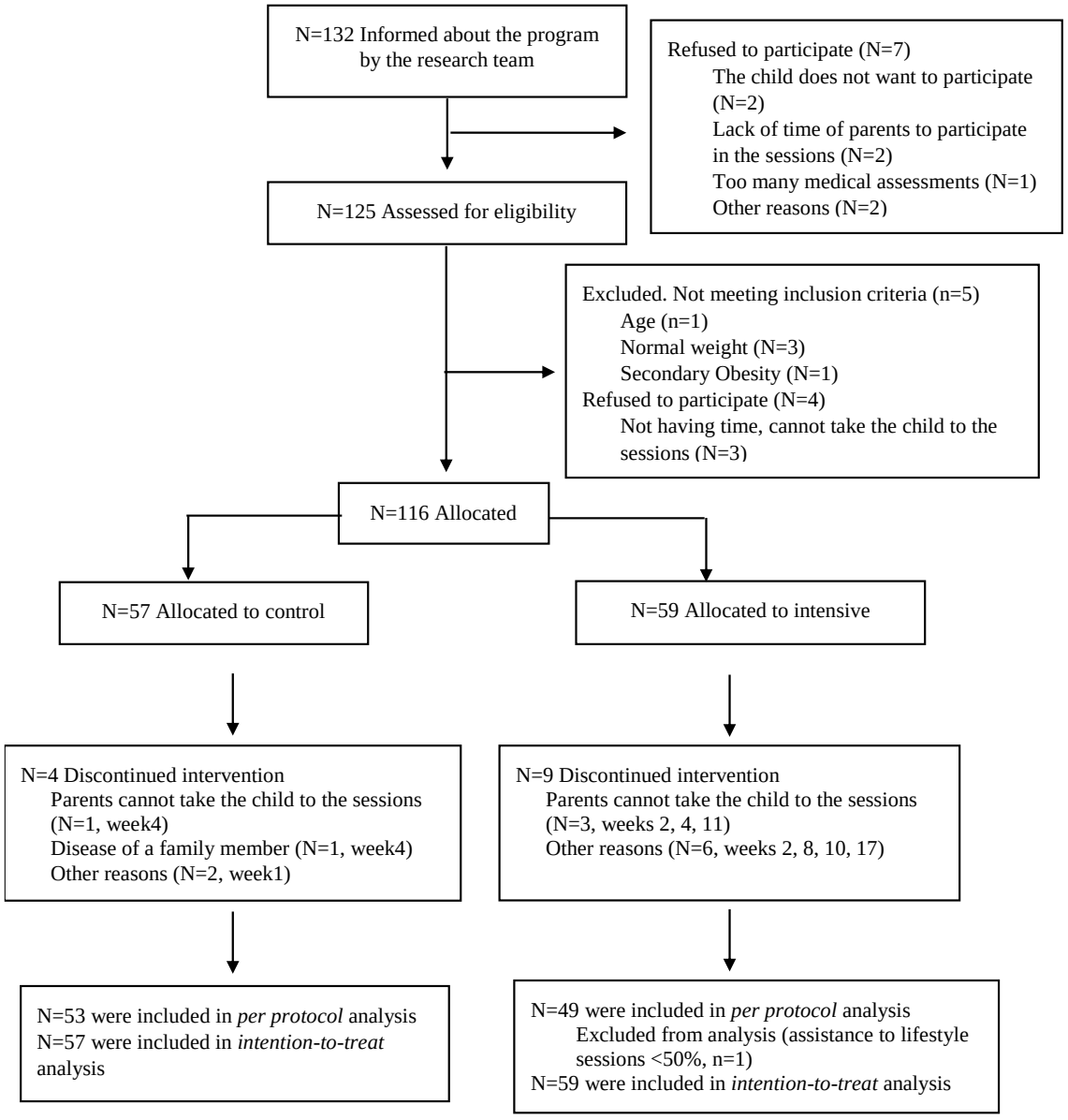


Figure 1—Flowchart showing enrollment into the study groups. Control group: children participating in the family-based lifestyle- and psycho-educational program; Intensive group: children participating in the latter plus exercise training.

Table 1 shows the participants' baseline characteristics. Overall, there were no significant differences in the socioeconomic, sociodemographic, anthropometric or clinical characteristics between the CInt and II groups.

Table 1—Characteristics of the children participating in the study

	Total			Control group (CInt)			Intensive group (II)			
	N	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*P*
Age (years)	116	10.6	1.1	57	10.6	1.1	59	10.5	1.0	0.701
Girls (*N*, %)	116	62	53.4	57	30	52.6	59	32	54.2	0.862
Non-Spanish origin of the mother (*N*, %)	116	18	15.5	57	6	10.5	59	12	20.3	0.114
High maternal educational level (*N*, %)	115	84	73.0	57	46	80.7	58	38	65.5	0.092
Family history of diabetes (*N*, %)	115	9	7.8	57	7	11.3	58	2	4.0	0.094
Tanner stage (*N*, %)										
Telarche or gonadarche	108			55			53			0.277
I		38	34.3		20	36.4		22	41.7	
II		30	38.9		20	36.4		12	22.6	
III		19	15.7		12	21.0		11	20.8	
IV-V		10	11.1		3	5.4		8	15.1	
Pubarche	108			55			53			0.865
I		37	34.3		20, 36.4			17	32.1	
II		42	38.9		22, 40.0			20	37.7	
III		17	15.7		7, 12.7			10	18.9	
IV-V		12	11.1		6, 10.9			6	11.3	
Weight status	116			57			59			0.727
Overweight (*N*, %)		49	42.3		25	43.9		24	40.7	
Obesity grade I (*N*, %)		58	50.0		29	50.9		29	49.2	
Obesity grade II (*N*, %)		6	5.2		2	3.5		4	6.8	
Obesity grade III (*N*, %)		3	2.6		1	1.8		2	3.4	
High blood pressure (*N*, %)	116	8	6.9	57	3	5.3	59	5	8.5	0.350
Hypertriglyceridaemia (*N*, %)	115	9	7.8	57	3	5.3	58	6	10.3	0.490
High TG/HDLc (*N*, %)	114	37	32.	56	17	30.4	58	20	34.5	0.692
Hepatic steatosis (*N*, %)	115	41	35.7	57	18	31.6	58	23	39.7	0.239
Insulin resistance (*N*, %)	114	51	44.7	55	24	42.9	58	27	46.6	0.705

Control group: children participating in the family-based lifestyle and psycho-educational program; Intensive group: children participating in the same program plus the supervised exercise program.

A total of 102 of the original 116 subjects (87.9%; *N*=53 in the CInt group; *N*=49 in the II group) successfully completed the trial, attending at least 50% of the sessions. Their data were included in the per protocol analyses. The data for those children that discontinued the intervention (*N*=4 in the CInt group; *N*=9 in the II group), or who did not attend at least 50% of the educational program sessions (*N*=1 in the II group), were also included in the intention-to-treat analyses.

Attendance, adverse events and main characteristics of the program

No significant difference was seen between the CInt and II groups in terms of attendance at the lifestyle- and psychoeducation program sessions, either for the parents/caregivers (86.4±12.9% vs. 80.6±15.3%; *P*=0.334) or the children (87.2±12.0% vs. 82.5±14.6%; *P*=0.496). The mean attendance rate for the II subjects with respect to the exercise program was 72.0±16.1% sessions. Exercise-related adverse events included knee and ankle pain (*N*=2); no adverse events were recorded for the lifestyle and psycho-education programs.

In the exercise program, the mean HR per session was 146±16 bpm. High-intensity exercise was maintained for 49±23% of the time, and moderate-intensity exercise for 32±15% of the time.

Effects of the intervention on primary outcome

Percentage hepatic fat

No significant difference in percentage of HF was seen between the CInt and II groups at baseline (**Table 2** and **Supplementary Table 1**, for the per protocol and intention-to-treat analyses, respectively). Although there were no significant differences in postintervention values of the percentage of HF between the two groups (Table 2 and Supplementary Table 1), only children in the intensive group reduced the percentage of

HF after the intervention (Table 2 and Supplementary Table 1, for the per protocol and intention-to-treat analyses, respectively). The difference in the change in percentage of HF between the two groups (per protocol analysis) was significant (P<0.02) (**Fig. 2** and **Supplementary Fig. 1**); these results persisted after adjustment for baseline values for percentage of HF and changes in height (-1.20±0.31% vs. 0.04±0.30% for the II and CInt groups, respectively; P=0.006), and when changes in FMI (-1.13±0.27% vs. -0.02±0.27% for the II and CInt groups respectively; P=0.004) and abdominal fat (-1.19±0.26% vs. -0.03±0.28% for the II and CInt groups, respectively; P=0.004) were adjusted for instead of changes in height (Supplementary Fig. 1). Further adjustment for baseline VO_2 peak (measured via the treadmill test) did not substantially alter the results (P<0.05). The intention-to-treat analyses for differences between groups in terms of the change in percentage of HF returned similar results (**Supplementary Fig. 2**).

Table 2. Percentage hepatic fat, body composition, cardiometabolic and diabetes risk factors, and physical fitness before (Pre) and after (Post) participation in the family-based lifestyle and psycho-educational intervention program (control group, CI) or the plus exercise training (intensive group, II) in children with overweight/obesity (*per protocol* analysis).

	Cint group						II group						CI vs. II	
	Pre			Post			Pre			Post				
	N	Mean	SD	Mean	SD	*P*	*N*	Mean	SD	Mean	SD	*P*	P_{Pre}	P_{post}
Primary outcome														
HF (%)*	50	5.2	2.8	5.2	2.9	0.769	49	5.6	4.5	4.5	3.6	**0.006**	0.836	0.193
Secondary outcomes														
Body composition														
Body mass (kg)	53	53.4	8.9	53.9	9.8	0.122	49	55.9	11.3	54.9	11.3	**0.011**	0.224	0.491
Height (cm)	53	145.5	7.9	148.1	7.8	**<0.001**	49	146.9	8.3	149.4	8.5	**<0.001**	0.350	0.430
Body mass index (kg/m^2)	53	25.1	2.8	24.4	3.2	**<0.001**	49	25.7	3.3	24.4	3.3	**<0.001**	0.325	0.813
Fat mass index (kg/m^2)	53	9.8	2.1	9.0	2.4	**<0.001**	48	10.4	2.3	9.2	2.2	**<0.001**	0.191	0.345
Lean mass index (kg/m^2)	53	14.6	1.1	14.5	1.3	0.141	48	14.4	1.2	14.3	1.3	0.242	0.408	0.869
Abdominal fat (kg)	53	5.7	2.1	5.3	2.2	**0.001**	48	6.4	2.5	5.7	2.1	**<0.001**	0.151	0.215
Cardiometabolic and diabetes risk factors														
SBP (mmHg)	52	96.7	10.6	97.1	10.6	0.835	49	95.6	10.2	96.0	11.9	0.815	0.586	0.738
DBP (mmHg)	52	62.4	7.5	61.5	6.6	0.354	49	60.1	7.8	62.5	7.6	**0.049**	0.162	0.356
MAP (mmHg)	52	85.3	8.5	85.3	7.9	0.982	49	83.8	8.5	84.9	9.1	0.408	0.386	0.973
TG (mg/dL)*	51	81.4	35.3	79.8	28.4	0.876	48	78.6	37.6	69.3	26.4	0.079	0.907	0.122
HDLc (mg/dL)	51	50.3	10.7	49.0	9.4	0.235	48	53.7	11.8	50.3	10.9	**0.003**	0.198	0.627
TG/HDLc	51	1.72	0.89	1.72	0.81	0.992	48	1.61	1.08	1.52	0.93	0.460	0.882	0.299
LDLc (mg/dL)	51	102.8	24.7	99.2	23.9	0.052	48	102.9	22.9	90.9	18.7	**<0.001**	0.711	**0.046**
Insulin (IU/mL)*	50	11.7	4.4	10.5	4.6	**0.008**	47	12.3	4.5	11.4	5.4	**0.027**	0.400	0.334
Glucose (mg/dL)	51	85.3	5.6	85.2	5.4	0.910	48	85.5	5.1	84.9	6.7	0.446	0.813	0.846
HOMA*	50	2.50	1.06	2.23	1.05	**0.017**	48	2.64	1.12	2.44	1.35	0.054	0.438	0.354
Gamma-GT (units/L)	51	15.3	3.9	15.3	4.1	0.894	46	17.0	4.9	15.4	4.4	**0.010**	0.079	0.878
Physical fitness														
VO_2 peak treadmill test (mL/kg/min)	41	35.9	6.3	38.7	6.8	**0.017**	44	32.2	5.0	37.6	5.6	**<0.001**	**0.010**	0.422
End-time treadmill test (sg)	41	598	155	674	165	**0.004**	44	569	136	693	141	**<0.001**	0.365	0.567
20msrt (laps)	44	24	12	30	11	**0.001**	47	20	11	29	16	**<0.001**	0.284	0.912
Handgrip strength (kg/body mass)	52	0.34	0.06	0.37	0.07	**<0.001**	49	0.32	0.06	0.37	0.07	**<0.001**	0.229	0.732
Standing broad jump (cm)	46	103.6	16.0	110.0	24.5	**0.042**	46	98.4	19.0	111.0	20.7	**<0.001**	0.130	0.807

DBP, diastolic blood pressure; MAP, mean arterial pressure calculated as diastolic pressure + [0.333 3 x (systolic blood pressure - diastolic pressure)]; Post, after; Ppost indicates statistical differences in Postintervention values between the control and the II groups (Student *t* test); Ppre indicates statistical differences in baseline values (Student *t* test); Pre, before; SBP, systolic blood pressure. *Analyzed with log (ln)-transformed values, but nontransformed data are shown. *P* indicates statistical differences between Pre and Post values (paired Student *t* test). Bold type values indicate statistically significant differences ($P<0.05$).

	Control Mean (SD)	Intensive Mean (SD)	P
Δ Hepatic fat (%)	0.0 (1.9)	-1.1 (2.5)	**0.008**
Δ Body mass (kg)	0.5 (2.4)	-1.0 (2.7)	**0.003**
Δ Body mass index (kg/m^2)	-0.7 (1.0)	-1.4 (1.1)	**0.002**
Δ Fat mass index (kg/m^2)	-0.8 (0.9)	-1.1 (0.8)	0.076
Δ Lean mass index (kg/m^2)	-0.1 (0.5)	-0.1 (0.5)	0.763
Δ Abdominal fat (kg)	-0.4 (0.8)	-0.7 (1.0)	0.125
Δ Mean arterial pressure (mm Hg)	-0.1 (10.0)	1.1 (9.1)	0.554
Δ Triglycerides (mg/dL)	-1.6 (30.5)	-9.1 (32.9)	0.246
Δ HDLc (mg/dL) *	-0.9 (7.9)	-2.7 (7.6)	0.258
Δ TG/HDL	0.00 (0.68)	-0.10 (0.88)	0.557
Δ LDLc (mg/dL)	-3.5 (13.1)	-11.9 (15.6)	**0.005**
Δ Insulin (IU/mL)	-1.3 (4.6)	-1.0 (5.2)	0.746
Δ Glucose (mg/dL)	-0.1 (5.0)	-0.6 (5.4)	0.640
Δ HOMA	-0.28 (1.03)	-0.21 (1.31)	0.777
Δ Gamma-GT (U/L)	-0.1 (3.1)	-1.6 (4.1)	**0.037**
Δ VO_{2peak} (ml/kg/min) *	2.8 (7.1)	5.2 (5.6)	0.080
Δ 20msrt (laps) *	6 (11)	9 (12)	0.194
Δ Handgrip strength (kg/body weight) *	0.04 (0.04)	0.04 (0.05)	0.555
Δ Standing broad jump (cm) *	6.4 (20.6)	12.7 (14.7)	0.094

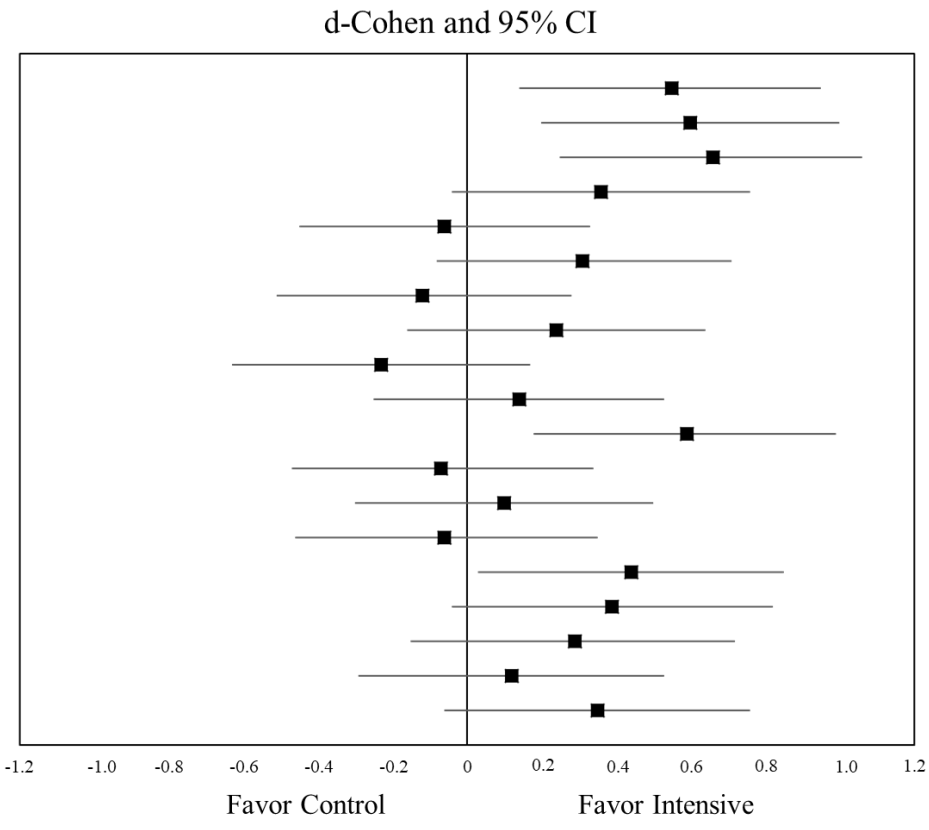


Figure 2—Unadjusted effect size (Cohen's d and 95% CI) and differences in changes (Δ) in percentage of HF, body mass and composition, cardiometabolic and diabetes risk factors between the control group (children participating in the family-based lifestyle and psycho-educational program), and intensive groups (children participating in the multicomponent intervention, including lifestyle and psycho-educational program). Differences between the control and the II groups in terms of changes in primary and secondary outcomes were examined (per protocol) by ANOVA. Changes were calculated as postintervention minus preintervention values. Data are the mean (SD). *Negative Cohen's d values obtained for the differences in the changes in adiposity and cardiometabolic and diabetes risk between the two groups (in favor of the II group) were multiplied by -1 for illustrative purposes.

Effects of the intervention on secondary outcomes

Adiposity and CVD and type 2 diabetes risk factors

No significant differences were seen between the CInt and II groups in terms of any of the studied cardiometabolic or type 2 diabetes risk factors at baseline (Table 2 and Supplementary Table 1). Both groups returned significantly reduced BMI, FMI, and abdominal fat values after the intervention ($P<0.01$), while no significant changes were seen in LMI (Table 2 and Supplementary Table 1 for the per protocol and intention-to-treat analyses, respectively). No significant differences were seen between the groups in terms of postintervention in any adiposity estimate (Table 2 and Supplementary Table 1). The reduction in BMI was significantly greater in the II group ($P<0.01$), but no significant differences were seen between the groups in terms of the change in FMI or abdominal fat (Fig. 2). These results did not substantially differ after adjusting for baseline values (Supplementary Fig. 2). The intention-to-treat analyses returned similar results (**Supplementary Fig. 3**).

Per protocol analysis showed the insulin concentration and HOMA value to be significantly smaller ($P<0.01$ and $P<0.05$, respectively) in the CInt group after the intervention (Table 2). These reductions were not significant, however, in the intention-to-treat analysis ($P<0.09$) (Supplementary Table 1). In the II group, the HDLc ($P<0.01$), LDLc ($P<0.001$), insulin ($P<0.05$), and GGT ($P<0.05$) concentrations all decreased significantly by the end of the intervention program (per protocol analysis). Diastolic blood pressure increased significantly in the II group, whereas no significant change was observed in the control group (Table 2). The intention-to-treat analysis returned similar results, but only the changes in TG ($P<0.05$), insulin ($P<0.001$), and GGT ($P<0.01$) remained significant (Supplementary Table 1).

No significant differences were seen in terms of postintervention cardiometabolic and diabetes risk factors between the two groups (Table 2 and Supplementary Table 1), except with respect to the LDLc level (P<0.05; both per protocol and intention to treat). However, greater reductions in LDLc level were seen in the II group than in the CInt group after the intervention; the per protocol and the intention-to-treat analyses returned similar results (Fig. 2 and Supplementary Figs. 2 and 3).

In the per protocol analysis, significant difference was seen between the groups in terms of the effect of the interventions on TG (P<0.05) (Table 2), but this was attenuated in the intention-to-treat analyses (P<0.07) (Supplementary Table 1). However, the differences in the change in TG levels were not consistent between the per protocol (Fig. 2 and Supplementary Fig. 2) and intention-to-treat analyses (Supplementary Fig. 3). Similarly, analysis-inconsistent differences were seen between groups in terms of the effect of the interventions on GGT levels (Fig. 2 and Supplementary Figs. 2 and 3).

Physical fitness

At baseline, VO_2 peak as measured by the treadmill test was lower in the II group than in the CInt group (P<0.05), but no significant differences were seen in the rest of the fitness variables (Table 2 and Supplementary Table 1). Significant increases were seen in both groups for all physical fitness variables after the intervention (P≤0.001) (Table 2 and Supplementary Table 1), but no significant differences were seen between them in terms of the changes in these values (Fig. 2), even after adjustment for baseline values (Supplementary Fig. 2). The intention-to-treat analysis returned similar results (Supplementary Fig. 3).

The increase in CRF measured in the 20msrt was significantly associated with the reduction in percentage of HF content in the II group subjects–at least in the per protocol

analysis (Supplementary Table 2). This relationship was attenuated and became nonsignificant (P<0.06) in the intention-to-treat analysis (Supplementary Table 3).

Effects of the intervention on lifestyle and psychological well-being factors

All comparisons were per protocol (**Supplementary Table 4**). No significant differences in dietary and physical activity variables were seen between the two groups at baseline. By the end of the intervention, both groups had significantly reduced their energy and fat intake and increased their intake of fruits and vegetables. No significant changes were seen, however, in the consumption of sugar and sugar-sweetened beverages. Neither moderate-to-vigorous physical activity nor sleep time changed after the intervention in either group, although sedentary time was significantly reduced in the II group (P<0.05). No significant differences in dietary or physical activity variables were seen between the two groups at the end of the intervention.

No significant differences were seen in psychological variables between the two groups at baseline (Supplementary Table 4). By the end of the intervention, the subjects of both groups had experienced a significant increase in terms of emotional self-concept (P<0.05). Improvements were also seen in physical self-concept, total depression, dysphoria, and anxiety, although they were only significant in the CInt group (P<0.05 and P<0.09, for the CInt and II intervention groups, respectively). No significant differences in the improvement in psychological wellbeing were seen between the two group at the end of the intervention.

Conclusions

The present results reveal the II subjects to have experienced a clinically important reduction (nearly 20%) in percentage of HF; no such reduction was seen in the CInt group. Both the CInt and II subjects experienced a reduction in total and abdominal fat and insulin resistance. The II subjects also enjoyed a significant reduction in LDLc. Given the importance of treating pediatric hepatic steatosis early, not only to prevent future liver damage, but also to prevent type 2 diabetes, these findings should be taken into account in pediatric obesity management programs.

Family-based lifestyle education programs accompanied with psychological support are recommended for the treatment and prevention of pediatric obesity and related comorbidities (24). However, the current study shows that while the educational program followed was able to effectively reduce adiposity and insulin resistance, it was unable to reduce the percentage of HF in the children. HF was only reduced in those who participated in the supervised exercise program in addition to the lifestyle education program. These results agree with those of a study involving obese adolescents (25), in which a 4-month intervention focused on changing the quality of carbohydrate intake resulted in significant improvements in insulin sensitivity, but only a combined lifestyle, psycho-education, and resistance exercise training program resulted in a reduction in percentage of HF.

As far as we are aware, the present work is the first to report the additional effect of supervised exercise on HF as measured by MRI in children. Other studies have reported significant reductions in HF after aerobic or resistance exercise training interventions, but in adolescents (13,26–28). In a recent systematic review and meta-analysis, it was reported that exercise training at moderate-to-vigorous or vigorous intensity, in sessions

of at least 60 min, and a frequency ≥3 sessions per week are effective reducing HF content in youth (12). The present findings are in agreement with these recommendations.

In adults and adolescents with overweight or obesity, lifestyle interventions including exercise, or not, have been reported to significantly reduce HF (29,30). However, these programs were based on hypocaloric diets that caused large body mass losses (30,31). In the current study, which involved preadolescent children, the lifestyle education program was not designed to achieve body weight loss in the short term, and certainly the CInt subjects lost no body mass or lean mass.

A systematic review and meta-analysis comparing the effect of exercise training and a hypocaloric diet on visceral adiposity (32) reported the latter to result in a larger body mass loss, whereas exercise training tended to induce larger reductions in visceral adiposity. Interestingly, in the present work, the effects of exercise on the percentage of HF were independent of the change in total or abdominal fat, suggesting a direct metabolic effect of exercise training on HF metabolism.

Family-based, structured lifestyle modification programs combined with behavioral strategies for treating obesity have been associated with adiposity reductions in children (33). In the current study, the children's dietary habits were improved with respect to the development of obesity after the 22-week intervention. Improvements were also seen in several components of self-concept, depression, stress, and anxiety in children. These improvements in both dietary habits and psychological health are quite notable.

Physical activity was not increased at the end of the intervention in either the CInt or II group; indeed, in the CInt group there was even a trend toward a reduction in total physical activity. It should also be noted that several families declined to participate in

the II arm given the relatively large number of sessions involved. A possible solution might be to reduce the number of sessions per week or the duration of each session. Future studies examining the effectiveness of less intensive exercise programs on the percentage of HF and cardiometabolic risk in overweight/obese children are warranted.

One of the strengths of this study is the use of MRI for measuring percentage of HF. Moreover, the sample size was also larger, and the duration of the intervention program was longer than in previous studies (13,26,27). Of note, however, is that several families did not agree to participate in the II due to the relatively elevated number of sessions, which may comprise the feasibility of the intensive program. There are, however, some limitations that should be mentioned. The most important limitation of the study is its not entirely strict randomization (14). This may limit the validity of the results. However, the participants in both groups were comparable at baseline, and adjustment for potential baseline differences between groups were made in analyses. Finally, the results were not adjusted for multiple comparisons.

Summary

A family-based, multicomponent intervention program including supervised exercise training, and lifestyle and psycho-education designed following international recommendations for obesity prevention and health promotion in children was shown to have the potential to reduce HF, total and abdominal fat, and insulin resistance, and to improve dietary habits and psychological well-being in preadolescent children with overweight/obesity. These findings highlight the importance of promoting such programs as part of pediatric obesity treatment: improvements may be achieved not only in total and abdominal fat and insulin resistance, but also in hepatic steatosis, a metabolic abnormality that increases the risk for type 2 diabetes and CVD.

Acknowledgments

The authors thank the pediatricians who took part in the recruitment of subjects, and all those research assistants involved in assessment duties. The authors also thank Siemens Medical Systems for supplying the software for quantifying the percentage of HF, and the assistants who helped run the exercise training sessions and educational program.

Funding

This project was funded by the Spanish Ministry of Health "Fondos de Investigación Sanitaria del Instituto de Salud Carlos III" (PI13/01335), the Spanish Ministry of Industry and Competitiveness (DEP2016-78377-R), and by EU Fondos Estructurales de la Unión Europea (FEDER) funds ("Una manera de hacer Europa"). Support was also provided by grants from the Spanish Ministry of Education, Culture and Sports (FPU14/03329), the Education Department of the Government of the Basque Country (PRE_2016_1_0057, PRE_2017_2_0224, PRE_2018_2_0057), the University of Granada Plan Propio de Investigación 2016-Excellence actions: Unit of Excellence on Exercise and Health (UCEES)—and the Junta de Andalucía, Consejería de Conocimiento, Investigación y Universidades (FEDER, ref. SOMM17/6107/UGR).

Funding sources were not involved in the collection, analysis, or interpretation of any data; in the writing of the report; or in the decision to submit the article for publication.

Duality of Interest

No potential conflicts of interest relevant to this article were reported.

Author contributions

I.L. designed the study, analyzed the data, and drafted the manuscript. I.L. takes full responsibility for the integrity of the data analyses. I.L., V.M.-V., J.R.R., and F.B.O. participated in the interpretation of the results. M.M., L.A., E.M., and M.O. collected the data and critically revised the manuscript. I.L., M.M., L.A., E.M., M.O., V.M.-V., J.R.R., and F.B.O. critically revised the manuscript for its intellectual content and approved the final version. I.L. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Abarca-Gómez L, Abdeen ZA, Hamid ZA, et al.; NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. Lancet 2017;390:2627–2642

2. Steinberger J, Daniels SR, Eckel RH, et al.; American Heart Association Atherosclerosis, Hypertension, and Obesity in the Young Committee of the Council on Cardiovascular Disease in the Young; Council on Cardiovascular Nursing; and Council on Nutrition, Physical Activity, and Metabolism. Progress and challenges in metabolic syndrome in children and adolescents: a scientific statement from the American Heart Association Atherosclerosis, Hypertension, and Obesity in the Young Committee of the Council on Cardiovascular Disease in the Young. Circulation 2009;119:628–647

3. Chung ST, Onuzuruike AU, Magge SN. Cardiometabolic risk in obese children. Ann N Y Acad Sci 2018;1411:166–183

4. Anderson EL, Howe LD, Jones HE, Higgins JPT, Lawlor DA, Fraser A. The prevalence of non-alcoholic fatty liver disease in children and adolescents: a systematic review and meta-analysis. PLoS One 2015;10:e0140908

5. Lobstein T, Jackson-Leach R. Planning for the worst: estimates of obesity and comorbidities in school-age children in 2025. Pediatr Obes 2016;11:321–325

6. Yki-Järvinen H. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. Lancet Diabetes Endocrinol 2014;2:901–910

7. Stefan N, Haring H-U, Cusi K. Non-alcoholic fatty liver disease: causes, diagnosis, cardiometabolic consequences, and treatment strategies. Lancet Diabetes Endocrinol 2019;7:313-324

8. Zimmermann E, Gamborg M, Holst C, Baker JL, Sørensen TIA, Berentzen TL. Body mass index in school-aged children and the risk of routinely diagnosed non-alcoholic fatty liver disease in adulthood: a prospective study based on the Copenhagen School Health Records Register. BMJ Open 2015;5:e006998

9. Bjerregaard LG, Baker JL. Change in overweight from childhood to early adulthood and risk of type 2 diabetes. N Engl J Med 2018;378:2537–2538

10. Vos MB, Abrams SH, Barlow SE, et al. NASPGHAN clinical practice guideline for the diagnosis and treatment of nonalcoholic fatty liver disease in children: recommendations from the Expert Committee on NAFLD (ECON) and the North American Society of Paediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr 2017;64:319–334

11. Mead E, Brown T, Rees K, et al. Diet, physical activity and behavioural interventions for the treatment of overweight or obese children from the age of 6 to 11 years. Cochrane Database Syst Rev 2017;6:CD012651

12. Medrano M, Cadenas-Sanchez C, Álvarez-Bueno C, et al. Evidence-based exercise recommendations to reduce hepatic fat content in youth- a systematic review and meta-analysis. Prog Cardiovasc Dis 2018;61:222–231

13. Davis JN, Gyllenhammer LE, Vanni AA, et al. Startup circuit training program reduces metabolic risk in Latino adolescents. Med Sci Sports Exerc 2011;43:2195–2203

14. Medrano M, Maiz E, Maldonado-Martín S, et al. The effect of a multidisciplinary intervention program on hepatic adiposity in overweight-obese children: protocol of the EFIGRO study. Contemp Clin Trials 2015;45(Pt B):346–355

15. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatr Obes 2012;7:284–294

16. Riebe D, Ehrman JK, Liguori G, Magal M; American College of Sports Medicine. *ACSM's Guidelines for Exercise Testing and Prescription.* Philadelphia, Wolters Kluwer, 2018

17. Kukuk GM, Hittatiya K, Sprinkart AM, et al. Comparison between modified Dixon MRI techniques, MR spectroscopic relaxometry, and different histologic quantification methods in the assessment of hepatic steatosis. Eur Radiol 2015;25:2869–2879

18. Browning JD, Szczepaniak LS, Dobbins R, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 2004;40:1387–1395

19. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child 1976;51:170–179

20. Flynn JT, Kaelber DC, Baker-Smith CM, et al.; SUBCOMMITTEE ON SCREENING AND MANAGEMENT OF HIGH BLOOD PRESSURE IN CHILDREN. Clinical practice guideline for screening and management of high blood pressure in children and adolescents. Pediatrics 2017;140:e20171904

21. Ruiz JR, Castro-Pinero J, Espana-Romero V, et al. Field-based fitness assessment in young people: the ALPHA health-related fitness test battery for children and adolescents. Br J Sports Med 2011;45:518–524

22. Nogueira-de-Almeida CA, de Mello ED. Different Criteria for the Definition of Insulin Resistance and Its Relation with Dyslipidemia in Overweight and Obese Children and Adolescents. Pediatr Gastroenterol Hepatol Nutr 2018;21:59–67

23. Di Bonito P, Moio N, Scilla C, et al. Usefulness of the high triglyceride-to-HDL cholesterol ratio to identify cardiometabolic risk factors and preclinical signs of organ damage in outpatient children. Diabetes Care 2012;35:158–162

24. Spear BA, Barlow SE, Ervin C, et al. Recommendations for treatment of child and adolescent overweight and obesity. Pediatrics 2007;120(Suppl. 4):S254-S288

25. Hasson RE, Adam TC, Davis JN, et al. Randomized controlled trial to improve adiposity, inflammation, and insulin resistance in obese African-American and Latino youth. Obesity (Silver Spring) 2012;20:811-818

26. Lee S, Deldin AR, White D, et al. Aerobic exercise but not resistance exercise reduces intrahepatic lipid content and visceral fat and improves insulin sensitivity in obese adolescent girls: a randomized controlled trial. Am J Physiol Endocrinol Metab 2013;305:E1222-E1229

27. Lee S, Bacha F, Hannon T, Kuk JL, Boesch C, Arslanian S. Effects of aerobic versus resistance exercise without caloric restriction on abdominal fat, intrahepatic lipid, and insulin sensitivity in obese adolescent boys a randomized, controlled trial. Diabetes 2012;61:2787–2795

28. Hay J, Wittmeier K, MacIntosh A, et al. Physical activity intensity and type 2 diabetes risk in overweight youth: a randomized trial. Int J Obes 2016;40:607-614

39. Hens W, Vissers D, Hansen D, et al. The effect of diet or exercise on ectopic adiposity in children and adolescents with obesity: a systematic review and meta-analysis. Obes Rev 2017;18:1310-1322

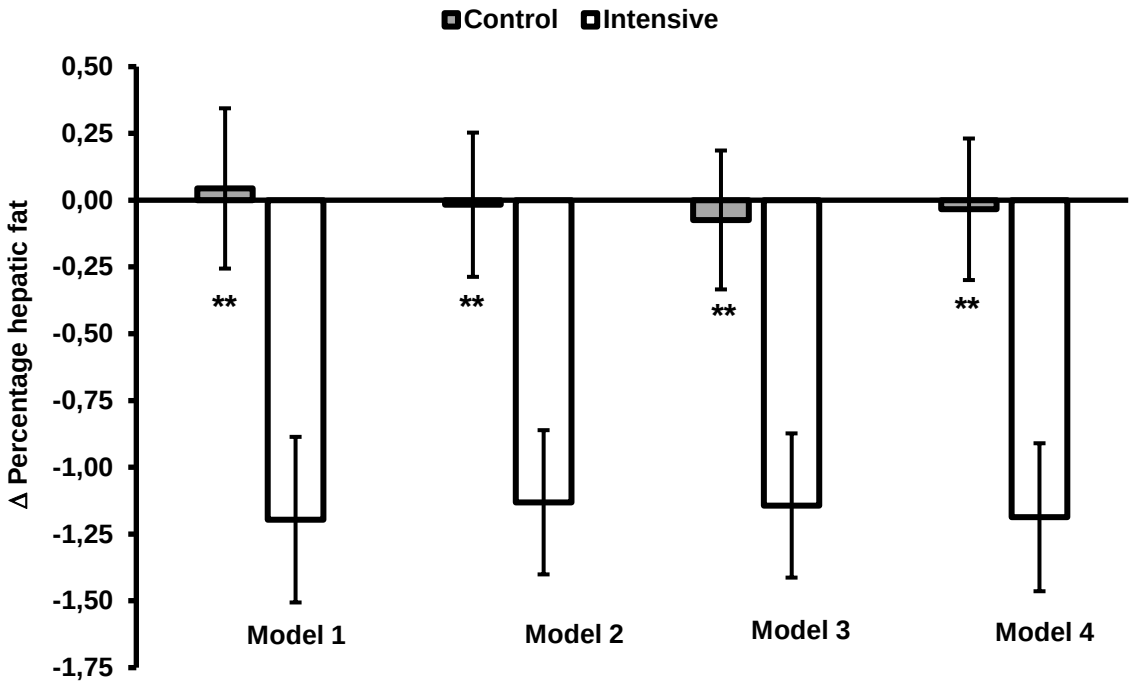
30. Hens W, Taeyman J, Cornelis J, Gielen J, Van Gaal L, Vissers D. The effect of lifestyle interventions on excess ectopic fat deposition measured by noninvasive techniques in overweight and obese adults: a systematic review and meta-analysis. J Phys Act Health 2016;13:671–694

31. Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, et al. Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis. Gastroenterology 2015;149:367–378.e5; quiz e14–e15

32. Verheggen RJHM, Maessen MFH, Green DJ, Hermus ARMM, Hopman MTE, Thijssen DHT. A systematic review and meta-analysis on the effects of exercise training versus hypocaloric diet: distinct effects on body weight and visceral adipose tissue. Obes Rev 2016;17:664–690

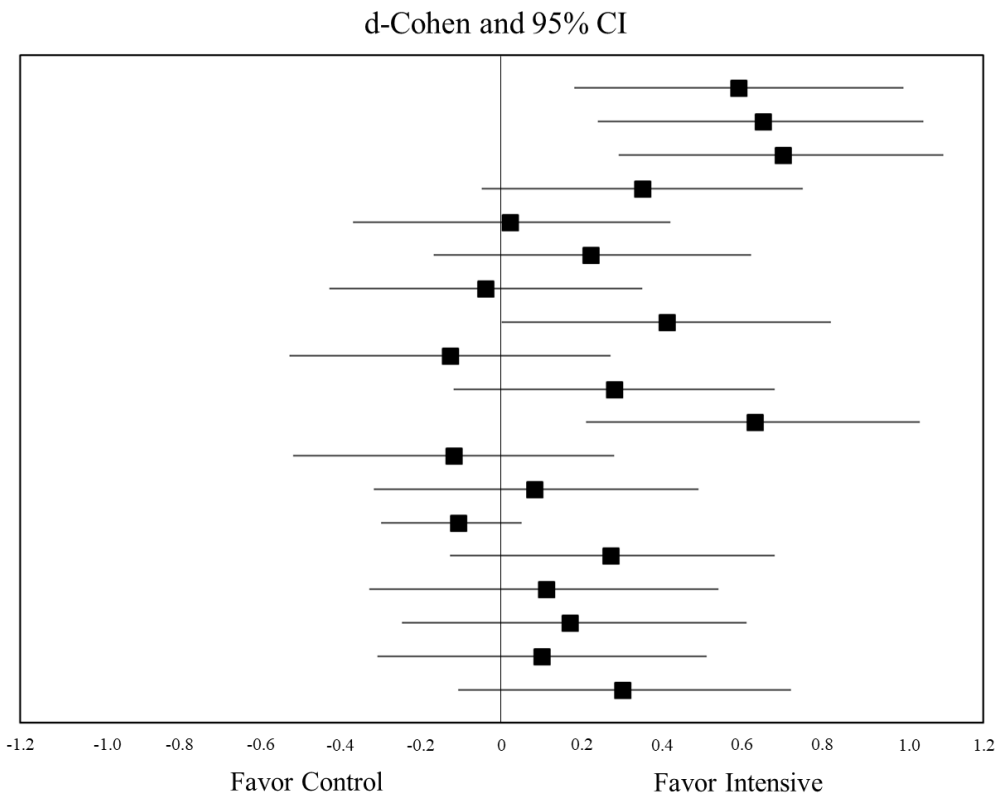
33. Sung-Chan P, Sung YW, Zhao X, Brownson RC. Family-based models for childhood-obesity intervention: a systematic review of randomized controlled trials. Obes Rev 2013;14:265–278

Supplemental Material



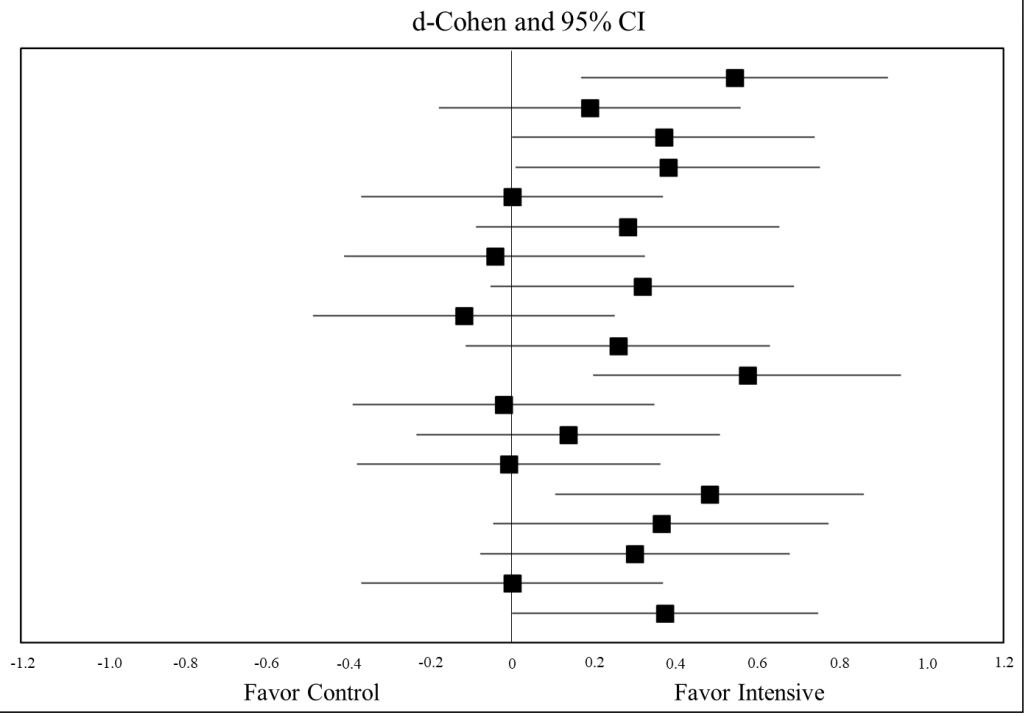
Supplemental Figure 1. Change in percentage hepatic fat in the group of children participating in the lifestyle- and psycho-educational program (grey bars, control), and in those participating in the latter plus exercise training (white bars, intensive). Model 1: unadjusted; Model 2: analyses were adjusted with baseline values and changes in height; Model 3: analyses were adjusted with baseline values and changes in fat mass index; Model 4: analyses were adjusted with baseline values and changes in abdominal fat. **: P<0.01.

	Control Mean±SEM	Intensive Mean±SEM	P
Δ Hepatic fat (%)	-0.0±0.3	-1.1±0.3	**0.005**
Δ Body mass (kg)	0.5±0.3	-1.1±0.4	**0.002**
Δ Body mass index (kg/m^2)	-0.7±0.1	-1.4±0.2	**0.001**
Δ Fat mass index (kg/m^2)	-0.8±0.1	-1.2±0.1	0.089
Δ Lean mass index (kg/m^2)	-0.1±0.1	-0.1±0.1	0.899
Δ Abdominal fat (kg)	-0.4±0.1	-0.6±0.1	0.264
Δ Mean arterial pressure (mm Hg)	0.4±0.1	0.7±1.2	0.843
Δ Triglycerides (mg/dL)	-1.0±3.3	-10.8±3.4	**0.041**
Δ HDLc (mg/dL) *	-1.3±1.1	-2.2±1.1	0.529
Δ TG/HDL	0.02±0.09	-0.15±0.09	0.176
Δ LDLc (mg/dL)	-3.4±1.8	-11.5±1.9	**0.003**
Δ Insulin (IU/mL)	-1.5±0.6	-1.0±0.6	0.556
Δ Glucose (mg/dL)	-0.1±0.7	-0.5±0.7	0.683
Δ HOMA	-0.32±0.15	-0.20± 0.16	0.590
Δ Gamma-GT (U/L)	-0.4±0.5	-1.3±0.5	0.192
Δ VO_{2peak} (ml/kg/min) *	3.7±0.9	4.4±0.9	0.636
Δ 20msrt (laps) *	7±2	9±2	0.481
Δ Handgrip strength (kg/body weight) *	0.04±0.01	0.04±0.01	0.621
Δ Standing broad jump (cm) *	6.7±2.6	12.0±2.6	0.163



Supplemental Figure 2. Adjusted effect size (d-Cohen and 95 %CI) and differences in the change in percentage hepatic fat, body mass and composition, cardiometabolic and diabetes risk factors between the control group (children participating in the family based lifestyle and psycho-educational program) and the intensive group (children participating in the multicomponent intervention including lifestyle and psycho-educational program and exercise training). Differences between the control and the intensive intervention groups in terms of the changes in primary and secondary outcomes were examined per protocol by ANCOVA, adjusting for baseline values. *Negative Cohen's d values obtained for the differences in the changes in adiposity and cardiometabolic and diabetes risk between the two groups (in favor of the intensive group) were multiplied by -1 for illustrative purposes. HDL: high density lipoprotein; HOMA: homeostasis model assessment; TG/HDL: triglycerides to high density lipoprotein ratio; LDL: low density lipoprotein; GGT: gamma glutammyl transferase; VO_2peak: peak oxygen consumption in the treadmill test; 20msrt: performance in the 20 meters shuttle run test.

	Control Mean (SD)	Intensive Mean (SD)	P (unadjusted)	P (adjusted)
Δ Hepatic fat (%)	0.0 (1.7)	-1.2 (2.6)	**0.008**	**0.008**
Δ Body mass (kg)	0.2 (4.7)	-0.6 (3.7)	0.291	0.394
Δ Body mass index (kg/m^2)	-0.8 (1.3)	-1.3 (1.4)	**0.037**	0.053
Δ Fat mass index (kg/m^2)	-0.8 (1.0)	-1.2 (1.1)	0.056	0.120
Δ Lean mass index (kg/m^2)	-0.1 (0.5)	-0.1 (0.5)	0.573	0.579
Δ Abdominal fat (kg)	-0.4 (0.9)	-0.7 (1.2)	0.058	0.327
Δ Mean arterial pressure (mm Hg)	0.0 (9.6)	0.4 (9.2)	0.798	0.889
Δ Triglycerides (mg/dL)	-2.0 (32.8)	-13.2 (37.4)	0.091	0.064
Δ HDLc (mg/dL) *	-0.8 (7.7)	-1.7 (7.6)	0.514	0.571
Δ TG/HDL	-0.01 (0.76)	-0.24 (1.00)	0.176	0.231
Δ LDLc (mg/dL)	-3.8 (13.0)	-11.9 (15.1)	**0.003**	**0.001**
Δ Insulin (IU/mL)	-1.2 (4.6)	-1.1 (5.0)	0.942	0.488
Δ Glucose (mg/dL)	0.1 (4.9)	-0.6 (5.3)	0.453	0.462
Δ HOMA	-0.25 (1.05)	-0.24 (1.24)	0.978	0.548
Δ Gamma-GT (U/L)	-0.2 (3.1)	-1.9 (3.9)	**0.010**	0.056
Δ VO_{2peak} (ml/kg/min) *	2.9 (6.8)	5.1 (5.3)	0.075	0.688
Δ 20msrt (laps) *	6 (10)	9 (10)	0.159	0.307
Δ Handgrip strength (kg/body weight) *	0.04 (0.04)	0.04 (0.06)	0.770	0.922
Δ Standing broad jump (cm) *	7.0 (18.2)	13.0 (13.8)	0.056	0.121



Supplemental Figure 3. Effect size (d-Cohen and 95 %CI) and differences in the change in percentage hepatic fat, body mass and composition, cardiometabolic and diabetes risk factors between the control group (children participating in the family based lifestyle and psycho-educational program) and the intensive group (children participating in the multicomponent intervention including lifestyle and psycho-educational program and exercise training). Differences between the control and the intensive intervention groups in terms of the changes in primary and secondary outcomes were examined on an intention to treat basis ANOVA (unadjusted P) and ANCOVA adjusting for baseline values (adjusted P). Changes were calculated as post-intervention minus pre-intervention values. Data are means (standard deviation). *Negative Cohen's d values obtained for the differences in the changes in adiposity and cardiometabolic and diabetes risk between the two groups (in favor of the intensive group) were multiplied by -1 for illustrative purposes. HDL: high density lipoprotein; HOMA: homeostasis model assessment; TG/HDL: triglycerides to high density lipoprotein ratio; LDL: low density lipoprotein; GGT: gamma glutamyl transferase; VO_2peak: peak oxygen consumption in the treadmill test; 20msrt: performance in the 20 meters shuttle run test.

Supplemental Table 1. Percentage hepatic fat, body composition, cardiometabolic and diabetes risk factors, and physical fitness before (Pre) and after (Post) participation in the family-based lifestyle and psycho-educational intervention program (control group, CI) or the plus exercise training (intensive group, II) in children with overweight/obesity (*intention-to-treat* analysis).

	Control group (CI)						Intensive group (II)						CI *vs*. II	
		Pre		Post		P		Pre		Post		P	P_{pre}	P_{post}
	N	Mean	SD	Mean	SD		N	Mean	SD	Mean	SD			
Primary outcome														
Hepatic fat (%)*	57	5.3	2.9	5.2	2.6	0.901	59	6.0	4.8	4.8	3.4	**0.001**	0.346	0.303
Secondary outcomes														
Body composition														
Body mass (kg)	57	53.8	9.7	54.0	9.5	0.716	59	55.6	11.4	55.0	11.1	0.211	0.361	0.613
Body mass index (kg/m^2)	57	25.2	2.8	24.5	3.1	**<0.001**	59	25.8	3.7	24.5	3.5	**<0.001**	0.355	0.976
Fat mass index (kg/m^2)	57	9.8	2.1	9.0	2.3	**<0.001**	59	10.5	2.6	9.3	2.4	**<0.001**	0.124	0.464
Lean mass index (kg/m^2)	57	14.7	1.2	14.6	1.3	0.134	59	14.5	1.3	14.4	1.2	0.380	0.289	0.508
Abdominal fat (kg)	57	5.7	2.1	5.4	2.1	**0.003**	59	6.5	2.7	5.9	2.3	**<0.001**	0.085	0.260
Cardiometabolic and diabetes risk factors														
SBP (mm Hg)	57	96.8	10.3	97.3	10. 3	0.799	59	96.1	10.3	95.7	11.3	0.841	0.681	0.453
DBP (mm Hg)	57	62.7	7.5	61.7	6.5	0.329	59	61.2	9.0	63.0	7.6	0.083	0.334	0.327
MAP (mm Hg)	57	85.5	8.2	85.4	7.8	0.976	59	84.4	9.0	84.8	8.8	0.733	0.522	0.707
Triglycerides (mg/dL)*	57	80.9	37.9	78.9	27.3	0.648	58	85.0	40.7	71.8	28.5	**0.010**	0.577	0.166
HDLc (mg/dL)	57	50.4	10.6	49.6	10.0	0.431	58	51.1	12.0	49.4	11.9	0.089	0.741	0.841
TG/HDLc	56	1.71	0.95	1.69	0.77	0.893	58	1.84	1.16	1.60	0.89	0.074	0.507	0.516
LDLc (mg/dL)	57	104.4	25.4	100.6	24.0	**0.030**	58	104.4	22.5	92.5	18.3	**<0.001**	0.998	**0.035**
Insulin (IU/mL)*	57	11.7	4.8	10.5	4.3	0.054	58	12.6	5.0	11.5	5.1	0.090	0.323	0.306
Glucose (mg/dL)	56	85.3	5.5	85.4	5.3	0.897	58	85.5	5.3	84.9	6.2	0.364	0.860	0.534
HOMA*	56	2.48	1.08	2.23	1.00	0.086	58	2.69	1.16	2.45	1.28	0.148	0.305	0.343
Gamma-GT (U/L)	57	15.5	4.0	15.4	3.9	0.677	56	17.1	5.2	15.2	4.3	**0.001**	0.070	0.618
Physical fitness														
VO_2 peak treadmill test (ml/kg/min)	44	35.5	6.4	38.4	6.7	**0.008**	51	31.9	5.1	37.0	5.6	**<0.001**	**0.003**	0.184
Endtime treadmill test (sg)	44	597	153	663	164	**0.004**	51	549	143	673	141	**<0.001**	0.116	0.759
20msrt (laps)	55	23	12	29	11	**0.001**	56	20	12	29	15	**<0.001**	0.152	0.975
Handgrip strength (kg/body mass)	56	0.34	0.06	0.38	0.07	**<0.001**	59	0.32	0.06	0.36	0.07	**<0.001**	0.173	0.348
Standing broad jump (cm)	55	103.5	15.7	110.5	23.1	**0.008**	59	96.6	18.8	109.7	20.1	**<0.001**	0.100	0.794

SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure calculated as diastolic pressure + [0.333 × (systolic blood pressure - diastolic pressure)]; HDLc: high density lipoprotein cholesterol; LDLc: low density lipoprotein cholesterol; TG/HDL: triglycerides to high density lipoprotein cholesterol ratio; HOMA: homeostasis model assessment; Gamma-GT: gamma-glutamyl transferase. 20msrt: 20 meters shuttle run test.

*Analyzed with log (ln) transformed values, but non-transformed data are shown in table. P indicates statistical differences between Pre and Post values (paired Student t test). P_{pre} indicates statistical differences in baseline values (Student t test). P_{post} indicates statistical differences in Post-intervention values between the control and the intensive intervention groups (Student t test).

Supplemental Table 2. Associations of changes in physical fitness with changes in percentage hepatic fat in children with overweight/obesity after their participation in the family-based lifestyle and psycho-educational intervention program (control group, CI) and the same plus exercise training (intensive group, II) (*per protocol* analyses).

	Δ Percentage hepatic fat									
	Control group (CI)					Intensive group (II)				
	Unadjusted Model			Adjusted Model*		Unadjusted Model			Adjusted Model*	
	N	β	P	β	P	N	β	P	N β	P
ΔVO$_2$ peak treadmill test (ml/kg/min)	41	-0.002	0.988	-0.007	0.972	44	0.061	0.697	-0.057	0.696
Δ 20msrt (laps)	44	0.098	0.541	-0.012	0.949	47	-0.359	**0.023**	-0.291	**0.006**
Δ Handgrip strength (kg/body mass)	52	-0.294	0.057	-0.270	0.064	49	0.152	0.318	-0.069	0.589
Δ Standing broad jump (cm)	46	0.050	0.748	-0.041	0.796	46	0.007	0.964	-0.135	0.272

*Regression analyses were adjusted with baseline physical fitness and percentage hepatic fat. β: unstandardized regression coefficient. Δ: Changes calculated as Post intervention-Pre intervention values.

Supplemental Table 3. Associations of changes in physical fitness with changes in percentage hepatic fat in children with overweight/obesity after their participation in the family-based lifestyle and psycho-educational intervention program (control group, CI) and the same plus exercise training (intensive group, II) (*intention-to-treat* analyses).

	Δ Percentage hepatic fat									
	Control group (CI)					Intensive group (II)				
	Unadjusted Model			Adjusted Model*		Unadjusted Model			Adjusted Model*	
	N	β	P	β	P	N	β	P	N	β	P
ΔVO_2 peak treadmill test (ml/kg/min)	44	-0.011	0.943	0.002	0.991	51	0.024	0.869	-0.038	0.734
Δ 20msrt (laps)	55	0.049	0.727	-0.056	0.697	56	-0.219	0.122	-0.180	0.051
Δ Handgrip strength (kg/body mass)	56	-0.267	0.048	-0.203	0.107	59	0.174	0.187	-0.004	0.968
Δ Standing broad jump (cm)	55	0.058	0.678	-0.062	0.620	59	0.036	0.785	-0.077	0.417

*Regression analyses were adjusted with baseline physical fitness and baseline percentage hepatic fat. β: unstandardized regression coefficient. Δ: Changes calculated as Post intervention-Pre intervention values.

Supplemental Table 4. Main lifestyle and psychological variables before (Pre) and after (Post) participation in the family-based lifestyle and psycho-educational intervention program (control group, CI), and the same plus exercise training (intensive group, II) in children with overweight/obesity (*per protocol* analysis).

	Control group (CI)				Intensive group (II)				CI vs. II		
	N	Pre	Post	P	N	Pre	Post	P	P_{pre}	P_{post}	$P_{group*time}$
Dietary and physical activity outcomes											
Energy intake (kcal/day)*	53	1828 (428)	1695 (425)	**0.039**	49	1785 (408)	1595 (332)	**0.007**	0.625	0.165	0.641
Fat intake (g/day)*	53	81 (26)	72 (27)	**0.033**	49	81 (26)	65 (21)	**<0.001**	0.929	0.193	0.224
Fruits and vegetables (g/day)*	53	239 (142)	343 (210)	**0.001**	49	237 (189)	314 (216)	**0.034**	0.590	0.283	0.402
Sugar intake (g/day)*	53	88 (32)	79 (26)	0.061	49	81 (30)	72 (25)	0.055	0.194	0.118	0.953
Sugar-sweetened beverages (g/day)*	53	80 (144)	50 (92)	0.179	49	57 (90)	25 (84)	0.073	0.392	0.097	0.962
Physical activity (counts/min)	47	3689 (697)	3575 (827)	0.126	35	3536 (620)	3615 (699)	0.370	0.606	0.825	0.394
MVPA (min/day)	47	92 (26)	89 (29)	0.392	35	88 (22)	90 (26)	0.618	0.930	0.929	0.348
Sedentary time (min/day)	47	515 (63)	517 (76)	0.744	35	531 (78)	496 (76)	**0.026**	0.313	0.212	**0.018**
Sleep time (min/day)	50	464 (31)	458 (44)	0.259	43	460 (45)	457 (30)	0.561	0.710	0.882	0.697
Psychological outcomes											
Self-concept											
Academic self-concept	37	8.3 (1.9)	8.0 (1.7)	0.316	37	8.1 (1.0)	7.9 (1.7)	0.300	0.762	0.717	0.980
Social self-concept	37	7.8 (1.5)	8.1 (1.5)	0.202	38	7.9 (1.4)	7.8 (1.7)	0.902	0.812	0.381	0.284
Emotional self-concept	37	5.9 (2.1)	6.8 (2.1)	**0.032**	38	5.1 (2.1)	5.9 (2.4)	**0.036**	0.168	0.122	0.838
Family self-concept	37	9.3 (1.0)	9.4 (0.7)	0.325	38	8.9 (1.4)	9.2 (1.1)	0.150	0.138	0.273	0.497
Physical self-concept	37	6.6 (1.9)	7.2 (2.1)	**0.036**	38	5.8 (2.0)	6.4 (2.3)	0.089	0.068	0.126	0.893
Total Depression	35	10.3 (6.6)	7.9 (4.6)	**0.008**	33	6.2 (3.8)	5.6 (3.1)	0.071	0.917	0.489	0.601
Dysphoria	36	4.0 (3.4)	2.7 (2.4)	**0.004**	33	4.1 (3.6)	3.1 (3.0)	0.056	0.919	0.521	0.638
Negative Self-Esteem	35	6.2 (3.7)	5.2 (2.7)	0.056	37	6.2 (3.8)	5.6 (3.1)	0.223	0.892	0.459	0.585
Total Stress	34	6.1 (3.3)	5.2 (3.1)	0.073	31	6.2 (4.3)	6.0 (4.0)	0.629	0.923	0.732	0.343
Health problems	36	2.4 (1.8)	2.0 (1.7)	0.094	34	2.8 (1.9)	2.2 (1.9)	0.063	0.557	0.614	0.829
School stress	36	2.0 (1.5)	1.9 (1.5)	0.906	34	1.9 (1.6)	2.3 (1.5)	0.276	0.888	0.764	0.335
Family stress	36	1.9 (1.3)	1.4 (1.0)	**0.017**	34	1.5 (1.5)	1.5 (1.4)	0.891	0.319	0.722	0.130
Trait-Anxiety	35	34.0 (7.6)	31.9 (6.3)	**0.030**	36	34.5 (7.8)	32.8 (8.7)	0.083	0.481	0.746	0.786

*Analyzed with logarithmically transformed data.

P indicates statistical differences between Pre and Post values (paired Student t test). P_{pre} indicates statistical differences in baseline values between the control and the intensive intervention groups (Student t test); P_{post} indicates statistical differences in Post-intervention values between the control and the intensive intervention groups (Student t test). $P_{group*time}$ indicates statistical differences between groups in changes (before and after values) (ANOVA).

3.5. Study V

Status: submitted.

Prevalence of responders for hepatic fat, overall and abdominal adiposity and gamma-glutamyl transferase in response to a lifestyle intervention with or without supervised exercise in children with overweight/obesity: secondary analyses of the EFIGRO randomized controlled trial

María Medrano[a,b], Lide Arenaza[a,b], Robinson Ramírez-Vélez[a,c], Francisco B. Ortega[d,e], Jonatan R. Ruiz[d,e], Idoia Labayen[a,b]

[a]Department of Health Sciences, Public University of Navarra, Pamplona, Spain

[b]Institute for Innovation & Sustainable Development in Food Chain (IS-FOOD), Public University of Navarra, Campus de Arrosadía, Pamplona, Spain.

[c]Department of Health Sciences, Public University of Navarra, Navarrabiomed, Pamplona, Navarra, Spain

[d]PROFITH ''PROmoting FITness and Health through physical activity'' Research Group, Department of Physical Education and Sport, Faculty of Sport Sciences, University of Granada, Granada, Spain.

[e]Department of Biosciences and Nutrition at NOVUM, Karolinska Institutet, Huddinge, Sweden.

Contact Author: María Medrano, Department of Health Sciences, Public University of Navarra, Avenida Barañaín s/n, 31007 Pamplona, Spain. Phone: +34 629558766. e-mail: maria.medrano@unavarra.es

ClinicalTrials.gov Identifier: NCT02258126

Running title: Responders for hepatic fat to an exercise program

Abstract

Background: Exercise and lifestyle interventions have been shown to reduce hepatic fat (HF) and adiposity in children. However, the interindividual response in HF after a lifestyle intervention with or without exercise in children is unknown.

Objectives: To compare interindividual variability for HF, overall and central adiposity, and gamma-glutamyl transferase (GGT) in children with overweight/obesity (N=102, 55% girls, 9-12 years) participating in a 22-week lifestyle intervention with (intensive intervention) or without exercise (control intervention).

Methods: HF (magnetic resonance imaging), weight, body and fat mass index (BMI and FMI), GGT, and cardiorespiratory fitness (CRF, 20 meters shuttle run test, laps) were assessed.

Results: The prevalence of responders for HF (54% *vs*. 34%), weight (27% *vs*. 11%), BMI (71% *vs*. 47%), FMI (90% *vs*. 60%), and GGT (69% *vs*. 39%) was higher in the intensive than in the control group (P<0.05). Responders for weight (16±3 *vs*. 6±2 laps) and BMI (11±2 *vs*. 3±4 laps) improved more CRF levels than non-responders in the intensive group (*Ps*<0.05).

Conclusions: The addition of exercise to a lifestyle intervention may increase the rates of responders for HF, adiposity, and GGT in children with overweight/obesity. Improvements in CRF may explain differences between responders and non-responders for weight and BMI.

Key words: interindividual variability, NAFLD, cardiorespiratory fitness, muscular strength, pediatric obesity.

Abbreviations

HF, hepatic fat

NAFLD, non-alcoholic fatty liver disease

GGT, gamma-glutamyl transferase

BMI, body mass index

FMI, fat mass index

CRF, cardiorespiratory fitness

MF, musculoskeletal fitness

DEXA, dual energy X-ray absorptiometry

20mSRT, 20m shuttle run test

SDs, standard deviations

ANCOVA, univariate lineal models

HIIT, high-intensity interval training

Introduction

Exercise and lifestyle interventions reduce fat mass[1] and hepatic fat (HF),[2] as well as the prevalence of non-alcoholic fatty liver disease (NAFLD)[2] in children and adolescents, and have been recommended as a treatment option for pediatric obesity[1,3] and hepatic steatosis.[4] Improvements of cardiorespiratory fitness (CRF) or/and musculoskeletal fitness (MF) after lifestyle5 or exercise[2] intervention programs are associated with greater reductions in both HF2 and fat mass.[2,5]

There is a large interindividual variability in the response to the same intervention;[6] whereas some participants improve in one or several outcomes after an intervention (responders), others do not change or even a worsen their response (non-responders) to the interventions.[6] However, this information is rarely provided, and the vast majority of published studies only report the results as overall mean values.[7] Interindividual variability exist after pharmacological,[8] nutritional,[9] or exercise[10–12] interventions. Occasionally, some studies have compared the prevalence of responders or non-responders between different exercise intervention programs[13] with the aim of knowing the most appropriate an effective design for the prevention/treatment of obesity or other obesity-related comorbidities. Several studies in adults provided data of the interindividual variability in variables such as body composition[11,12] or other health outcomes,[14] while there is scarce evidence in the pediatric population.[10,13,15] Our group has shown that the addition of supervised exercise to a family-based lifestyle education program resulted in significantly greater reduction of percentage HF (≈20%) in children with overweight/obesity.[16] Other studies also reported the overall mean change in the percentage HF[2] after exercise-based programs in youths. However, the interindividual variability on changes in HF, as well as the prevalence of responders/non-responders to lifestyle or exercise interventions in children is unknown.

Previous studies have identified genes,[17] metabolic or behavioral responses,[12] or diet and physical activity changes[11,14] as potential determinants of interindividual variability. However, little is known about the role of changes in physical fitness after an intervention on interindividual variability. Therefore, the aims of the present study were: (1) to compare the prevalence of responders regarding percentage HF, overall and abdominal adiposity, and GGT levels among children with overweight/obesity participating in a 22-week lifestyle intervention with or without supervised exercise and, (2) to analyze whether physical fitness may explain differences in the interindividual variability between responders and non-responders.

Methods

Study design and participants

The present study was conducted at the University of the Basque Country in Vitoria-Gasteiz (northern Spain), from September 2014 to June 2017. The Euskadi Clinical Research Ethics Committee approved the study protocol (PI2014045), which complies with the ethical guidelines of the Declaration of Helsinki (2013 revision). All parents/legal guardians gave their informed, written consent for their children to be included in the study; the children also gave their assent before enrolment.

The current study is a secondary analysis of the EFIGRO study (ClinicalTrials.gov ID: NCT02258126).[18] The main aim of the EFIGRO study was to analyze whether the addition of supervised exercise to a 22 weeks family-based lifestyle- and psycho-educational intervention program resulted in greater reduction of HF, fat mass, and cardiometabolic risk factors in children with overweight/obesity. The primary results of the EFIGRO study (i.e., overall mean changes after the intervention and differences between groups in HF, adiposity and cardiometabolic risk factors) have been previously

published. The study design and methodology of the EFIGRO study have been published elsewhere.[18] The design of the EIFIGRO project was conceived as a randomized clinical trial; however, it was not possible to perform the assignment to the control or the intensive intervention groups strictly at random. The research team decided not to exclude some children (and families) who did not have time availability to attend exercise sessions, and they were allocated in the control group for this reason (N=11). This decision was adopted because children/families were recruited and encouraged to participate in the study by their pediatricians arguing the probable health benefits associated to the program.

Children included in the study were between 8 to 12 years and had primary overweight or obesity according to the World Obesity Federation.[19] Additionally, at least one parent or caregiver should have participated in the lifestyle and psycho-educational program. Children whose medical condition or medication limited physical activity or could affect the results of the study were excluded from the study. Children who completed the intervention and whose adherence to the lifestyle and psycho-educational program was at least 50% of the sessions were included in the current analysis (N=102) (**Figure S1**).

Intervention

Children were assigned to a lifestyle-and psycho-educational program (control group) or to a lifestyle-and psycho-educational plus exercise program (intensive group). All the families from both groups (control and intensive) attended a lifestyle-and psycho-educational program composed by eleven sessions of 90 minutes (once every two weeks). In these sessions, experienced nutritionists and psychologists worked with families to improve their dietary habits and physical activity patterns as well as children's and parents' psychological well-being. Children of the intensive group, additionally

participated in a supervised exercise program that included aerobic games and strength exercises 3 days per week, 90 mins per session.[18]

All measurements were carried out before and after the intervention by the same trained researchers. Post-intervention measurements were assessed within three days following the last lifestyle-and psycho-educational or exercise session.

Hepatic fat, adiposity and GGT levels

Percentage HF was assessed by magnetic resonance imaging (Magnetom Avanto, Siemens Healthcare, Erlangen, Germany) as previously reported.[18] Weight (kg) and height (m) were measured in duplicate using standard procedures in light indoor clothes and without shoes. Total and abdominal fat were assessed by dual energy X-ray absorptiometry (DEXA, Hologic QDR 4500W; Hologic Inc., Waltham, MA). Thereafter, body mass index (BMI; weight (kg)/height (m^2)) and fat mass index (FMI; fat mass (kg)/height (m^2)) were calculated. Then, BMI z-score was calculated with the World Health Organization reference values.[20] Serum concentration of GGT was measured using standard protocols from blood samples collected by experienced nurses after an overnight fast. Details of blood extraction and collection methods have been published previously.[18]

Physical fitness

Cardiorespiratory fitness was estimated from the number of laps obtained in the 20m shuttle run test (20mSRT).[21] The handgrip test (kg) was employed to evaluate upper muscular strength of children. Both tests are part of the Alpha-fitness test battery, which was validated for assessing health-related physical fitness components in children of this age.[22]

Classification of responders and non-responders

The individual response to the control or to the intensive group was categorized based on the effect size (d-Cohen) into responders and non-responders for each outcome. The minimum change after the intervention required in each variable to be considered the participant as responders was an effect size (d-Cohen) ≥0.2. Thus, children were considered as non-responders when the change in the outcome after the intervention was <0.2. Other different methodological classification criteria have been employed in previous studies to define responders,[23] such as to achieve: 1) a clinical meaningful change, 2) any improvement (change) from baseline value,[24] or 3) a change above the technical error of the test.[10] However, we have selected the minimum change required according the effect size as definition of responders and non-responders because: 1) not all variables have a cut-off point that determine a meaningful clinical change in children, 2) a change above 0 does not consider other sources of variability such as the random variability,[23] and 3) the technical error of the test was not available for all the measurements in our study (i.e., measurements from external laboratory tests). Moreover, this method allow us to define responders and non-responders in a more similar and standard way for all the different variables. Participants were categorized as responders or non-responders for six different outcomes: weight, BMI, FMI, abdominal fat, HF and GGT levels. Likewise, the same children could be classified as responder for one of the variables, and as non-responder for others.

Statistical analysis

Data were analyzed to detect extreme values and then, were winsorized (i.e., FMI, GGT). The normality of data distribution was tested by Kurtosis. Transformation to their natural logarithms was used for variables non-normally distributed (i.e. HF). However, non-transformed data are presented in tables and figures. Changes (Δ) in physical fitness

were calculated as Post-intervention *minus* Pre-intervention values. Categorical variables were presented as absolute frequencies and percentages, whereas continuous variables are presented as means (standard deviations, SDs).

Baseline differences between control and intensive interventions were calculated using chi-square (categorical variables) and Student's t-tests (continuous variables). Chi-square tests were performed to analyze the difference in the prevalence of responders between the control and the intensive groups. The comparison of the likelihood of being non-responders for the different outcomes between intervention groups (control and intensive) was analyzed using logistic regression analysis.

Differences in physical fitness between responders and non-responders for the considered outcomes (i.e., weight, BMI, FMI, abdominal fat, HF and GGT levels) were examined by univariate lineal models (ANCOVA) adjusting for age, sex, baseline value and attendance to the program in each intervention group.

All the analyses were repeated after excluding those children who were not randomly assigned to an intervention group (N=11).

All the analyses were performed using the Statistical Package for the Social Sciences (SPSS, IBM, version 23.0 for WINDOWS; SPSS Inc., Chicago), and the level of significance was set at $\alpha = 0.05$.

Results

Flow diagram of participants through the study is provided in Figure S1. **Table 1** shows the baseline descriptive characteristics of the study sample according to the intervention group. There were no significant differences in any of the studies outcomes between groups.

Table 1. Baseline characteristics of children that completed the EFIGRO study participating in the lifestyle-and psycho-educational intervention (control group) or in the lifestyle-and psycho-educational *plus* exercise intervention (intensive group).

	Control group (N=53)	**Intensive group (N=49)**	***P***
Age (years)	10.6 (1.1)	10.5 (1.1)	0.691
Girls (N, %)	29, 55	27, 55	
Hepatic fat content (%)	5.2 (2.8)	5.6 (4.5)	0.836
Weight (kg)	53.4 (9.0)	55.9 (11.3)	0.224
Height (m)	1.45 (0.08)	1.47 (0.08)	0.350
Body mass index (kg/m^2)	25.1 (2.8)	25.7 (3.3)	0.325
Children with obesity (N, %)	29, 55	29, 59	
Body mass index z-score	2.5 (0.6)	2.6 (0.6)	0.613
Fat mass (%)	38.8 (4.7)	40.4 (4.4)	0.069
Fat mass index (kg/m^2)	9.8 (2.0)	10.3 (2.1)	0.184
Abdominal fat (kg)	5.7 (2.1)	6.4 (2.5)	0.151
GGT (U/L)	15 (4)	17 (5)	0.074
Physical fitness			
CRF (laps)	23 (12)	21 (12)	0.284
Handgrip strength (kg)	18 (4)	18 (4)	0.972

Values are means (standard deviation) unless otherwise indicated. *Abbreviations*: CRF: cardiorespiratory fitness; GGT: gamma-glutamyl transferase.

Missing data: hepatic fat content (N=1), fat mass and fat mass index (N=1), GGT (N=3), laps (N=4), handgrip strength (N=1). Reasons: it was not possible to perform the test (equipment problems, injuries), families did not attend the tests, or there were not enough valid days in accelerometer data collection or analysis. *P* values are baseline differences between control and intensive group.

Figure S2 shows the individual changes and the distribution of responders and non-responders in each intervention group ordered by their identification code. It can be observed that the same child could be classified as responder for some variables, but as non-responder for others (for example, the first participant of the control group was classified as BMI responder, but as non-responder for the rest of the variables). In the control group none participant was classified as responder for all the variables, whereas in the intensive group 6 children (12%) were responders for all the variables. Moreover, 12 children (23%) were non-responders for all the variables in the control group, whereas only 2 children (4%) were non-responders for all the variables in the intensive group.

The prevalence of responders was significantly higher in the intensive group compared with the control group for all the variables except for abdominal fat (**Figure 1**). The likelihood of being non-responder for HF (OR=2.294; CI-95%=1.015-5.184; *P*=0.046), BMI (OR=2.800; 95% CI, 1.231-6.367, *P*=0.014), FMI (OR=5.644; CI-95%=1.922-16.574; *P*=0.002) and GGT (OR=3.432; CI-95%=1.474-7.991; *P*=0.004) was higher in the control than intensive group.

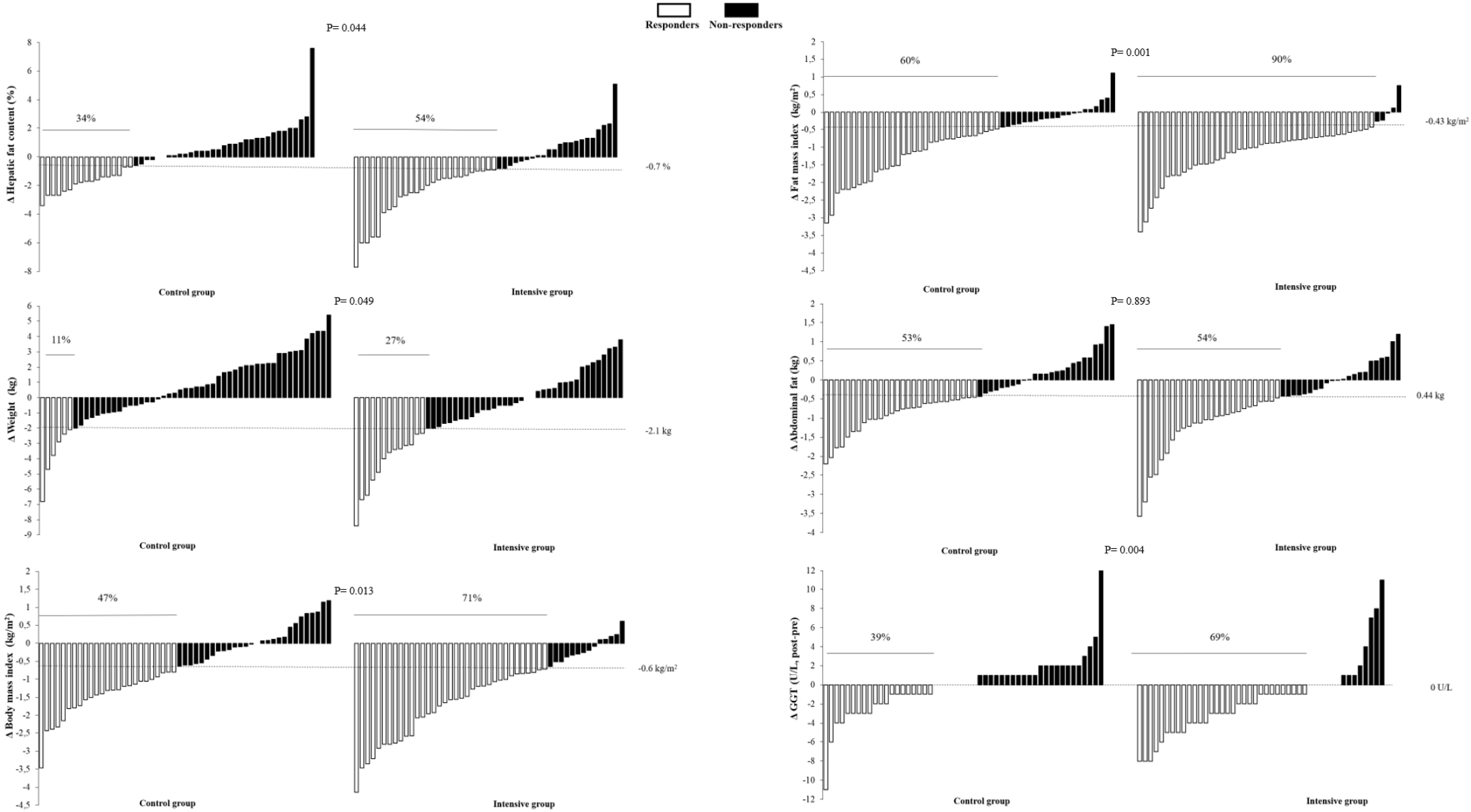


Figure 1: Prevalence of responders (d-Cohen>0.2) for hepatic fat content, weight, body mass index (BMI), fat mass index (FMI), abdominal fat and gamma-glutamyl transferase (GGT) in the lifestyle intervention group (control group) and lifestyle plus exercise intervention group (intensive group) after the EFIGRO intervention. The dotted line represents responder cut-off point (effect size=0.2) for each variable. The numbers represent the percentage of responders for each variable. P values indicate differences (chi-square) in the prevalence of responders between control and intensive groups. Δ: changes calculated as Post-intervention–Pre-intervention values.

Differences in the number of laps in the 20mSRT between responders and non-responders for the considered outcomes in each intervention group are depicted in **Figure 2**. In the intensive group, the increase in the number of laps after the intervention was higher in weight-responders (16±3 *vs*. 6±2 laps for responders and non-responders, $P<0.02$, respectively) and BMI-responders (11±2 *vs*. 3±4 laps for responders and non-responders, $P<0.05$, respectively) than in the non-responders. In the control group, only the BMI-responders increased more the number of laps than their non-responders peers (14±4 *vs*. 5±1 laps for responders and non-responders, $P<0.02$, respectively). There were no significant differences in the change of the number of laps between responders and non-responders for the rest of the outcomes (i.e., HF, FMI, abdominal fat, GGT), neither in the control, nor in the intensive group ($P<0.05$) (Figure 2). There were no significant differences in changes on the handgrip test at the end of the intervention between responders and non-responders in none of the variables in both groups (*Ps*>0.05, **Figure S3**).

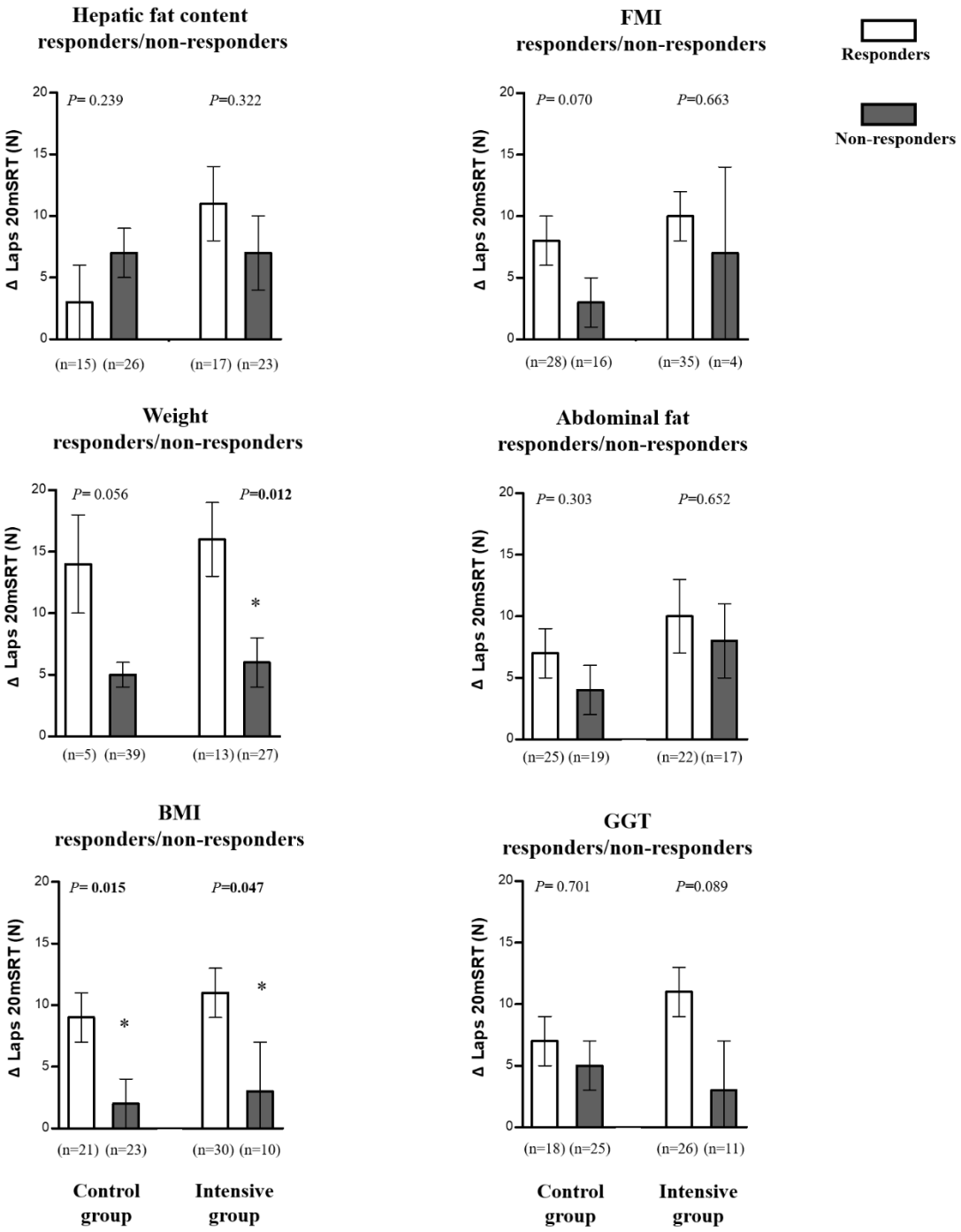


Figure 2: Differences in changes (Δ) in the number of laps in the 20mSRT between responders and non-responders for the considered outcomes (i.e., hepatic fat content, weight, BMI, FMI and GGT levels) in the lifestyle intervention group (control group) and lifestyle plus exercise intervention group (intensive group). ANCOVA was adjusted for age, sex, baseline value and attendance to the program. *Abbreviations*: 20mSRT: 20 meters shuttle run test; BMI: body mass index, FMI: fat mass index; GGT: gamma-glutamyl transferase. Δ: changes calculated as Post-intervention–Pre-intervention values.

In sensitivity analysis, after excluding those children who were not randomly assigned to an intervention group (N=11), we observed similar results except in the difference of the prevalence of responders for weight between groups that was attenuated and became non-significant (27% *vs*. 14% of responders in the intensive and the control group, respectively, *P*=0.137).

Discussion

The main findings of the present study were that (1) the number of responders for HF, weight, BMI, FMI, and GGT was significantly higher in the intensive than in the control group and, (2) weight and BMI responders increased more CRF than their non-responders peers. To our knowledge, this is the first study analyzing and comparing interindividual variability in HF, total and abdominal fat, and GGT after a lifestyle intervention including or not exercise in children with overweight/obesity. Hereby, the addition of supervised exercise to a lifestyle and psycho-education intervention program seems to reduce the number of children not achieving beneficial effects in HF, adiposity, and GGT after the intervention. These findings may have important implications in the design of intervention focused on the prevention/treatment of pediatric obesity and related comorbidities such as hepatic steatosis.

The most novel finding of the current study is that the prevalence of responders for percentage HF and GGT levels was significantly higher in the intensive group (54% and 68% of responders for HF and GGT, respectively) than in the control group (34% and 39% of responders for percentage HF and GGT levels, respectively). The lack of previous studies providing interindividual response to lifestyle intervention programs regarding HF or GGT hampers comparisons. Nevertheless, a recent published meta-analysis in children and adolescents showed that the prevalence of hepatic steatosis decreased[2] after an exercise intervention in combination with other lifestyle components.

For example, Koot et al.[25] observed a reduction of 19.4% in the prevalence of hepatic steatosis after a 6-months multidisciplinary intervention. Similarly, Damaso et al.[26] reported a significant reduction in the prevalence of hepatic steatosis from 33% to 10% after a one-year of aerobic and resistance exercise training plus a nutritional intervention. Of note that in the aforementioned studies, hepatic steatosis was measured by ultrasound, which is a non-accurate method to measure percentage HF, and that the studies were performed in adolescents. Metabolic effects of exercise, such as the stimulation of fatty acid oxidation in the liver, the reduction of lipogenic enzymes, or insulin resistance, have been proposed as potential mechanisms to explain the beneficial effects of exercise in the reversion of hepatic steatosis.[27] This is clearly an area in need of more research.

Our results show that the number of responders for weight (27% *vs*. 11%), BMI (71% *vs*. 47%), and FMI (90% *vs*. 47%) was higher in the group of children who performed exercise in addition to the family-based lifestyle education program. One previous report in children and adolescents examining the interindividual variability after a multidisciplinary lifestyle intervention including exercise[15] reported a lower prevalence of BMI responders than in our group of children participating in the program that included exercise (50% *vs*. 71%). This difference may be explained by the different criteria used to define responders, or by differences in the design of the program. In another previous study, not designed to evaluate interindividual variability, the authors found a similar number of participants reducing their weight and BMI after two lifestyle interventions with and without exercise their both lifestyle interventions (with or without exercise); unfortunately, they did not provide statistical analyses.[24] In any case, the number of responders for weight and for BMI was higher after their dietary advice or dietary advice plus exercise programs than at the end of our control or intensive programs. However, our cut-off point to define the responsiveness is much strict than in the study of

Schwingshandl et al.[24] In this regard, Schwingshandl et al.[24] considered as "responders" those participants reducing their body weight or BMI, while our cut-off point is a minimum effect size of 0.2.

We observed that the risk of being non-responders for BMI, FMI, HF and GGT was higher in the control than in the intensive group. To the best of our knowledge, there are no previous studies analysing the likelihood of being non-responder for adiposity, HF, or GGT in children or adolescents after different lifestyle or exercise interventions which hampers comparisons. The risk of being non-responder for adiposity related outcomes has been previously reported in some studies in adults.[28,29] However, they compared the likelihood of being non-responder between different clinical status: women with high or low insulin resistance,[28] and women with normotension or pre-hypertension.[29]

Several mechanisms besides of genetic factors have been proposed to explain either interindividual variability or responsiveness to lifestyle and exercise intervention programs. Among them, the adherence to the programs[30] and behavioral and metabolic compensatory mechanisms.[12] Previous studies have observed that several individuals reduced their resting energy expenditure, increased energy intake, lowered physical activity levels or reduced fat oxidation rate in response to the exercise-associated increase of energy expenditure or energy intake restriction programs.[11,12,31,32] We did not measure energy and substrate metabolism in our study; nevertheless, as we did not observe any significant difference in the adherence to the lifestyle education program or in changes on dietary intake variables and physical activity levels between the two groups at the end of the intervention (data not shown), the higher number of responders in the intensive than in the control group may be explained by the addition of supervised exercise to the program.

The improvement of CRF has been proposed as a potential mediator of metabolic effects of exercise on adiposity and related comorbidities, such as hepatic steatosis.[2,33,34] In an experiment performed in rats, the level of CRF was associated with hepatic steatosis.[35] Rats with lower levels of CRF had a reduced hepatic mitochondrial oxidative capacity and a higher liver triglycerides content.[35] Therefore, we hypothesize that the improvement of CRF could be a mechanism that may explain the reduction of percentage hepatic fat and, similarly, the reduction of the adiposity in other tissues. Our data showed that responders for weight and BMI increased more their CRF than non-responders, which is in line with this hypothesis. There are no previous studies comparing physical fitness between responders and non-responders in children; anyway, in adults, De Luis et al.[14] observed that only weight responders, but not non-responders, increased their CRF after a diet plus exercise intervention. In contrast, a previous analysis that examined the prevalence of non-responders for different metabolic outcomes (i.e. fasting insulin, triglycerides, blood pressure) from various studies, did not observe greater improvements of CRF in responders than in non-responders.[36] Thus, further research is needed to elucidate whether responders for a certain outcome are those who experience greater improvements in CRF.

The current study presents some limitations. The main limitation, which is common to most reports in this field, is related to the vast variety of definitions of the response to an intervention in order to discern the systematic change and the interindividual variability from the intra-individual or the random variability.[23,37,38] Thus, these results must be interpreted with caution, and it would be important to search a consensus in the definition of responders and non-responders. On the other hand, genetic factors or energy metabolism that could be determinants of the interindividual variability were not measured in the present study. The strengths of the present study include the use

of magnetic resonance imaging for HF quantification, the homogeneity of the studied sample that includes children with a narrow age range and all of them with overweight or obesity status, as well as the strict control of the intervention and measurements. The inclusion of a non-exercise control group allow us to determine the exercise-induced improvements for the different parameters. Finally, this study constitutes the first study focused on a comparison between interventions groups of the interindividual response in after a 22-week lifestyle intervention including or not exercise in children with overweight/obesity.

In conclusion, the addition of supervised exercise to a family-based lifestyle intervention may increase the rates of responders for fat mass, percentage hepatic fat and gamma-GT levels in children with overweight/obesity. Improvements in CRF may explain differences between responders and non-responders for weight and BMI after a multidisciplinary lifestyle intervention program including supervised exercise. Further research exploring interindividual variability is needed in order to know the more effective intervention in the treatment and prevention of hepatic steatosis and obesity in children.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgements

This work was conducted under the umbrella of the EFIGRO study (ClinicalTrials.gov ID: NCT02258126) which was supported by the Spanish Ministry of Health, “Fondo de Investigación Sanitaria del Instituto de Salud Carlos III”, “Fondos de la Unión Europea (FEDER), una manera de hacer Europa” (PI13/01335) and by the University of the Basque Country (GIU14/21); by the University of Granada Plan Propio

de Investigación 2016 (Excellence actions: Unit of Excellence on Exercise and Health [UCEES]), by the Junta de Andalucía, Consejería de Conocimiento, Investigación y Universidades, and FEDER, ref. SOMM17/6107/UGR, by Redes Temáticas de Investigación Cooperativa RETIC grant Red SAMID RD16/0022, and the EXERNET Research Network on Exercise and Health in Special Populations (DEP2005-00046/ACTI). MM is supported by grant from the Spanish Ministry of Education, Culture and Sport (FPU14/03329) and EST17/00210. LA is supported by a grant from Education, Linguistic Policy and Culture Department of the Government of the Basque Country (PRE_2016_1_0057).

We would thank all the children and parents/caregivers for participating in the study. We acknowledge the paediatricians for their participation in the recruitment, the members involved in the assessments for their efforts and to Siemens Medical Systems for supplying the software to quantify hepatic fat content, and to the pre- and post-graduate students for their collaboration in the exercise training sessions and in the educational program.

Author contribution to manuscript

IL designed the study. MM analyzed the data and takes full responsibility for the integrity of the data analyses. MM and IL drafted the manuscript. MM generated the figures. MM, LA and IL collected the data. All the authors participated in the interpretation of the results, critically revised the manuscript for important intellectual content and approved the final version of the submitted manuscript.

References

1. Stoner L, Rowlands D, Morrison A, et al. Efficacy of Exercise Intervention for Weight Loss in Overweight and Obese Adolescents: Meta-Analysis and Implications. *Sports Med*. 2016; 46(11): 1737-1751.

2. Medrano M, Cadenas-Sanchez C, Álvarez-Bueno C, et al. Evidence-Based Exercise Recommendations to Reduce Hepatic Fat Content in Youth- a Systematic Review and Meta-Analysis. *Prog Cardiovasc Dis*. 2018; 61(2): 222-231.

3. Mead E, Brown T, Rees K, et al. Diet, physical activity and behavioural interventions for the treatment of overweight or obese children from the age of 6 to 11 years. *Cochrane Database Syst Rev*. 2017; 6: CD012651. doi:10.1002/14651858.CD012651.

4. Vos MB, Abrams SH, Barlow SE, et al. NASPGHAN Clinical Practice Guideline for the Diagnosis and Treatment of Nonalcoholic Fatty Liver Disease in Children: Recommendations from the Expert Committee on NAFLD (ECON) and the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr*. 2017; 64(2): 319-334.

5. Kantartzis K, Thamer C, Peter A, et al. High cardiorespiratory fitness is an independent predictor of the reduction in liver fat during a lifestyle intervention in non-alcoholic fatty liver disease. *Gut*. 2009; 58(9): 1281-1288.

6. Bouchard C, Rankinen T. Individual differences in response to regular physical activity. *Med Sci Sports Exerc*. 2001; 33(6 Suppl): S446-51; discussion S452-3.

7. Buford TW, Roberts MD, Church TS. Toward exercise as personalized medicine. *Sports Med*. 2013; 43(3): 157-165.

8. Turner RM, Park BK, Pirmohamed M. Parsing interindividual drug variability: an emerging role for systems pharmacology. *Wiley Interdiscip Rev Syst Biol Med*. 2015; 7(4): 221-241.

9. Healey GR, Murphy R, Brough L, Butts CA, Coad J. Interindividual variability in gut microbiota and host response to dietary interventions. *Nutr Rev*. 2017; 75(12): 1059-1080.

10. Álvarez C, Ramírez-campillo R, Ramírez-vélez R. Effects of 6-Weeks High-Intensity Interval Training in Schoolchildren with Insulin Resistance : Influence of Biological Maturation on Metabolic , Body Composition , Cardiovascular and Performance Non-responses. *Front Physiol*. 2017; 8: 444. doi:10.3389/fphys.2017.00444

11. Hammond BP, Stotz PJ, Brennan AM, Day AG, Ross R. Individual Variability in Waist Circumference and Body Weight in Response to Exercise. *Med Sci Sports Exerc*. 2019; 51(2): 315-322.

12. King NA, Hopkins M, Caudwell P, Stubbs RJ, Blundell JE. Individual variability following 12 weeks of supervised exercise: identification and characterization of compensation for exercise-induced weight loss. *Int J Obes (Lond)*. 2008; 32(1): 177-184.

13. Álvarez C, Martínez C, Izquierdo M. Metabolic effects of resistance or high-intensity interval training among glycemic control-nonresponsive children with insulin resistance. *Nature*. 2017; 42(1): 79-87.

14. De Luis DA, Aller R, Izaola O, Gonzalez-Sagrado M, Conde R, Perez-Castrillon JL. Effects of lifestyle modification on adipocytokine levels in obese patients. *Eur Rev Med Pharmacol Sci*. 2008; 12(1): 33-39.

15. Kinder M, Lotze M, Davids S, et al. Functional imaging in obese children responding to long-term sports therapy. *Behav Brain Res*. 2014; 272: 25-31.

16. Labayen I, Medrano M, Arenaza L, et al. Effects of exercise in addition to a family-based lifestyle intervention program on hepatic fat in children with overweight. *Diabetes Care*. 2019; Epub ahead of print. doi: 10.2337/dc19-0351.

17. Erskine RM, Williams AG, Jones DA, Stewart CE, Degens H. Do PTK2 gene polymorphisms contribute to the interindividual variability in muscle strength and the response to resistance training? A preliminary report. *J Appl Physiol*. 2012; 112(8): 1329-1334.

18. Medrano M, Maiz E, Maldonado-Martín S, et al. The effect of a multidisciplinary intervention program on hepatic adiposity in overweight-obese children: Protocol of the EFIGRO study. *Contemp Clin Trials*. 2015; 45(Pt B): 346-355.

19. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatr Obes*. 2012; 7(4): 284-294.

20. Growth reference 5-19 years. WHO - World Health Organization. https://www.who.int/growthref/en/. Accedded April 10, 2019.

21. Léger L a, Mercier D, Gadoury C, Lambert J. The multistage 20 metre shuttle run test for aerobic fitness. *J Sports Sci Med*. 1988; 6(2): 93-101.

22. Ruiz JR, Castro-Pinero J, Espana-Romero V, et al. Field-based fitness assessment in young people: the ALPHA health-related fitness test battery for children and adolescents. *Br J Sports Med*. 2011; 45(6): 518-524.

23. Hecksteden A, Kraushaar J, Scharhag-Rosenberger F, Theisen D, Senn S, Meyer T. Individual response to exercise training - a statistical perspective. *J Appl Physiol*. 2015; 118(12): 1450-1459.

24. Schwingshandl J, Sudi K, Eibl B, Wallner S, Borkenstein M. Effect of an individualised training programme during weight reduction on body composition: a randomised trial. *Arch Dis Child*. 1999; 81(5): 426-428.

25. Koot BGP, van der Baan-Slootweg OH, Tamminga-Smeulders CLJ, et al. Lifestyle intervention for non-alcoholic fatty liver disease: prospective cohort study of its efficacy and factors related to improvement. *Arch Dis Child*. 2011; 96(7): 669-674.

26. Dâmaso AR, de Piano A, da Silveira Campos RM, et al. Multidisciplinary approach to the treatment of obese adolescents: effects on cardiovascular risk factors, inflammatory profile, and neuroendocrine regulation of energy balance. *Int J Endocrinol*. 2013; 12(5): 237-241.

27. Ordonez R, Carbajo-Pescador S, Mauriz JL, Gonzalez-Gallego J. Understanding nutritional interventions and physical exercise in non-alcoholic fatty liver disease. *Curr Mol Med*. 2015; 15(1): 3-26.

28. Álvarez C, Ramírez-Campillo R, Ramírez-Vélez R. Prevalence of Non-responders for Glucose Control Markers after 10 Weeks of High-Intensity Interval Training in Adult Women with Higher and Lower Insulin Resistance. *Front Physiol*. 2017; 8: 479. doi:10.3389/fphys.2017.00479.

29. Álvarez C, Ramírez-campillo R, Cristi-montero C, Low DA. Prevalence of Non-responders for Blood Pressure and Cardiometabolic Risk Factors Among Prehypertensive Women After Long-Term High-Intensity Interval Training. *Front Physiol*. 2018; 9: 1443. doi:10.3389/fphys.2018.01443.

30. Solomon TPJ. Sources of inter-individual variability in the therapeutic response of blood glucose control to exercise in type 2 diabetes: Going beyond exercise dose. *Front Physiol*. 2018; 9: 896. doi:10.3389/fphys.2018.00896.

31. Ruiz JR, Ortega FB, Rodriguez G, Alkorta P, Labayen I. Validity of resting energy expenditure predictive equations before and after an energy-restricted diet intervention in obese women. *PLoS One*. 2011; 6(9): e23759. doi:10.1371/journal.pone.0023759.

32. Labayen I, Ortega FB, Ruiz JR, Lasa A, Simon E, Margareto J. Role of baseline leptin and ghrelin levels on body weight and fat mass changes after an energy-restricted diet intervention in obese women: effects on energy metabolism. *J Clin Endocrinol Metab*. 2011; 96(6): E996-1000. doi:10.1210/jc.2010-3006.

33. Senechal M, Rempel M, Duhamel TA, et al. Fitness is a determinant of the metabolic response to endurance training in adolescents at risk of type 2 diabetes mellitus. *Obesity*. 2015; 23(4): 823-832.

34. Borel AL, Nazare JA, Baillot A, et al. Cardiometabolic risk improvement in response to a 3-yr lifestyle modification program in men: contribution of improved cardiorespiratory fitness vs. weight loss. *Am J Physiol Endocrinol Metab*. 2017; 312(4): E273-E281. doi:10.1152/ajpendo.00278.2016

35. Thyfault JP, Rector RS, Uptergrove GM, et al. Rats selectively bred for low aerobic capacity have reduced hepatic mitochondrial oxidative capacity and susceptibility to hepatic steatosis and injury. *J Appl Physiol (1985).* 2009; 587(Pt 8): 1805-1816.

36. Bouchard C, Blair SN, Church TS, et al. Adverse Metabolic Response to Regular Exercise: Is It a Rare or Common Occurrence? *PLoS One*. 2012; 7(5): e37887. doi:10.1371/journal.pone.0037887.

37. Voisin S, Jacques M, Lucia A, Bishop DJ, Eynon N. Statistical Considerations for Exercise Protocols Aimed at Measuring Trainability. *Exerc Sport Sci Rev*. 2019; 47(1): 37-45.

38. Pickering C, Kiely J. Do Non - Responders to Exercise Exist — and If So, What Should We Do About Them? *Sports Med*. 2019; 49(1): 1-7. doi:10.1007/s40279-018-01041-1.

Supplemental Material

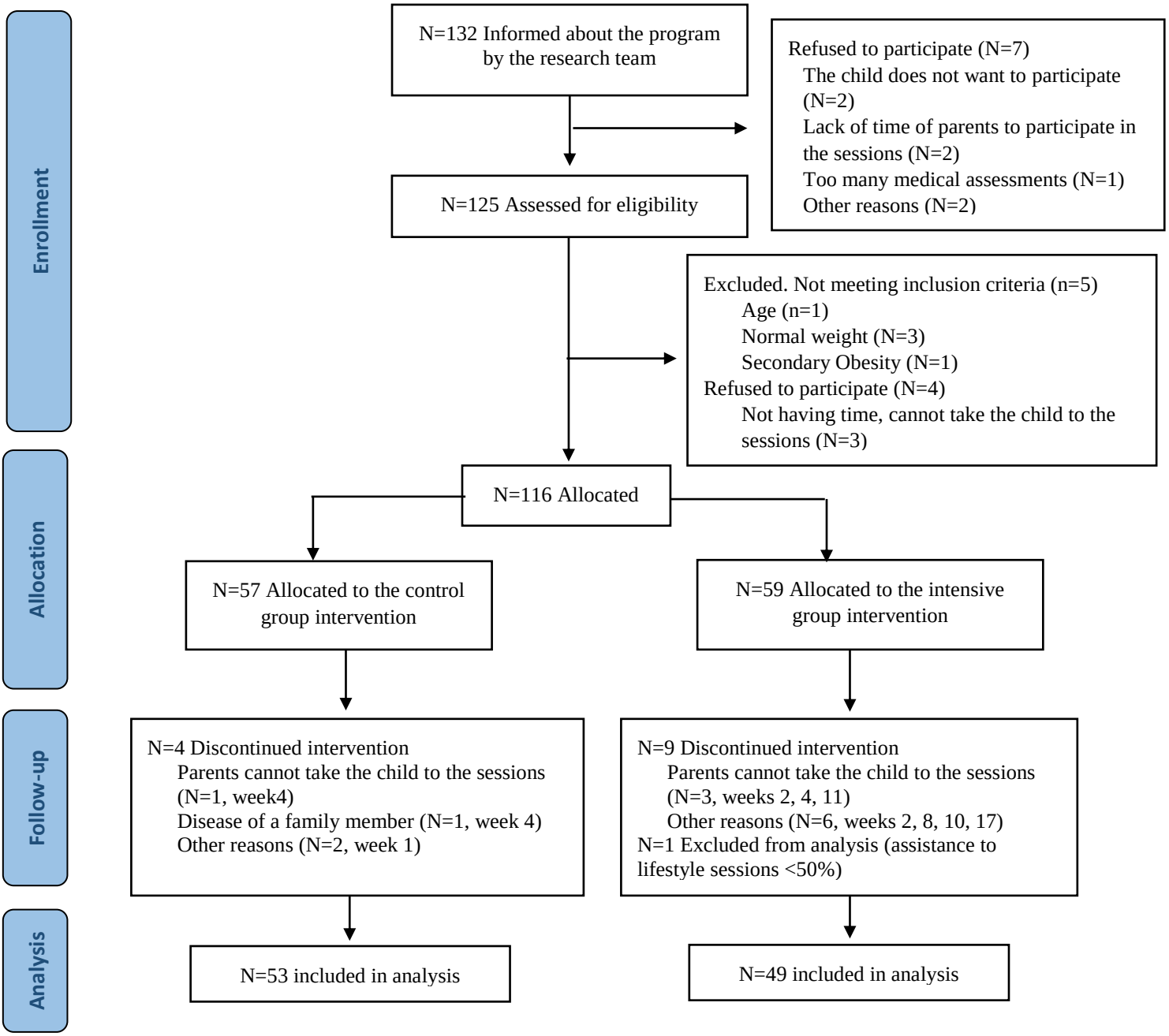


Figure S1. CONSORT flow diagram of the EFIGRO study.

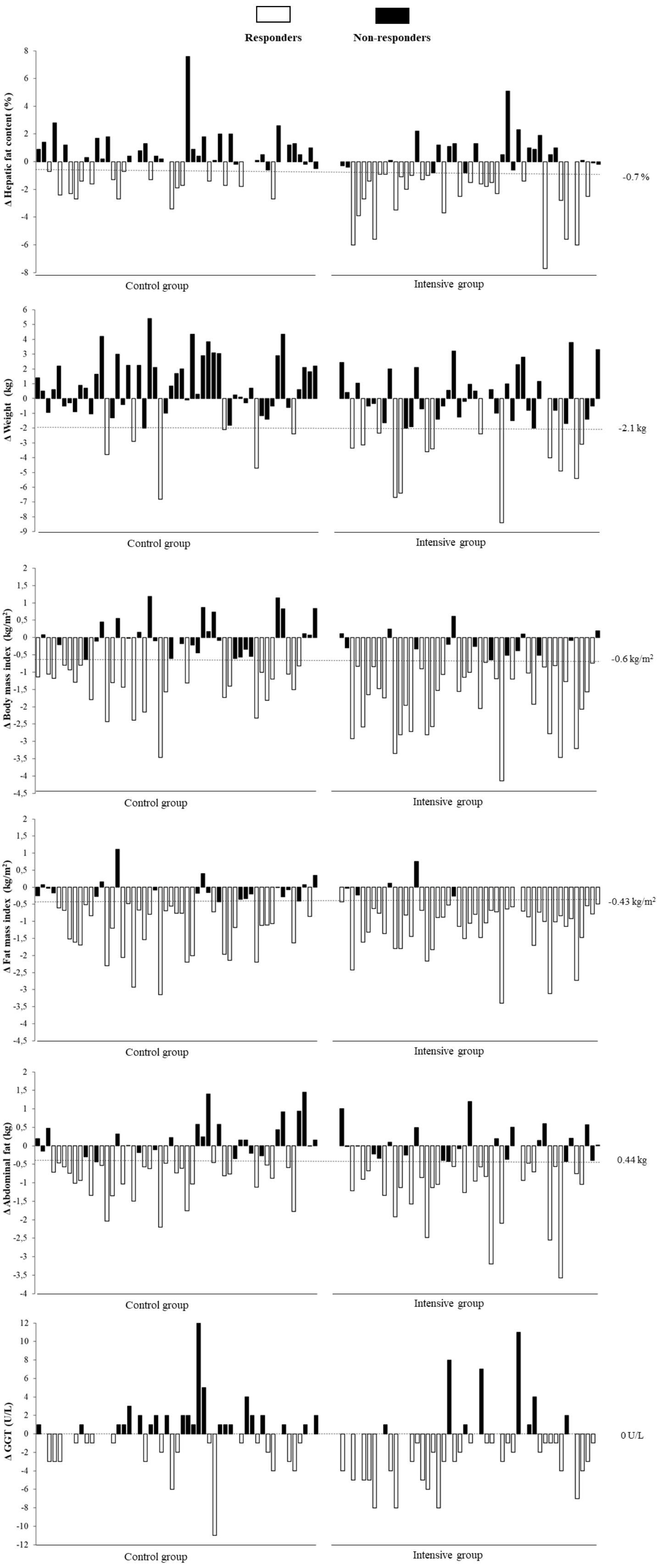


Figure S2. Responders and non-responders for hepatic fat content, weight, BMI, FMI and GGT level after the EFIGRO intervention ordered by their ID code in the lifestyle intervention group (control group) and lifestyle plus exercise intervention group (intensive group). The dotted line represents responder cut-off point (effect size=0.2) for each variable. *Abbreviations*: GGT: gamma-glutamyl transferase. Δ: changes calculated as Post-intervention–Pre-intervention values.

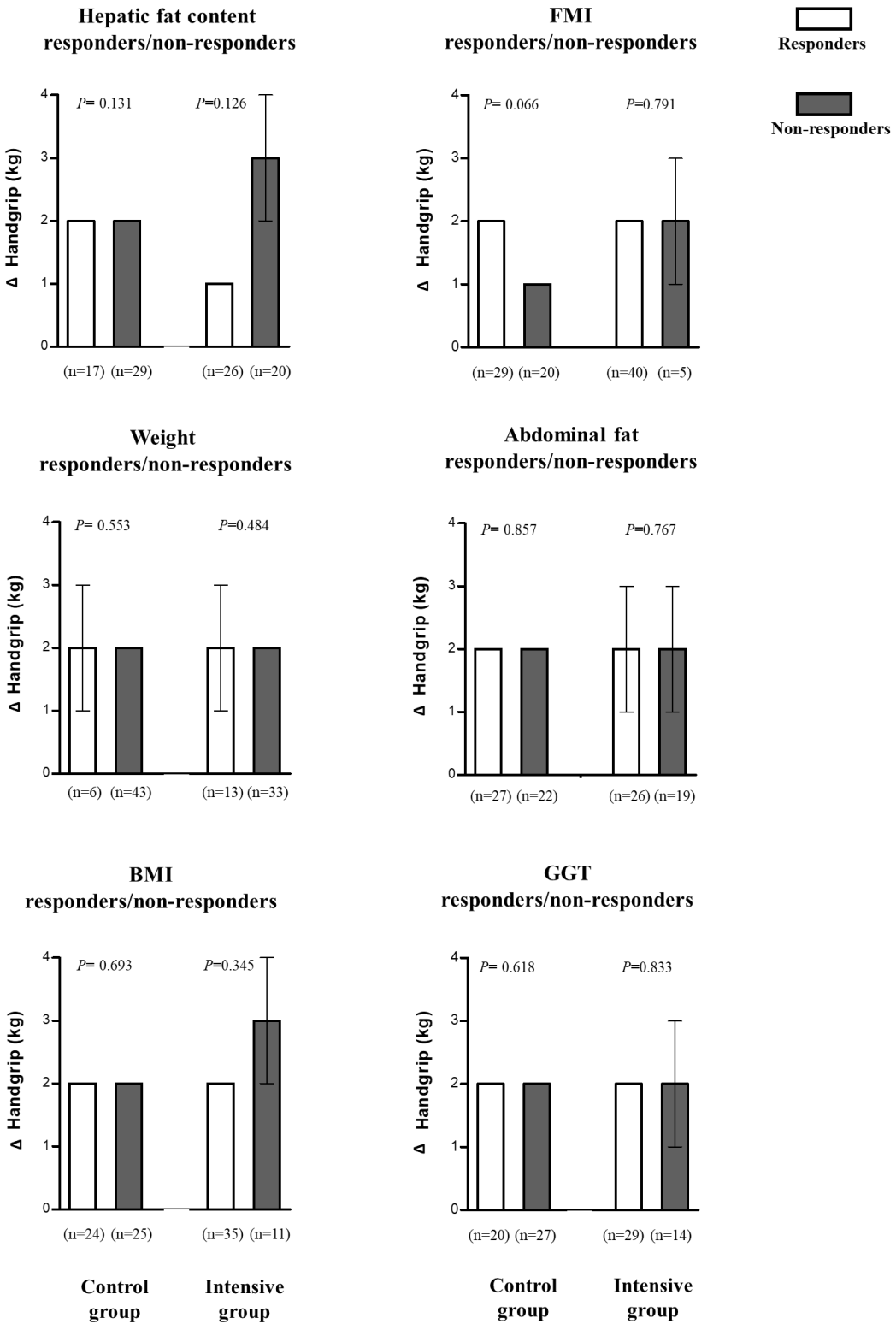


Figure S3. Differences in changes in the handgrip test (kg) between responders and non-responders for the considered outcomes (i.e., hepatic fat content, weight, BMI, FMI and GGT levels) in the lifestyle intervention group (control group) and lifestyle plus exercise intervention group (intensive group). ANCOVA was adjusted for age, sex, baseline value and attendance to the program. *Abbreviations*: BMI: body mass index, FMI: fat mass index; GGT: gamma-glutamyl transferase. Δ: changes calculated as Post-intervention–Pre-intervention values.

4. GENERAL DISCUSSION

The main findings of the studies included in the present International Doctoral Thesis are that: **I)** CRF is associated with liver enzymes levels in adolescents, although waist circumference is a stronger predictor of liver enzyme profile than CRF. Among adolescents with high waist circumference, those having high levels of CRF have lower levels of ALT and higher AST/ALT ratio than their unfit peers; **II)** higher levels of CRF are associated with lower percentage hepatic fat and a healthier profile of liver enzymes in preadolescent children with overweight/obesity. Moreover, fit children with overweight/obesity have lower insulin resistance and TG/HDL ratio than their unfit peers; **III)** supervised exercise interventions reduce percentage hepatic fat and hepatic steatosis prevalence in children and adolescents. Both aerobic and resistance exercise at vigorous or moderate-to-vigorous intensity, with a volume of ≥60 min/session and a minimum frequency of 3 sessions/week significantly reduce hepatic fat content. Moreover, higher increases in CRF and MF levels are associated with greater reductions in hepatic fat after supervised exercise interventions*;* **IV**) the addition of supervised exercise to a family-based lifestyle and psycho-educational program lead to a clinically relevant percentage hepatic fat reduction of nearly 20% in children with overweight/obesity. Percentage hepatic fat is only reduced in those children participating in the program that includes supervised exercise in addition to the educational intervention, but not in those children participating only in the lifestyle and psycho-educational program. Both intervention programs reduce total and abdominal adiposity, and insulin resistance; nonetheless, the addition of exercise training to the lifestyle and psycho-educational program results in larger decreases of adiposity, LDL and GGT in children with overweight/obesity; and **V)** the number of responders for percentage hepatic content, weight, BMI, FMI, and GGT is significantly higher in the lifestyle plus exercise intervention than in the lifestyle intervention alone. Weight and BMI responders increase more CRF than their non-responders peers.

4.1. Cross-sectional associations of physical fitness and physical activity with percentage hepatic fat, liver enzymes, insulin resistance and cardiometabolic risk in youth

Studies I and II of this Thesis analysed the cross-sectional associations of physical fitness components and/or PA levels with percentage hepatic fat, liver enzymes, insulin resistance, and cardiometabolic risk in European adolescents (Study I) or in preadolescent children with overweight/obesity (Study II).

4.1.1. Cross-sectional association of physical fitness with percentage hepatic fat and liver enzymes in youth

To our knowledge, the Study I is the first to examine the association of CRF with all liver enzymes levels and AST/ALT ratio in adolescents. Study II is the first to examine the associations of the main health-related physical fitness components with percentage hepatic fat and liver enzymes in children.

In the Study I, we observed that high CRF levels were associated with lower ALT and GGT plasma concentrations and higher AST and AST/ALT ratio. In the Study II, CRF, but not MF or speed-agility, was negatively associated with percentage hepatic fat, GGT and AST/ALT. In agreement with our results, Wittmeier et al.[68], in a sample of 137 adolescents that included both normal weight and overweight participants, observed that CRF (measured by a graded maximal cycle ergometer test and normalized to fat free mass) was negatively associated with percentage hepatic fat (determined by ^{1}H-MRS). With regard to liver enzymes levels, the National Health and Nutrition Examination Survey did not find any significant association between low CRF and ALT below the 80th percentile of ALT, while the relationships became significant above this cut-off in a large sample of adolescents (N=2844)[78]. In a smaller sample of adolescents

(N=95, 12-18 years old), CRF was also inversely correlated with ALT in both non-overweight and overweight youth[71]. As far as we are aware, no studies have examined the relationship between other physical fitness components and percentage hepatic fat and liver enzymes or between CRF and GGT or AST/ALT in young people, which hamper comparisons.

Among adolescents with high waist circumference (WC), those having high levels of CRF had lower levels of ALT and higher AST/ALT ratio than those who were unfit (Study I). In the Study II, CRF-fit children with overweight/obesity had lower GGT and higher AST/ALT, but not statistically significant lower percentage hepatic fat or ALT than their unfit counterparts. In agreement with our results, other study observed that youth classified in the higher categories of CRF (assessed by the 20mSRT) had lower levels of liver enzymes than those in the lower CRF categories[78]. We did not find any study in the scientific literature analysing differences in hepatic fat content (particularly, measured by MRI) between fit and unfit children. However, in a previous study with obese male adults, CRF-fit participants had lower levels of intrahepatocellular lipids (MRI) than CRF-unfit ones[79], which is consistent with our results.

The mechanisms underlying the relationship between CRF and hepatic fat and liver enzymes have not been clearly elucidated. A plausible mechanism that might explain this association could be that the intrinsic aerobic capacity is linked with hepatic mitochondrial oxidative capacity and susceptibility to hepatic steatosis[80]. An experiment performed in an animal model showed that rats with low aerobic fitness had a reduced hepatic mitochondrial content and a reduced mitochondrial oxidative capacity. The reductions in the hepatic mitochondrial content and oxidative activity were associated with a higher liver triglycerides content, percentage of hepatocytes with lipid droplets and lipid peroxidation in low CRF rats, which in turn was associated with hepatic steatosis.

4.1.2. Cross-sectional associations of physical activity with percentage hepatic fat and liver enzymes in children with overweight/obesity

Regarding PA, we did not find any significant association between PA levels and percentage hepatic fat or liver enzyme levels in children with overweight/obesity (Study II). Likewise, we did not find significant differences in percentage hepatic fat or liver enzymes levels between active and inactive children. To the best of our knowledge, the Study II is the first study analysing the relationship between objectively measured PA and percentage hepatic fat (measured by MRI) in preadolescent children. Nevertheless, other studies have reported significant associations with PA (questionnaires) and other hepatic health markers (US[69,81], liver enzymes[69,75]) in youth. In a longitudinal analysis with a large sample (N=1292), it was observed that objectively measured total PA and MVPA at 12 years was inversely associated with the risk of hepatic steatosis (US), and abnormal GGT levels, but not with ALT levels at 17.8 years of age[69]. Another study, performed exclusively in adolescents (both normal weight and overweight), reported significant associations of total PA and MVPA (measured by accelerometry) with AST/ALT, but not with ALT or GGT[75]. The authors also observed that active adolescents had a higher AST/ALT ratio than their inactive peers[75]. In summary, direct comparisons among studies are difficult because of differences in the age, or BMI categories of participants, as well as the different methodology used to assess PA or hepatic steatosis. A possible explanation for the lack of association between PA and percentage hepatic fat in Study II could be that our sample is exclusively composed by prepubertal children with overweight/obesity with a narrow range of PA levels.

4.1.3. Cross-sectional association of physical fitness and physical activity with insulin resistance and cardiometabolic risk in children with overweight/obesity

In the Study II, we observed inverse associations between physical fitness (CRF, lower body MF and speed-agility) and insulin resistance (HOMA-IR) in children with overweight/obesity. However, these associations disappeared after controlling for BMI. In line with our results, previous studies showed that associations of physical fitness with HOMA-IR disappeared after adjusting for BMI or other adiposity estimates[82,83]. However, in other reports, the associations between physical fitness components and HOMA-IR were significant irrespective of BMI[84]. Thereby, the potential influence of physical fitness on insulin resistance in youth, independently of BMI or adiposity, remains unclear due to the contradictory results. The non-significant association between physical fitness and HOMA-IR after controlling for BMI could be explained both by the influence of BMI on insulin resistance and by the association of physical fitness levels and BMI in children[85]. Differences in the results among studies could be related to the various adiposity variables used for adjustment, physical fitness tests used and children's characteristics.

In addition, we found that HOMA-IR levels were lower in CRF-fit than in CRF-unfit children. In line with our results, Llorente-Cantarero et al.[86] observed that Spanish prepubertal children (N=141) categorized in the higher CRF group had lower levels of HOMA-IR than those categorized in the lower CRF group (classified according to other reference values). When children were classified as MF-fit or MF-unfit according to both the handgrip or standing long jump results, we did not find differences in HOMA-IR levels across physical fitness categories. By contrast, other authors observed differences in HOMA-IR across handgrip[87,88] or standing long jump[88] categories.

Maturational state (adolescents[88] or both children and adolescents[87]), as well as ethnic and or regional differences[87] could be underlying discrepancies among studies' findings.

We did not observe any significant association of PA with insulin resistance in children with overweight/obesity. Nevertheless, other studies in youth reported significant associations between different PA intensities (measured by questionnaires) and HOMA-IR[89,90]. Moreover, we did not find differences in HOMA-IR levels between active and inactive children with overweight/obesity. In agreement with our findings, no significant differences in HOMA-IR levels were found between active and inactive children and adolescents (N=745) in week days PA (measured by accelerometry) in the study by Janssen et al.[91].

Several studies have examined the association between physical fitness or/and PA and cardiometabolic risk factors, included as individual risk factors[92,93] or in clustered scores[94] in children. However, we only found one study examining the relationship between physical fitness or PA and the TG/HDL ratio[95]. In line with our results, this study observed inverse correlations between CRF (VO_2peak calculated from a progressive cycle ergometer test) and the TG/HDL ratio in a similar sample size (N=155) of schoolers (10–14 years old) in all BMI categories. They also found that CRF-fit had a lower TG/HDL ratio than CRF-unfit children[95]. In contrast to our findings, this study did not observe any significant association between vigorous PA (VPA) or MVPA and the TG/HDL in the sample of both children and adolescents[95]. Moreover, these authors did not find significant differences in the TG/HDL ratio across PA categories. However, in the study of Bailey et al.[95], different model of accelerometers ((RT3® triaxial accelerometers) and different cut-offs for defining PA intensities were employed, which could have a large impact in the outcomes[60].

4.2. Effects of supervised exercise on hepatic steatosis and other cardiometabolic risk factors in youth

Studies III, IV and V analysed, from different perspectives, the effect of supervised exercise on hepatic steatosis and others cardiometabolic risk factors in youth. In the Study III, we systematically reviewed and meta-analysed the effects of supervised exercise on percentage hepatic fat and hepatic steatosis prevalence in children and adolescents. The Study IV provided the overall main effects of a supervised exercise program in addition to a 22-week family-based lifestyle- and psycho-educational intervention on hepatic steatosis and other cardiovascular risk factors in children with overweight/obesity. Finally, in the Study V we compared the interindividual variability and prevalence of responders regarding percentage hepatic fat, overall and abdominal adiposity, and GGT levels between the two different 22-week lifestyle interventions (with or without supervised exercise) of the EFIGRO study in children with overweight/obesity.

4.2.1. Systematic review and meta-analysis of the effects of supervised exercise on hepatic steatosis in children and adolescents

Our systematic review and meta-analysis (Study III) shows that supervised exercise alone has beneficial and strong effects on percentage hepatic fat reduction in youth, compared to a non-exercise control group. Likewise, supervised exercise interventions, in conjunction to other lifestyle components (nutritional and/or physiological activities), reduced hepatic steatosis prevalence in children and adolescents.

Findings of the Study III are consistent with another published systematic review and meta-analysis[47] which showed that exercise significantly reduced intrahepatic fat

content in youth. A narrative review[46] also suggested that exercise alone may improve hepatic steatosis in children and adolescents. Keating et al.[96] in adults, and Orci et al.[97] in adults and adolescents also demonstrated benefits of exercise alone or in addition to a dietary intervention on hepatic fat content and NAFLD management. Many of the studies reviewed in the Study III reported significant reductions in percentage hepatic fat after exercise training interventions[50,51,98,99]. However, it is important to note that the majority of the studies included in our systematic review were performed in adolescent[50,51,98–106], and that the remaining studies included both children and adolescents[107–111]. None of the studies were focused only in children (≤12 years). Hence, the EFIGRO study was the first research intervention examining the effect of supervised exercise on percentage hepatic fat (measured by MRI) exclusively in children (Studies IV and V).

4.2.1.1. Specific evidence-based exercise recommendations to reduce hepatic fat in youth

The subgroup analyses performed in the Study III revealed that both aerobic and resistance exercise training at vigorous and moderate-to-vigorous intensities, in sessions of ≥60 minutes and a frequency ≥3 sessions per week were effective reducing percentage hepatic fat in children and adolescents. Although lifestyle interventions involving diet and exercise were already considered a cornerstone of paediatric hepatic steatosis treat[12], this is the first time that evidence-based specific exercise recommendations, regarding the type, intensity, duration and frequency, were provided in adolescents and children.

Our results were in line with the international recommendations of PA for the promotion of health in young people[63,64]. Thus, these results suggested that the design of future exercise interventions for hepatic steatosis management and prevention in

children and adolescents should include both aerobic and resistance exercise, it should be performed at vigorous or moderate-to-vigorous intensity, with at least a volume of 60 min/session and a frequency of three sessions/week since they have shown greater benefits in reducing percentage hepatic fat and hepatic steatosis prevalence. However, these results should be taken with caution given the reduced number of studies included in the meta-analysis.

A recent systematic-review in adults concluded that both aerobic and resistance exercise similarly reduced hepatic steatosis[112], which concurs with our findings. In contrast, a meta-analysis in adults concluded that aerobic exercise was more effective than resistance training in the reduction of intrahepatic lipid content[97]. As far as we are aware, there is only one study comparing the effects between vigorous and moderate exercise intensities on percentage hepatic fat in youth[99], which is included in the meta-analysis of Study III. Only those children participating in the vigorous intensity group reduced their percentage hepatic fat; while youths of the control and moderate intensity groups increased their hepatic fat content; although there were no significant differences among groups after the intervention[99]. To the best of our knowledge, there are no more previous studies in humans comparing the effects of moderate-to-vigorous exercise with just vigorous exercise on percentage hepatic fat or hepatic steatosis prevalence; nevertheless, a study performed in mice showed that vigorous exercise was more effective than moderate exercise improving hepatic steatosis[113]. Finally, Hashida et al. in their meta-analysis[112] proposed as a median effective protocol to improve hepatic steatosis in adults a volume of 40 min/session, three times/week for aerobic exercise or 45 min/session and three times/week for resistance exercise, which is similar to our results.

4.2.2. Effects of supervised exercise in addition to a 22-week multidisciplinary family-based lifestyle intervention on hepatic fat and other cardiovascular risk factors in children with overweight/obesity

In line with the findings of the Study III, we observed that children with overweight/obesity participating in a 22-week family-based lifestyle plus exercise intervention significantly reduced percentage hepatic fat (Study IV). In contrast, those children participating in the lifestyle program but without exercise, did not reduce liver fat. Study IV results agree with those of a study involving obese adolescents[102], in which a 4-month intervention focused on changing the quality of carbohydrate intake resulted in significant improvements in insulin sensitivity -but only the combined lifestyle, psycho-education and resistance exercise training program resulted in a reduction in percentage hepatic fat. In adults and adolescents with overweight or obesity, lifestyle interventions including exercise -or not- have been reported to significantly reduce percentage hepatic fat[114,115]. However, these programs were based on hypocaloric diets that caused large body mass losses[115,116]. In our study, the lifestyle education program was not designed to achieve body weight loss in the short term, and certainly children participating only in the lifestyle and psycho-education program lost no body mass or lean mass. A systematic review and meta-analysis[117] comparing the effect of exercise training and a hypocaloric diet on visceral adiposity reported the latter to result in a larger body mass loss, whereas exercise training tended to induce larger reductions in visceral adiposity. Interestingly, in the Study IV, the effects of exercise on percentage hepatic fat were independent of the change in total or abdominal fat, suggesting a direct metabolic effect of exercise training on hepatic fat metabolism. Metabolic effects of exercise, such as the stimulation of fatty acid oxidation in the liver, the reduction of

lipogenic enzymes, or insulin resistance, have been proposed as potential mechanisms to explain the beneficial effects of exercise in the reversion of hepatic steatosis[33].

In regard with other cardiovascular risk factors, both lifestyle interventions (including or not supervised exercise) reduced total and abdominal fat and insulin resistance in children with overweight or obesity (Study IV). Children in the lifestyle plus exercise intervention also significantly reduced low-density lipoprotein cholesterol. In line with our results, other family-based, structured lifestyle modification programs combined with behavioural strategies for treating obesity have been associated with adiposity reductions in children[40,41]. Likewise, lifestyle interventions have beneficial effects on insulin resistance and other cardiovascular risk factors in children[42]. On the other hand, we observed significant increases in both groups for all physical fitness variables after the intervention, but no significant differences were seen between groups. In contrast with our study, statistically significant improvements in MF and speed, but not in CRF were observed in children with overweight after a similar exercise, nutritional and psychological intervention[43]. In another study[44], the authors observed that those children participating in the program that included exercise increased more their MT than the usual care group, whereas none of the groups improved CRF. Differences among findings of the studies could be explained by differences in the physical fitness measurements, the intervention design, or the participant characteristics.

4.2.3. Effects of supervised exercise in addition to a 22-week multidisciplinary family-based lifestyle intervention on interindividual variability for hepatic fat and other cardiovascular risk factors in children with overweight/obesity

The Study V reports the interindividual effect and the responder prevalence for percentage hepatic fat in the children with overweight/obesity of the EFIGRO project. The most novel finding of Study V is that the prevalence of responders for percentage hepatic fat was significantly higher in the group of children who performed exercise in addition to the family-based lifestyle education program (54% of responders), than in those who only participated in the lifestyle program (34% of responders). There are no studies providing the interindividual variability in hepatic steatosis in response to a lifestyle and exercise intervention, which hampers comparisons. Thus, this is clearly an area in need of more research.

The number of responders for weight (27% *vs*. 11%), BMI (71% *vs*. 47%), FMI (90% *vs*. 47%) and GGT (68% *vs.* 39%) was higher in the group of children who performed exercise in addition to the family-based lifestyle education program, than in those who only participated in the lifestyle program (Study V). One previous report in children and adolescents examining the interindividual variability after a multidisciplinary lifestyle intervention including exercise[118] reported a lower prevalence of BMI responders than in our group of children participating in the program that included exercise (50% *vs*. 71%). This difference may be explained by the different *criteria* used to define responders, or by differences in the design of the programs. In another previous study, not designed to evaluate interindividual variability, the authors found a similar number of participants reducing their weight and BMI after two different lifestyle interventions, with or without exercise; unfortunately, they did not provide

statistical analyses[119]. In any case, the number of responders for weight and for BMI was higher after their dietary advice or dietary advice plus exercise programs, than at the end of our programs. However, our cut-off point to define the responsiveness is much strict than in the study of Schwingshandl et al.[119] In this regard, Schwingshandl et al.[119] considered as "responders" those participants reducing their body weight or BMI, while our cut-off point is a minimum effect size of 0.2.

Several mechanisms besides of genetic factors have been proposed to explain either interindividual variability or responsiveness to lifestyle and exercise interventions. Among them, the adherence to the programs[120] and behavioural and metabolic compensatory mechanisms[121]. Previous studies have observed that several individuals reduced their resting energy expenditure, increased energy intake, lowered physical activity levels or reduced fat oxidation rate in response to the exercise-associated increase of energy expenditure or energy intake restriction programs[121–124]. We did not measure energy and substrate metabolism in the EFIGRO study; nevertheless, as we did not observe any significant difference in the adherence to the lifestyle education program or in changes on dietary intake variables and physical activity levels between the two groups at the end of the intervention, the higher number of responders in the intensive than in the control group may be explained by the addition of supervised exercise to the program.

4.3. Role of fitness improvements on changes in percentage hepatic fat and adiposity after supervised exercise interventions in youth

Studies III, IV and V analysed the role of improvements in CRF and MF on percentage hepatic fat and/or on adiposity changes after exercise programs in youths.

The meta-regression analyses showed that higher improvements in CRF and MT are associated with greater reductions in percentage hepatic fat (Study III). Similarly, in the main analysis of the EFIGRO study (Study IV), we observed that the increase in CRF (measured by the 20msrt) was significantly associated with the reduction in percentage hepatic fat in the lifestyle plus exercise intervention group subjects (just in the per protocol analysis). Study V reports that the increment of CRF was higher in weight and BMI responders than in their non-responders peers. Thus, altogether, these results suggest a relevant role of physical fitness improvements, particularly CRF, on the reduction of hepatic fat and adiposity after an exercise intervention in youths. Our findings are in accordance with those reported by Senechal et al.[77], which showed that CRF was the best predictor of the reduction of percentage hepatic triglycerides in adolescents at risk of T2D after an exercise intervention. In adults, Kantartzis et al.[76] reported a significant correlation between changes in VO_2peak and percentage hepatic fat after a lifestyle intervention that included unsupervised aerobic exercise and sports.

In the Study III, we also observed that the association of changes in CRF levels and changes in percentage hepatic fat was stronger when aerobic exercise at a moderate intensity was excluded from the analyses. This suggests that exercise interventions aiming to improve CRF levels in the management and prevention of hepatic steatosis should be performed at least at a moderate-to-vigorous intensity.

There are no previous studies comparing physical fitness between responders and non-responders in children. Anyway, in adults, De Luis et al.[125] observed that only weight responders, but not non-responders, increased their CRF after a diet plus exercise intervention. In contrast, a previous analysis that examined the prevalence of non-responders for different metabolic outcomes (i.e. fasting insulin, triglycerides, blood pressure) from various studies, did not observe greater improvements of CRF in responders than in non-responders[126]. Thus, further research is needed to elucidate whether responders for a certain outcome are those who experience greater improvements in CRF.

The improvement of CRF has been proposed as a potential mediator of metabolic effects of exercise on adiposity and related comorbidities, such as hepatic steatosis[77,127]. In an experiment performed in rats, it was observed that lower levels of CRF were related to lower reduced hepatic mitochondrial oxidative capacity and a higher liver triglycerides content[80]. Therefore, we hypothesize that the improvement of CRF could be a mechanism that may explain the reduction of hepatic fat content and, similarly, the reduction of the adiposity in other tissues. Our results are in line with this hypothesis.

5. CONCLUSIONS AND CLINICAL HEALTH IMPLICATIONS/

CONCLUSIONES E IMPLICACIONES CLÍNICAS

5.1. Conclusions

The conclusions of the present International Doctoral Thesis are:

I. High cardiorespiratory fitness levels may attenuate the deleterious consequences associated with a high waist circumference on liver enzymes in adolescents.

II. High levels of cardiorespiratory fitness seem to have specific protective effects on hepatic fat, liver enzyme levels, insulin resistance, and cardiometabolic risk in children with overweight/obesity.

III. Supervised exercise alone reduces percentage hepatic fat in children and adolescents and, its combination with lifestyle activities, is an effective approach to reduce hepatic steatosis prevalence in youth. Both aerobic and resistance exercise at vigorous or moderate-to-vigorous intensities in sessions of at least 60 minutes and with a minimum of 3 sessions per week, focused on increasing cardiorespiratory fitness and musculoskeletal fitness, should be included in the design of exercise programs for the prevention and management of hepatic steatosis in children and adolescents.

IV. A 22-week family-based multicomponent intervention program that included supervised exercise in addition to a lifestyle- and psycho-education program has the potential to reduce hepatic steatosis, total and abdominal fat, and insulin resistance in preadolescent children with overweight/obesity. These findings highlight the importance of including supervised exercise in addition to other lifestyle components for the management of hepatic steatosis and related health risks in children with overweight/obesity.

V. The addition of supervised exercise to a 22-week family-based lifestyle educational intervention seems to increase the rates of responders for percentage hepatic fat, adiposity, and gamma-glutamil-transferase levels in children with

overweight/obesity after the intervention. Improvements in cardiorespiratory fitness may explain the differences between responders and non-responders for weight and body mass index.

5.2. Clinical health implications

Altogether, the findings of this Thesis may have practical and clinical implications for the design of future lifestyle interventions for the prevention/treatment of paediatric obesity and hepatic steatosis, and other related comorbidities such as type 2 diabetes and cardiovascular diseases. Specifically, supervised exercise, both aerobic and resistance exercise at least at moderate-to-vigorous intensity, in sessions of 60 minutes and 3 sessions per week and focused on the improvements of cardiorespiratory, should be included in the programs for the management of hepatic steatosis and related health risks in youth, and particularly in children with overweight/obesity.

5.1. Conclusiones

Las conclusiones de esta Tesis Doctoral Internacional son:

I. Una elevada capacidad cardiorrespiratoria podría atenuar las consecuencias negativas de la circunferencia de la cintura sobre las concentraciones plasmáticas de las enzimas hepáticas en los adolescentes.

II. Una elevada capacidad cardiorrespiratoria podría tener efectos beneficiosos específicos sobre la grasa hepática, las concentraciones plasmáticas de las enzimas hepáticas, la resistencia a la insulina y el riesgo cardiometabólico en niñas y niños con sobrepeso u obesidad.

III. El ejercicio físico supervisado reduce el porcentaje de grasa hepática en niños y adolescentes, y conjuntamente con otras actividades basadas en los cambios del estilo de vida, es una estrategia eficaz para reducir la prevalencia de esteatosis hepática en los jóvenes. Tanto el ejercicio aeróbico como el de fuerza, a intensidades vigorosas o de moderadas a vigorosas, en sesiones de al menos 60 minutos de duración, con un mínimo de 3 sesiones por semana, con el objetivo de aumentar la capacidad cardiorrespiratoria y la fuerza muscular, deberían ser incluidos en el diseño de los programas de ejercicio para la prevención y el tratamiento de la esteatosis hepática en niños y adolescentes.

IV. Un programa de intervención familiar multicomponente de 22 semanas de duración que incluye ejercicio físico supervisado, además de un programa basado en educación familiar en estilos de vida saludable y psicoeducación, es capaz de reducir la esteatosis hepática, la adiposidad total y abdominal, y la resistencia a la insulina en niñas y niños preadolescentes con sobrepeso u obesidad.

V. La adición de ejercicio físico supervisado a un programa de educación familiar de 22 semanas de duración, basado en educación familiar en estilos de vida saludable y

psicoeducación, parece aumentar la tasa de respondedores en cuanto al porcentaje de grasa hepática, la adiposidad y las concentraciones plasmáticas de gamma-glutamil--transferasa en niñas y niños con sobrepeso u obesidad tras la intervención. Las mejoras en la capacidad cardiorrespiratoria podrían explicar las diferencias entre respondedores y no respondedores en relación con el peso y el índice de masa corporal.

5.2. Implicaciones clínicas

Los resultados de esta Tesis pueden tener implicaciones clínicas y prácticas a la hora de diseñar intervenciones basadas en los estilos de vida y el ejercicio físico para la prevención o el tratamiento de la obesidad y la esteatosis hepática pediátrica, así como de otras comorbilidades asociadas como la diabetes tipo 2 o las enfermedades cardiovasculares. Concretamente, el ejercicio físico supervisado, tanto el aeróbico como el de fuerza, realizado al menos a intensidad moderada a vigorosa, en sesiones de 60 minutos y 3 sesiones por semana, y cuyo objetivo sea el aumento de la condición física cardiorrespiratoria, debería formar parte de los programas de prevención y tratamiento de la esteatosis hepática y de los factores de riesgo cardiovascular en jóvenes, particularmente en niñas y niños con sobrepeso u obesidad.

REFERENCES

References

1. Abarca-Gómez L, Abdeen ZA, Hamid ZA, et al. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. *Lancet*. 2017;390(10113):2627-2642.

2. World Health Organization. Childhood overweight and obesity. https://www.who.int/dietphysicalactivity/childhood/en/. Published 2019. Accessed May 8, 2019.

3. World Health Organization. Obesity and overweight. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight. Published 2018. Accessed May 8, 2019.

4. Sánchez-Cruz JJ, Jiménez-Moleón JJ, Fernández-Quesada F, Sánchez MJ. Prevalencia de obesidad infantil y juvenil en España en 2012. *Rev Esp Cardiol*. 2013;66(5):371-376.

5. Agencia Española de consumo seguridad alimentaria y nutrición (AECOSAN). Estudio ALADINO 2015. Estudio de Vigilancia del Crecimiento, Alimentación, Actividad Física, Desarrollo Infantil y Obesidad en España. Published 2015. Accessed June 8, 2019.

6. World Health Organization. Data and statistics-The challenge of obesity - quick statistics. http://www.euro.who.int/en/health-topics/noncommunicable-diseases/obesity/data-and-statistics. Published 2019. Accessed May 8, 2019.

7. Lobstein T, Jackson-Leach R. Planning for the worst: estimates of obesity and comorbidities in school-age children in 2025. *Pediatr Obes*. 2016;11(5):321-325.

8. Chung ST, Onuzuruike AU, Magge SN. Cardiometabolic risk in obese children. *Ann N Y Acad Sci*. 2018;1411(1):166-183.

9. Steinberger J, Daniels SR, Eckel RH, et al. Progress and challenges in metabolic syndrome in children and adolescents: a scientific statement from the American Heart Association Atherosclerosis, Hypertension, and Obesity in the Young Committee of the Council on Cardiovascular Disease in the Young. *Circulation*. 2009;119(4):628-647.

10. World Obesity-Global Obesity Observatory. Obesity prevalence worldwide - Girls. https://www.worldobesitydata.org/map/overview-girls#. Published 2017. Accessed June 17, 2019.

11. Ayer J, Charakida M, Deanfield JE, Celermajer DS. Lifetime risk: childhood obesity and cardiovascular risk. *Eur Heart J*. 2015;36(22):1371-1376.

12. Vos MB, Abrams SH, Barlow SE, et al. NASPGHAN Clinical Practice Guideline for the Diagnosis and Treatment of Nonalcoholic Fatty Liver Disease in Children:

Recommendations from the Expert Committee on NAFLD (ECON) and the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN). *J Pediatr Gastroenterol Nutr*. 2017;64(2):319-334.

13. Lim S, Oh TJ, Koh KK. Mechanistic link between nonalcoholic fatty liver disease and cardiometabolic disorders. *Int J Cardiol*. 2015;201:408-414.

14. Angulo P. Long-term mortality in nonalcoholic fatty liver disease: is liver histology of any prognostic significance? *Hepatology*. 2010;51(2):373-375.

15. Dowman JK, Tomlinson JW, Newsome PN. Pathogenesis of non-alcoholic fatty liver disease. *QJM*. 2010;103(2):71-83.

16. Bhala N, Angulo P, van der Poorten D, et al. The natural history of nonalcoholic fatty liver disease with advanced fibrosis or cirrhosis: an international collaborative study. *Hepatology*. 2011;54(4):1208-1216.

17. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: Practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Am J Gastroenterol*. 2012;107(6):811-826.

18. Pacifico L, Martino MD, Catalano C, et al. T1-weighted dual-echo MRI for fat quantification in pediatric nonalcoholic fatty liver disease. *World J Gastroenterol*. 2011;17(25):3012-3019.

19. Browning JD, Szczepaniak LS, Dobbins R, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology*. 2004;40(6):1387-1395.

20. Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics*. 2006;118(4):1388-1393.

21. Anderson EL, Howe LD, Jones HE, Higgins PT, Lawlor DA, Fraser A. The prevalence of non-alcoholic fatty liver disease in children and adolescents: a systematic review and meta-analysis. *PLoS One*. 2015;10(10):e0140908.

22. Blachier M, Leleu H, Peck-Radosavljevic M, Valla D, Roudot-Thoraval F. The burden of liver disease in Europe : A review of available epidemiological data. *J Hepatol*. 2013;58(3):593-608.

23. Caballeria L, Pera G, Auladell MA, et al. Prevalence and factors associated with the presence of nonalcoholic fatty liver disease in an adult population in Spain. *Eur J Gastroenterol Hepatol*. 2010;22(1):24-32.

24. Younossi ZM, Blissett D, Blissett R, et al. The economic and clinical burden of nonalcoholic fatty liver disease in the United States and Europe. *Hepatology*. 2016;64(5):1577-1586.

25. Temple JL, Cordero P, Li J, Nguyen V, Oben JA. A guide to non-alcoholic fatty liver disease in childhood and adolescence. *Int J Mol Sci*. 2016;17(6):E947.

26. Vajro P, Lenta S, Socha P, et al. Diagnosis of nonalcoholic fatty liver disease in children and adolescents: position paper of the ESPGHAN Hepatology Committee. *J Pediatr Gastroenterol Nutr*. 2012;54(5):700-713.

27. Sanyal AJ, Brunt EM, Kleiner DE, et al. Endpoints and clinical trial design for nonalcoholic steatohepatitis. *Hepatology*. 2011;54(1):344-353.

28. Mazhar SM, Shiehmorteza M, Sirlin CB. Noninvasive assessment of hepatic steatosis. *Clin Gastroenterol Hepatol*. 2009;7(2):135-140.

29. Zand KA, Shah A, Heba E, et al. Accuracy of multiecho magnitude-based MRI (M-MRI) for estimation of hepatic proton density fat fraction (PDFF) in children. *J Magn Reson Imaging*. 2015;42(5):1223-1232.

30. Labayen I, Ruiz JR, Ortega FB, et al. Liver enzymes and clustering cardiometabolic risk factors in European adolescents: The HELENA study. *Pediatr Obes*. 2015;10(5):361-370.

31. Schwimmer JB, Pardee PE, Lavine JE, Blumkin AK, Cook S. Cardiovascular risk factors and the metabolic syndrome in pediatric nonalcoholic fatty liver disease. *Circulation*. 2008;118(3):277-283.

32. Pacifico L, Nobili V, Anania C, Verdecchia P, Chiesa C. Pediatric nonalcoholic fatty liver disease, metabolic syndrome and cardiovascular risk. *World J Gastroenterol*. 2011;17(26):3082-3091.

33. Ordonez R, Carbajo-Pescador S, Mauriz JL, Gonzalez-Gallego J. Understanding nutritional interventions and physical exercise in non-alcoholic fatty liver disease. *Curr Mol Med*. 2015;15(1):3-26.

34. Than NN, Newsome PN. A concise review of non-alcoholic fatty liver disease. *Atherosclerosis*. 2015;239(1):192-202.

35. Botros M, Sikaris KA. The de ritis ratio: the test of time. *Clin Biochem Rev*. 2013;34(3):117-130.

36. Rahmani J, Miri A, Namjoo I, et al. Elevated liver enzymes and cardiovascular mortality: a systematic review and dose-response meta-analysis of more than one million participants. *Eur J Gastroenterol Hepatol*. 2019;31(5):555-562.

37. Di Bonito P, Moio N, Scilla C, et al. Usefulness of the high triglyceride-to-HDL cholesterol ratio to identify cardiometabolic risk factors and preclinical signs of organ damage in outpatient children. *Diabetes Care*. 2012;35(1):158-162.

38. Pacifico L, Bonci E, Andreoli G, et al. Association of serum triglyceride-to-HDL cholesterol ratio with carotid artery intima-media thickness, insulin resistance and nonalcoholic fatty liver disease in children and adolescents. *Nutr Metab*

Cardiovasc Dis. 2014;24(7):737-743.

39. Deldin AR, Lee S. Role of physical activity in the treatment of nonalcoholic fatty liver disease in children and adolescents. *Appl Physiol Nutr Metab*. 2013;38(8):805-812.

40. Mead E, Brown T, Rees K, et al. Diet, physical activity and behavioural interventions for the treatment of overweight or obese children from the age of 6 to 11 years. *Cochrane Database Syst Rev*. 2017;6:CD012651.

41. O'Connor EA, Evans C V, Burda BU, Walsh ES, Eder M, Lozano P. Screening for obesity and intervention for weight management in children and adolescents: evidence report and systematic review for the US Preventive Services Task Force. *JAMA*. 2017;317(23):2427-2444.

42. Van Buren DJ, Tibbs TL. Lifestyle interventions to reduce diabetes and cardiovascular disease risk among children. *Curr Diab Rep*. 2014;14(12):557.

43. Ranucci C, Pippi R, Buratta L, et al. Effects of an intensive lifestyle intervention to treat overweight/obese children and adolescents. *Biomed Res Int*. 2017;2017:8573725.

44. Seo YG, Lim H, Kim Y, et al. The effect of a multidisciplinary lifestyle intervention on obesity status, body composition, physical fitness, and cardiometabolic risk markers in children and adolescents with obesity. *Nutrients*. 2019:11(1):E137.

45. Ho M, Garnett SP, Baur LA, et al. Impact of dietary and exercise interventions on weight change and metabolic outcomes in obese children and adolescents: a systematic review and meta-analysis of randomized trials. *JAMA Pediatr*. 2013;167(8):759-768.

46. Africa JA, Newton KP, Schwimmer JB. Lifestyle interventions including nutrition, exercise, and supplements for nonalcoholic fatty liver disease in children. *Dig Dis Sci*. 2016;61(5):1375-1386.

47. Gonzalez-Ruiz K, Ramirez-Velez R, Correa-Bautista JE, Peterson MD, Garcia-Hermoso A. The effects of exercise on abdominal fat and liver enzymes in pediatric obesity: a systematic review and meta-analysis. *Child Obes*. 2017;13(4):272-282.

48. Buford TW, Roberts MD, Church TS. Toward exercise as personalized medicine. *Sports Med*. 2013;43(3):157-165.

49. Bouchard C, Rankinen T. Individual differences in response to regular physical activity. *Med Sci Sports Exerc*. 2001;33(6 Suppl):S446-451;discussion S452-453.

50. Davis JN, Gyllenhammer LE, Vanni AA, et al. Startup circuit training program reduces metabolic risk in Latino adolescents. *Med Sci Sports Exerc*.

2011;43(11):2195-2203.

51. Lee S, Deldin AR, White D, et al. Aerobic exercise but not resistance exercise reduces intrahepatic lipid content and visceral fat and improves insulin sensitivity in obese adolescent girls: a randomized controlled trial. *Am J Physiol Endocrinol Metab*. 2013;305(10):E1222-1229.

52. Ortega FB, Ruiz JR, Castillo MJ, Sjöström M. Physical fitness in childhood and adolescence: a powerful marker of health. *Int J Obes (Lond)*. 2008;32(1):1-11.

53. Caspersen CJ, Christenson GM. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. *Public Health Rep*. 1985;100(2):126-131.

54. Ruiz JR, Castro-Pinero J, Espana-Romero V, et al. Field-based fitness assessment in young people: the ALPHA health-related fitness test battery for children and adolescents. *Br J Sports Med*. 2011;45(6):518-524.

55. Artero EG, España-Romero V, Castro-Pinero J, et al. Criterion-related validity of field-based muscular fitness tests in youth. *J Sport Med Phys Fit*. 2012;52(3):263-272.

56. Takken T, Bongers BC, van Brussel M, Haapala EA, Hulzebos EHJ. Cardiopulmonary exercise testing in pediatrics. *Ann Am Thorac Soc*. 2017;14(Supplement_1):S123-S128.

57. Rice MH, Howell CC. Measurement of physical activity, exercise, and physical fitness in children: issues and concerns. *J Pediatr Nurs*. 2000;15(3):148-156.

58. Welk GJ, Corbin CB, Dale D. Measurement issues in the assessment of physical activity in children. *Res Q Exerc Sport*. 2000;71(2 Suppl):S59-73.

59. Sirard JR, Pate RR. Physical activity assessment in children and adolescents. *Sports Med*. 2001;31(6):439-454.

60. Migueles JH, Cadenas-Sanchez C, Ekelund U, et al. Accelerometer data collection and processing criteria to assess physical activity and other outcomes: a systematic review and practical considerations. *Sports Med*. 2017;47(9):1821-1845.

61. Blair SN, Kohl HW 3rd, Paffenbarger RS Jr, Clark DG, Cooper KH, Gibbons LW. Physical fitness and all-cause mortality. A prospective study of healthy men and women. *JAMA*. 1989;262(17):2395-2401.

62. Ruiz JR, Huybrechts I, Cuenca-García M, et al. Cardiorespiratory fitness and ideal cardiovascular health in European adolescents. *Heart*. 2015:101(10):766-773.

63. World Health Organization. Recommended levels of physical activity for children aged 5 - 17 years. Global recommendations on physical activity for

health. http://www.who.int/dietphysicalactivity/factsheet_young_people/en/. Published 2010. Accessed May 25, 2017.

64. Centers for Disease Control and Prevention. How much physical activity do children need? Physical Activity Guidelines. https://www.cdc.gov/physicalactivity/basics/children/index.htm. Published 2015. Accessed May 25, 2017.

65. Katzmarzyk PT, Lee IM, Martin CK, Blair SN. Epidemiology of physical activity and exercise training in the United States. *Prog Cardiovasc Dis*. 2017;60(1):3-10.

66. Mielgo-Ayuso J, Aparicio-Ugarriza R, Castillo A, et al. Physical activity patterns of the Spanish population are mostly determined by sex and age: findings in the ANIBES Study. *PLoS One*. 2016;11(2):e0149969.

67. Raitakari OT, Porkka KV, Taimela S, Telama R, Rasanen L, Viikari JS. Effects of persistent physical activity and inactivity on coronary risk factors in children and young adults. The Cardiovascular Risk in Young Finns Study. *Am J Epidemiol*. 1994;140(3):195-205.

68. Wittmeier KD, Wicklow BA, MacIntosh AC, et al. Hepatic steatosis and low cardiorespiratory fitness in youth with type 2 diabetes. *Obesity (Silver Spring)*. 2012;20(5):1034-1040.

69. Anderson EL, Fraser A, Howe LD, et al. Physical activity is prospectively associated with adolescent nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr*. 2016;62(1):110-117.

70. Mager DR, Patterson C, So S, Rogenstein CD, Wykes LJ, Roberts EA. Dietary and physical activity patterns in children with fatty liver. *Eur J Clin Nutr*. 2010;64(6):628-635.

71. Kelishadi R, Cook SR, Amra B, Adibi A. Factors associated with insulin resistance and non-alcoholic fatty liver disease among youths. *Atherosclerosis*. 2009;204(2):538-543.

72. Martins C, Freitas IJ, Pizarro A, et al. Cardiorespiratory fitness, but not central obesity or C-reactive protein, is related to liver function in obese children. *Pediatr Exerc Sci*. 2013;25(1):3-11.

73. Martins C, Pizarro A, Aires L, et al. Fitness and metabolic syndrome in obese fatty liver children. *Ann Hum Biol*. 2013;40(1):99-101.

74. Wicklow BA, Wittmeier KD, MacIntosh AC, et al. Metabolic consequences of hepatic steatosis in overweight and obese adolescents. *Diabetes Care*. 2012;35(4):905-910.

75. Ruiz JR, Labayen I, Ortega FB, et al. Physical activity, sedentary time, and liver enzymes in adolescents: the HELENA study. *Pediatr Res*. 2014;75(6):798-802.

76. Kantartzis K, Thamer C, Peter A, et al. High cardiorespiratory fitness is an independent predictor of the reduction in liver fat during a lifestyle intervention in non-alcoholic fatty liver disease. *Gut*. 2009;58(9):1281-1288.

77. Senechal M, Rempel M, Duhamel TA, et al. Fitness is a determinant of the metabolic response to endurance training in adolescents at risk of type 2 diabetes mellitus. *Obesity (Silver Spring)*. 2015;23(4):823-832.

78. Trilk JL, Ortaglia A, Blair SN, Bottai M, Church TS, Pate RR. Cardiorespiratory fitness, waist circumference, and alanine aminotransferase in youth. *Med Sci Sports Exerc* . 2013;45(4):722-727.

79. O'Donovan G, Thomas EL, McCarthy JP, et al. Fat distribution in men of different waist girth, fitness level and exercise habit. *Int J Obes (Lond)*. 2009;33(12):1356-1362.

80. Thyfault JP, Rector RS, Uptergrove GM, et al. Rats selectively bred for low aerobic capacity have reduced hepatic mitochondrial oxidative capacity and susceptibility to hepatic steatosis and injury. *J Physiol*. 2009;587(Pt 8):1805-1816.

81. Nobili V, Liccardo D, Bedogni G, et al. Influence of dietary pattern, physical activity, and I148M PNPLA3 on steatosis severity in at-risk adolescents. *Genes Nutr*. 2014;9(3):392.

82. Slinger JD, van Breda E, Keizer H, Rump P, Hornstra G, Kuipers H. Insulin resistance, physical fitness, body composition and leptin concentration in 7-8 year-old children. *J Sci Med Sport*. 2008;11(2):132-138.

83. Krekoukia M, Nassis GP, Psarra G, Skenderi K, Chrousos GP, Sidossis LS. Elevated total and central adiposity and low physical activity are associated with insulin resistance in children. *Metab Clin Ex*. 2007;56(2):206-213.

84. Li S, Zhang R, Pan G, Zheng L, Li C. Handgrip strength is associated with insulin resistance and glucose metabolism in adolescents: Evidence from National Health and Nutrition Examination Survey 2011 to 2014. *Pediatr Diabetes*. 2018;19(3):375-380.

85. Huang YC, Malina RM. BMI and health-related physical fitness in Taiwanese youth 9-18 years. *Med Sci Sports Exerc*. 2007;39(4):701-708.

86. Llorente-Cantarero FJ, Perez-Navero JL, de Dios Benitez-Sillero J, Muñoz-Villanueva MC, Guillen-del Castillo M, Gil-Campos M. Non-traditional markers of metabolic risk in prepubertal children with different levels of cardiorespiratory fitness. *Public Health Nutr*. 2012;15(10):1827-1834.

87. Cohen DD, Gomez-Arbelaez D, Camacho PA, et al. Low muscle strength is associated with metabolic risk factors in Colombian children: the ACFIES study. *PLoS One*. 2014;9(4):e93150.

88. Jiménez-Pavón D, Ortega FB, Valtuena J, et al. Muscular strength and markers of insulin resistance in European adolescents: the HELENA Study. *Eur J Appl Physiol*. 2012;112(7):2455-2465.

89. Rizzo NS, Ruiz JR, Oja L, Veidebaum T, Sjostrom M. Associations between physical activity, body fat, and insulin resistance (homeostasis model assessment) in adolescents: the European Youth Heart Study. *Am J Clin Nutr*. 2008;87(3):586-592.

90. Owen CG, Nightingale CM, Rudnicka AR, et al. Physical activity, obesity and cardiometabolic risk factors in 9- to 10-year-old UK children of white European, South Asian and black African-Caribbean origin: the Child Heart And health Study in England (CHASE). *Diabetologia*. 2010;53(8):1620-1630.

91. Janssen I, Wong SL, Colley R, Tremblay MS. The fractionalization of physical activity throughout the week is associated with the cardiometabolic health of children and youth. *BMC Public Health*. 2013;13:554.

92. Puder JJ, Schindler C, Zahner L, Kriemler S. Adiposity, fitness and metabolic risk in children: a cross-sectional and longitudinal study. *Int J Pediatr Obes*. 2011;6(2-2):e297-306.

93. Chaput JP, Saunders TJ, Mathieu MÈ, et al. Combined associations between moderate to vigorous physical activity and sedentary behaviour with cardiometabolic risk factors in children. *Appl Physiol Nutr Metab*. 2013;38(5):477-483.

94. Andersen LB, Sardinha LB, Froberg K, Riddoch CJ, Page AS, Anderssen SA. Fitness, fatness and clustering of cardiovascular risk factors in children from Denmark, Estonia and Portugal: the European Youth Heart Study. *Int J Pediatr Obes*. 2008;3(Suppl 1):58-66.

95. Bailey DP, Savory LA, Denton SJ, Davies BR, Kerr CJ. The triglyceride to high-density lipoprotein ratio identifies children who may be at risk of developing cardiometabolic disease. *Acta Paediatr*. 2014;103(8):e349-353.

96. Keating SE, Hackett DA, George J, Johnson NA. Exercise and non-alcoholic fatty liver disease: a systematic review and meta-analysis. *J Hepatol*. 2012;57(1):157-166.

97. Orci LA, Gariani K, Oldani G, et al. Exercise-based interventions for nonalcoholic fatty liver disease: a meta-analysis and meta-regression. *Clin Gastroenterol Hepatol*. 2016;14(10):1398-1411.

98. Lee S, Bacha F, Hannon T, Kuk JL, Boesch C, Arslanian S. Effects of aerobic versus resistance exercise without caloric restriction on abdominal fat, intrahepatic lipid, and insulin sensitivity in obese adolescent boys a randomized, controlled trial. *Diabetes*. 2012;61(11):2787-2795.

99. Hay J, Wittmeier K, Macintosh A, et al. Physical activity intensity and type 2 diabetes risk in overweight youth: a randomized trial. *Int J Obes (Lond)*. 2016;40(4):607-614.

100. Dâmaso AR, Tock L, Tufik S, et al. Multidisciplinary treatment reduces visceral adiposity tissue, leptin, ghrelin and the prevalence of non-alcoholic fat liver disease (NAFLD) in obese adolescents. *Rev Bras Med Esporte*. 2006;12(5):263-267.

101. Tock L, Prado WL, Caranti DA, et al. Nonalcoholic fatty liver disease decrease in obese adolescents after multidisciplinary therapy. *Eur J Gastroenterol Hepatol*. 2006;18(12):1241-1245.

102. Hasson RE, Adam TC, Davis JN, et al. Randomized controlled trial to improve adiposity, inflammation, and insulin resistance in obese African-American and Latino youth. *Obesity (Silver Spring)*. 2012;20(4):811-818.

103. Dâmaso AR, de Piano A, Campos RM, et al. Multidisciplinary approach to the treatment of obese adolescents: effects on cardiovascular risk factors, inflammatory profile, and neuroendocrine regulation of energy balance. *Int J Endocrinol*. 2013;12(5):237-241.

104. Van der Heijden GJ, Wang ZJ, Chu ZD, et al. A 12-week aerobic exercise program reduces hepatic fat accumulation and insulin resistance in obese, Hispanic adolescents. *Obesity (Silver Spring)*. 2010;18(2):384-390.

105. Van Der Heijden GJ, Wang ZJ, Chu Z, et al. Strength exercise improves muscle mass and hepatic insulin sensitivity in obese youth. *Med Sci Sports Exerc*. 2010;42(11):1973-1980.

106. de Piano A, Prado WL, Caranti DA, et al. Metabolic and nutritional profile of obese adolescents with nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr*. 2007;44(4):446-452.

107. Gronbaek H, Lange A, Birkebaek NH, et al. Effect of a 10-week weight loss camp on fatty liver disease and insulin sensitivity in obese Danish children. *J Pediatr Gastroenterol Nutr*. 2012;54(2):223-228.

108. Koot BG, van der Baan-Slootweg OH, Tamminga-Smeulders CL, et al. Lifestyle intervention for non-alcoholic fatty liver disease: prospective cohort study of its efficacy and factors related to improvement. *Arch Dis Child*. 2011;96(7):669-674.

109. Reinehr T, Schmidt C, Toschke AM, Andler W. Lifestyle intervention in obese children with non-alcoholic fatty liver disease: 2-year follow-up study. *Arch Dis Child*. 2009;94(6):437-442.

110. Reinehr T, Schmidt C, de Sousa G, Andler W. Association between leptin and transaminases: 1-year follow-up study in 180 overweight children. *Metabolism*.

2009;58(4):497-503.

111. Reinehr T, Roth CL. Fetuin-A and its relation to metabolic syndrome and fatty liver disease in obese children before and after weight loss. *J Clin Endocrinol Metab*. 2008;93(11):4479-4485.

112. Hashida R, Kawaguchi T, Bekki M, et al. Aerobic vs. resistance exercise in non-alcoholic fatty liver disease: a systematic review. *J Hepatol*. 2017;66(1):142-152.

113. Cho J, Kim S, Lee S, Kang H. Effect of training intensity on nonalcoholic fatty liver disease. *Med Sci Sports Exerc*. 2015;47(8):1624-1634.

114. Hens W, Vissers D, Hansen D, et al. The effect of diet or exercise on ectopic adiposity in children and adolescents with obesity: a systematic review and meta-analysis. *Obes Rev*. 2017;18(11):1310-1322.

115. Hens W, Taeyman J, Cornelis J, Gielen J, Van Gaal L, Vissers D. The effect of lifestyle interventions on excess ectopic fat deposition measured by noninvasive techniques in overweight and obese adults: a systematic review and meta-analysis. *J Phys Act Health*. 2016;13(6):671-694.

116. Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, et al. Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis. *Gastroenterology*. 2015;149(2):365-378.

117. Verheggen RJ, Maessen MF, Green DJ, Hermus AR, Hopman MT, Thijssen DH. A systematic review and meta-analysis on the effects of exercise training versus hypocaloric diet: distinct effects on body weight and visceral adipose tissue. *Obes Rev*. 2016;17(8):664-690.

118. Kinder M, Lotze M, Davids S, et al. Functional imaging in obese children responding to long-term sports therapy. *Behav Brain Res*. 2014;272:25-31.

119. Schwingshandl J, Sudi K, Eibl B, Wallner S, Borkenstein M. Effect of an individualised training programme during weight reduction on body composition : a randomised trial. *Arch Dis Child*. 1999;81(5):426-428.

120. Solomon TPJ. Sources of inter-individual variability in the therapeutic response of blood glucose control to exercise in type 2 diabetes: Going beyond exercise dose. *Front Physiol*. 2018;9:896.

121. King NA, Hopkins M, Caudwell P, Stubbs RJ, Blundell JE. Individual variability following 12 weeks of supervised exercise: identification and characterization of compensation for exercise-induced weight loss. *Int J Obes (Lond)*. 2008;32(1):177-184.

122. Hammond BP, Stotz PJ, Brennan AM, Day AG, Ross R. Individual variability in waist circumference and body weight in response to exercise. *Med Sci Sports Exerc*. 2019;51(2):315-322.

123. Ruiz JR, Ortega FB, Rodriguez G, Alkorta P, Labayen I. Validity of resting energy expenditure predictive equations before and after an energy-restricted diet intervention in obese women. *PLoS One*. 2011;6(9):e23759.

124. Labayen I, Ortega FB, Ruiz JR, Lasa A, Simon E, Margareto J. Role of baseline leptin and ghrelin levels on body weight and fat mass changes after an energy-restricted diet intervention in obese women: effects on energy metabolism. *J Clin Endocrinol Metab*. 2011;96(6):E996-1000.

125. De Luis DA, Aller R, Izaola O, Gonzalez-Sagrado M, Conde R, Perez-Castrillon JL. Effects of lifestyle modification on adipocytokine levels in obese patients. *Eur Rev Med Pharmacol Sci.* 2008;12(1):33-39.

126. Bouchard C, Blair SN, Church TS, et al. Adverse metabolic response to regular exercise: is it a rare or common occurrence? *PLos One*. 2012;7(5):e37887.

127. Borel AL, Nazare JA, Baillot A, et al. Cardiometabolic risk improvement in response to a 3-yr lifestyle modification program in men: contribution of improved cardiorespiratory fitness vs. weight loss. *Am J Physiol Endocrinol Metab*. 2017;312(4):E273-E281.

ANEXES

Justification of the contribution to the publications

This is the author's implication for each Study of the Thesis, where the contribution of the author of the Thesis (MM) is specified for each study:

Study I

IL designed the study. **MM** and IL analyzed the data. **MM** and IL drafted the manuscript. **MM** generated the figures. LA, JRR, GR, CB, MC, RP, KW, AK, YM, DM, MG-G, FBO and LAM collected the data. All the authors participated in the interpretation of the results, critically revised the manuscript for important intellectual content and approved the final version of the submitted manuscript.

Study II

IL designed the study. **MM** analyzed the data and takes full responsibility for the integrity of the data analyses. **MM** and IL drafted the manuscript. **MM** generated the figures. **MM**, LA, BRV, and IL collected the data. All the authors participated in the interpretation of the results, critically revised the manuscript for important intellectual content and approved the final version of the submitted manuscript.

Study III

MM: Study concept and design, Acquisition, Statistical analysis, Drafting of the manuscript and Critical revision of the manuscript for important intellectual content. She is the guarantor. CC-S: Study concept and design, Acquisition, Statistical analysis, Drafting of the manuscript and Critical revision of the manuscript for important intellectual content. CA-B: Acquisition, analysis, or interpretation of data, Statistical analysis, Critical revision of the manuscript for important intellectual content and Administrative, technical, or material support. IC-R: Acquisition, analysis, or interpretation of data, Statistical analysis, Critical revision of the manuscript for important intellectual content and Administrative, technical, or material support. JRR:

Acquisition, analysis, or interpretation of data, Critical revision of the manuscript for important intellectual content and Supervision. FBO: Acquisition, analysis, or interpretation of data, Critical revision of the manuscript for important intellectual content and Supervision. IL: Study concept and design, Acquisition, analysis, or interpretation of data, Drafting of the manuscript, Critical revision of the manuscript for important intellectual content and Supervision.

Study IV

I.L. designed the study, analyzed the data, and drafted the manuscript. IL takes full responsibility for the integrity of the data analyses. IL, VMV, JRR, and FBO participated in the interpretation of the results. **MM,** LA, EM, and MO collected the data and critically revised the manuscript. IL, **MM**, LA, EM, MO, VMV, JRR, and FBO critically revised the manuscript for its intellectual content and approved the final version. IL is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study V

IL designed the study. **MM** analyzed the data and takes full responsibility for the integrity of the data analyses. **MM** and IL drafted the manuscript. **MM** generated the figures. **MM**, LA and IL collected the data. All the authors participated in the interpretation of the results, critically revised the manuscript for important intellectual content and approved the final version of the submitted manuscript.

Justificación de la contribución de la doctoranda a los estudios de la Tesis

A continuación, se expone la implicación de los autores para cada uno de los Estudios incluidos en la Tesis, en donde la contribución de la doctoranda (MM) se especifica para cada uno de los estudios:

Estudio I

IL diseñó el estudio. **MM** e IL analizaron los datos. **MM** e IL redactaron el manuscrito. **MM** generó las figuras. LA, JRR, GR, CB, MC, RP, KW, AK, YM, DM, MG-G, FBO and LAM recogieron los datos. Todos los autores participaron en la interpretación de los resultados, revisaron críticamente el contenido intelectual relevante del manuscrito y aprobaron la versión final del manuscrito presentado.

Estudio II

IL diseñó el estudio. **MM** analizó los datos y asume la total responsabilidad de la integridad de los análisis. **MM** e IL redactaron el manuscrito. **MM** generó las figuras. **MM**, LA, BRV, and IL recogieron los datos. Todos los autores participaron en la interpretación de los resultados, revisaron críticamente el contenido intelectual relevante del manuscrito y aprobaron la versión final del manuscrito presentado.

Estudio III

MM: diseño del estudio y del concepto, adquisición, análisis estadístico, redacción del manuscrito y revisión crítica del manuscrito para contenido intelectual importante. Ella es la responsable.CC-S: diseño del estudio y del concepto, adquisición, análisis estadístico, redacción del manuscrito y revisión crítica del manuscrito para contenido intelectual importante. CA-B: adquisición, análisis o interpretación de los datos, análisis estadístico, revisión crítica del manuscrito para contenido intelectual

importante y apoyo administrativo, técnico o material. IC-R: adquisición, análisis o interpretación de los datos, análisis estadístico, revisión crítica del manuscrito para contenido intelectual importante y apoyo administrativo, técnico o material. JRR: adquisición, análisis o interpretación de los datos, revisión crítica del manuscrito para contenido intelectual importante y supervisión. FBO: adquisición, análisis o interpretación de los datos, revisión crítica del manuscrito para contenido intelectual importante y supervisión. IL: diseño del estudio y del concepto, adquisición, análisis o interpretación de los datos, redacción del manuscrito, revisión crítica del manuscrito para contenido intelectual importante y supervisión.

Estudio IV

I.L. diseñó el estudio, analizó los datos y redactó el manuscrito. IL asume la total responsabilidad de la integridad de los análisis. IL, VMV, JRR, y FBO participaron en la interpretación de los resultados. **MM**, LA, EM, y MO recogieron los datos y revisaron de manera crítica el manuscrito. IL, **MM**, LA, EM, MO, VMV, JRR, y FBO revisaron críticamente el contenido intelectual y aprobaron la versión final. IL es la responsable de este trabajo, y por la tanto, tuvo acceso total a todos los datos del estudio y asume la responsabilidad de la integridad de los datos y de su exactitud en los análisis.

Estudio V

IL diseñó el estudio. **MM** analizó los datos y asume la total responsabilidad de la integridad de los análisis. **MM** e IL redactaron el manuscrito. **MM** generó las figuras. **MM**, LA y IL recogieron los datos. Todos los autores participaron en la interpretación de los resultados, revisaron críticamente el contenido intelectual relevante del manuscrito y aprobaron la versión final del manuscrito presentado.

SHORT *CURRICULUM VITAE*

MARÍA MEDRANO ECHEVERRÍA

Date of birth: 10/09/1992

Email: maria.medrano@unavarra.es

Academic training

- 2010-2014. Graduate degree in Sciences of Physical Activity and Sports (2504171). Faculty of Sciences of Physical Activity and Sports, University of Basque Country, Spain.
- 2014-2015. University Master degree in Nutrition and Health. Faculty of Farmacy, University of Basque Country, Spain.

Grants

- 2013-2014. Grant "Beca de colaboración 2013-2014" from the Spanish Ministry of Education, Culture and Sport.
- 2014-2019. Research Fellowship "Ayuda de Formación de Profesorado Universitario (FPU14/03329)" as part of the program "Formación y Movilidad dentro del Programa Estatal de Promoción del Talento y su Empleabilidad" from the Spanish Ministry of Education, Culture and Sport.
- 2017. Stay research fellowship "Ayuda de Movilidad para Estancias Breves en otros Centros Españoles y Extranjeros (EST17/00210)" from the Ministry of Science, Innovation and Universities.
- 2019. Grant "Ayuda a la Movilidad Internacional de Doctorandos" in the modality of "Ayudas para asistencias a Congresos" from the Public University of Navarra.

Awards

- 2014. "Premio Extraordinario de Fin de Carrera" in the "Grado de Ciencias de la Actividad Física y del Deporte" by the University of Basque Country.
- 2017. Award to the best poster presentation entitled "Associations of physical fitness with hepatic fat content in children with overweight/obesity" at the International Meeting of Research in Physical Activity and Health, in Cuenca from the 13th to 14th December 2017.
- 2019. "Premio Estrategia NAOS en el ámbito sanitario" for the project "Efecto adicional del ejercicio físico sobre la esteatosis hepática no alcohólica, la adiposidad y los factores de resigo cardiovascular en niños y niñas preadolescentes con sobrepeso que participan en un programa de educación familiar en estilos de vida saludables; proyecto EFIGRO" from the Spanish Ministry of Health, Cosumption and Social Well-being.

Research stays

- 2018. Centro de Estudios en Medición de la Actividad Física (CEMA). Universidad del Rosario. Bogotá, Colombia
  Duration: 3 month
- 2018. Instituto Mixto de Deporte de Deporte y Salud (i-MUDS). Universidad de Granada. Granada, Andalucía, Spain.
  Duration: 7 days
- 2016. Centro de Estudios Socio-Sanitarios (CESC). Universidad Castilla la Mancha. Cuenca, Castilla la Mancha, Spain.
  Duration: 7 days
- 2016. Karolinska Institutet. Stockholm, Sweden.
  Duration: 7 days.
- 2015. Instituto Mixto de Deporte de Deporte y Salud (i-MUDS). Universidad de Granada. Granada, Andalucía, Spain.
  Duration: 7 days

Research projects

María Medrano has participated in the following research projects:

- Project: The EFIGRO Study, "The Effect of Exercise on Hepatic Fat on Overweight Children Study".
  Founding source: Spanish Ministry of Health, "Fondo de Investigación Sanitaria del Instituto de Salud Carlos III", "Fondos de la Unión Europea (FEDER), una manera de hacer Europa" (PI13/01335), by the University of the Basque Country (GIU14/21) and by the Public University of Navarra "Ayudas a Grupos de Investigación".
  Principal investigator: Idoia Labayen Goñi
  Duration: 09/10/2014 - 08/10/2015
  Funding: 136.972€
  Role of the candidate: project manager, design and address of the exercise intervention sessions, data collection, data analysis, interpretation and scientific dissemination of results.
- Project: The PREDIKID Study "The PREvention of DIabetes in KIDs"
  Founding source: Spanish Ministry of Economy, Competition and Competitiveness, grant "Programa Estatal de Investigación, Desarrollo e Innovación Orientada a los Retos de la Sociedad" (DEP2016-78377-R).
  Principal investigator: Idoia Labayen Goñi
  Duration: 2016-2019
  Funding: 108.900€
  Role of the candidate: project manager, design and address of the exercise intervention sessions, data collection, data analysis, interpretation and scientific dissemination of results.

- Project: The HELENA Study "Healthy Lifestyle in Europe by Nutrition in Adolescence".
  Founding source: European Community Sixth RTD Framework Program (Contract FOODCT-2005-007034).
  Principal investigator: Luis Moreno Aznar
  Duration: 2005-2008
  Funding: 4.994.194,90€
  Role of the candidate: data analysis, interpretation and scientific dissemination of results of one article related to the Study.
- Project: PREFIT Study "Evaluación del FITness en PREescolares".
  Founding source: Ayudas a investigadores Ramón y Cajal a la iniciación de líneas de investigación (MINECO).
  Duration: 2013 to now
  Principal investigator: Francisco B. Ortega Porcel
  Role of the candidate: data collection, data analysis, interpretation and scientific dissemination of part of the results.
- Project: "Efecto combinado del ejercicio físico interválico de alta intensidad y la educación nutricional en la salud cardiovascular de niños y niñas de 9-10 años: estudio piloto".
  Founding source: "Ayudas a grupos de Investigación" from the Public University of Navarra.
  Duration: 2018
  Principal investigator: Idoia Labayen Goñi
  Role of the candidate: project manager, design and address of the exercise intervention sessions, data collection.
- Project: Estudio HEPAFIT, "Efectos de un programa de ejercicio sobre el metabolismo hepático, la grasa hepática y la salud cardiovascular en adolescentes con sobrepeso y obesidad de Bogotá, Colombia".
  Foundin Source: Instituto Colombiano para el Desarrollo de la Ciencia y la Tecnología "Francisco José de Caldas" COLCIENCIAS (code 59700 and no 122277757900).
  Duration: 2017-2019
  Principal investigator: Robinson Ramírez-Velez
  Role of the candidate: participation in their research stay, interpretation and scientific dissemination of results of one article related to the study.
- Project: Estudio PASOS, "Physical Activity, Sedentarism and Obesity in Spanish Youth".
  Foundin Source: Gasol Foundation and "Instituto Hospital del Mar de Investigaciones", Fundación Probitas, Banco Santander and Barça Foundation.
  Duration: 2019 to now
  Principal investigator: Santiago Felipe Gómez Santos
  Role of the candidate: project manager of the Public University of Navarra, and data collection.

- Project: EXERDIET-HTA Study, “Efectos de la tensión arterial, condición cardiorrespiratoria y riesgo cardiovascular de diferentes programas de ejercicio físico con intervención nutricional en personas con hipertensión arterial primaria”.
  Foundin Source: Vicerrectorado de investigación de la Universidad del País Vasco UPV-EHU
  Duration: 2013-2016
  Principal investigator: Sara Maldonado
  Role of the candidate: participation in the exercise sessions and data collection. Development of the grant “Beca de Colaboración 2013-2014”.

Publications

María Medrano has already published 14 scientific articles in Journals indexed in the JCR/Pubmed. To highlight, María is the first or second author in 50% of these papers. 71,4% of her publications are in the 1st quartile, with a mean impact factor of 5,146. The total number of citations is 50 (based on Google Scholar).

Scientific publications included in the present International Doctoral Thesis

1. Medrano M, Labayen I, Ruiz JR, Rodríguez G, Breidenassel C, Castillo M, Pedrero R, Widhalm K, Kafatos A, Manios Y, Molnar D, González-Gross M, Ortega FB, Moreno LA. Cardiorespiratory fitness, waist circumference and liver enzyme levels in European adolescents: The HELENA cross-sectional study. *Journal of Science and Medicine in Sport*. 2017;20(10): 932-936. Impact factor: 3.929 (Q1, Sport Sciences) (Study I of the Thesis).
2. Medrano M, Arenaza L, Hidalgo-Migueles J, Rodríguez-Vigil B, Ruiz JR, Labayen I. Associations of physical activity and fitness with hepatic steatosis, liver enzymes and insulin resistance in children. Submitted. (Study II of the Thesis).
3. Medrano M, Cadenas-Sánchez C, Álvarez-Bueno C, Cavero-Redondo I, Ruiz JR, Ortega FB, Labayen I. Evidence-based exercise recommendations to reduce hepatic fat content in youth- a systematic review and meta-analysis. *Progress in Cardiovascular Diseases*. 2018;61(2):222-231. Impact factor: 6.162 (Q1, Cardiac & Cardiovascular Systems) (Study III of the Thesis).
4. Labayen I, Medrano M, Arenaza L, Maíz E, Osés M, Martínez-Vizcaíno V, Ruiz JR, Ortega FB. Effects of exercise in addition to a family-based lifestyle intervention program on hepatic fat in children with overweight. *Diabetes Care*. 2019. Epub ahead of print. doi: 10.2337/dc19-0351. Impact factor: 15.270 (Q1, Endocrinology & Metabolism) (Study IV of the Thesis).
5. Medrano M, Arenaza L, Ramírez-Vélez R, Ortega FB, Ruiz JR, Labayen, I. Prevalence of responders for hepatic fat, overall and abdominal adiposity and gamma-glutamyl transferase in response to a lifestyle intervention with or without supervised exercise in children with overweight/obesity: secondary analyses of the EFIGRO randomized controlled trial. Submitted. (Study V of the Thesis).

Scientific publications related but not included in the present International Doctoral Thesis

1. González-Ruíz K, Medrano M, Correa-Bautista J, García-Hermoso A, Prieto-Benavides D, Tordecilla-Sanders A, Agostinis-Sobrinho C, Correa-Rodríguez M, Schmidt Rio-Valle J, González-Jiménez E, Ramírez-Vélez R. Comparison of bioelectrical impedance analysis, slaughter skinfold-thickness equations, and dual-energy x-ray absorptiometry for estimating body fat percentage in Colombian children and adolescents with excess of adiposity. *Nutrients*. 2018; 10(8):e1086. Impact factor: 4.171 (Q1, Nutrition and Dietetics).
2. Muñoz-Hernández V, Arenaza L, Gracia-Marco L, Medrano M, Merchan-Ramírez E, Martínez-Ávila D, Oses M, Ruiz JR, Ortega FB, Labayen I. Influence of Physical Activity on Bone Mineral Content and Density in Overweight and Obese Children with Low Adherence to the Mediterranean Dietary Pattern. *Nutrients*. 2018;10(8):e1075. Impact factor: 4.171 (Q1, Nutrition and Dietetics).
3. Arenaza L, Muñoz-Hernández V, Medrano M, Oses M, Amasene M, Merchán-Ramírez E, Cadenas-Sánchez C, Ortega FB, Ruiz JR, Labayen I. Association of breakfast quality and energy density with cardiometabolic risk factors in overweight/obese children: role of physical activity. *Nutrients*. 2018;10(8):e1066. Impact factor: 4.171 (Q1, Nutrition and Dietetics).
4. Labayen I, Ruiz JR, Arezana L, Medrano M, Tobalina I, Gracia-Marco L, Ortega FB, Rodríguez-Vigil B. Hepatic fat content and bone mineral density in children with overweight/obesity. *Pediatric Research*. 2018;84(5):684-688. Impact factor: 2.880 (Q1, Pediatrics).
5. Labayen I, Arenaza L, Medrano M, García N, Cadenas-Sánchez C, Ortega FB. Associations between the adherence to the Mediterranean diet and cardiorespiratory fitness with total and central obesity in preschool children: the PREFIT project. *European Journal of Nutrition*. 2017;57(8):2975-2983. Impact factor: 4.423 (Q1, Nutrition and Dietetics).
6. Delisle C, Henriksson P, Martínez-Vizcaíno V, Medrano M, Cadenas-Sánchez C, Arias-Palencia NM, Löf M, Ruiz JR, Labayen I, Sánchez-López M, Ortega FB. Does cardiorespiratory fitness attenuate the adverse effects of severe/morbid obesity on cardiometabolic risk and insulin resistance in children? A pooled analysis. *Diabetes Care*. 2017;40(11):1580–1587. Impact factor: 13.937 (Q1, Endocrinology & Metabolism).
7. Arenaza L, Medrano M, Amasene M, Rodríguez-Vigil B, Díez I, Graña M, Tobalina I, Maiz E, Arteche E, Larrarte E, Huybrechts I, Davis CL, Ruiz JR, Ortega FB, Margareto J, Labayen I. Prevention of diabetes in overweight/obese children through a family based intervention program including supervised exercise (PREDIKID project): study protocol for a randomized controlled trial. *Trials*. 2017;18(1):372. Impact factor: 2.067 (Q3, Medicine, Research & Experimental).

8. Medrano M, Maiz E, Maldonado-Martín S, Arenaza L, Rodríguez-Vigil B, Ortega FB, Ruiz JR, Larrarte E, Díez I, Sarasúa A, Tobalina I, Barrenechea L, Pérez-Asenjo J, Kannengiesser S, Manhães-Savio A, Echaniz O, Labayen I. The effect of a multidisciplinary intervention program on hepatic adiposity in overweight-obese children: protocol of the EFIGRO study. *Contemporary Clinical Trials*. 2015;45(Pt B):346-355. Impact factor: 2.052 (Q3, Medicine, Research & Experimental).

Scientific publications not related to the present International Doctoral Thesis

1. Arenaza L, Medrano M, Oses M, Huybrechts I, Diez I, Henriksson H, Labayen I. Dietary determinants of hepatic fat content and insulin resistance in children with overweight/obesity: a cross-sectional analysis of the PREDIKID study. *British Journal of Nutrition*. 2019;121(10):1158-1165. Impact factor: 3.319 (Q2, Nutrition and Dietetics).
2. Gómez-Butrón A, Arenaza L, Medrano M, Mora-González J, Cadenas-Sánchez C, Migueles JH, Muñoz-Hernández MV, Merchan-Ramirez E, Martínez-Avila D, Maldonado-Lozano J, Oses M, Tobalina I, Gracia-Marco L, Vicente-Rodríguez G, Ortega FB, Labayen I. Associations of dietary energy density with body composition and cardiometabolic risk in children with overweight and obesity: role of energy density calculations, under-reporting energy intake and physical activity. *British Journal of Nutrition*. 2019;121(9):1057-1068. Impact factor: 3.319 (Q2, Nutrition and Dietetics).
3. Labayen I, Margareto J, Maldonado-Martin S, Gorostegi I, Illera M, Medrano M, Barrenechea L and Larrarte E. Independent and combined influence of the FTO rs9939609 and MC4R rs17782313 polymorphisms on hypocaloric diet induced changes in body mass and composition and energy metabolism in non-morbid obese premenopausal women. *Nutrición Hospitalaria*. 2015;31(5):20-25. Impact factor: 1.497 (Q1, Nutrition and Dietetics).

Other merits

- Member of the ELIKOS ("ELikadura, arIKeta fisikoa eta Osasuna: Nutrición, Actividad Física y Salud") research group, EXERNET ("Red de Investigación en Ejercicio Físico y Salud para Poblaciones Especiales"), the Research Institute "Institute for Innovation & Sustainable Development in Food Chain (IS-FOOD)" and the "Instituto de Investigación Sanitaria de Navarra" (IDISNA).
- More than 35 contributions and participations in both national and international meeting or conferences.
- More than 15 participations in courses related with exercise and health.
- Participation in the organization of 2 meetings.
- 90 hours of teaching experience in both the University of Basque Country and the Public University of Navarra.