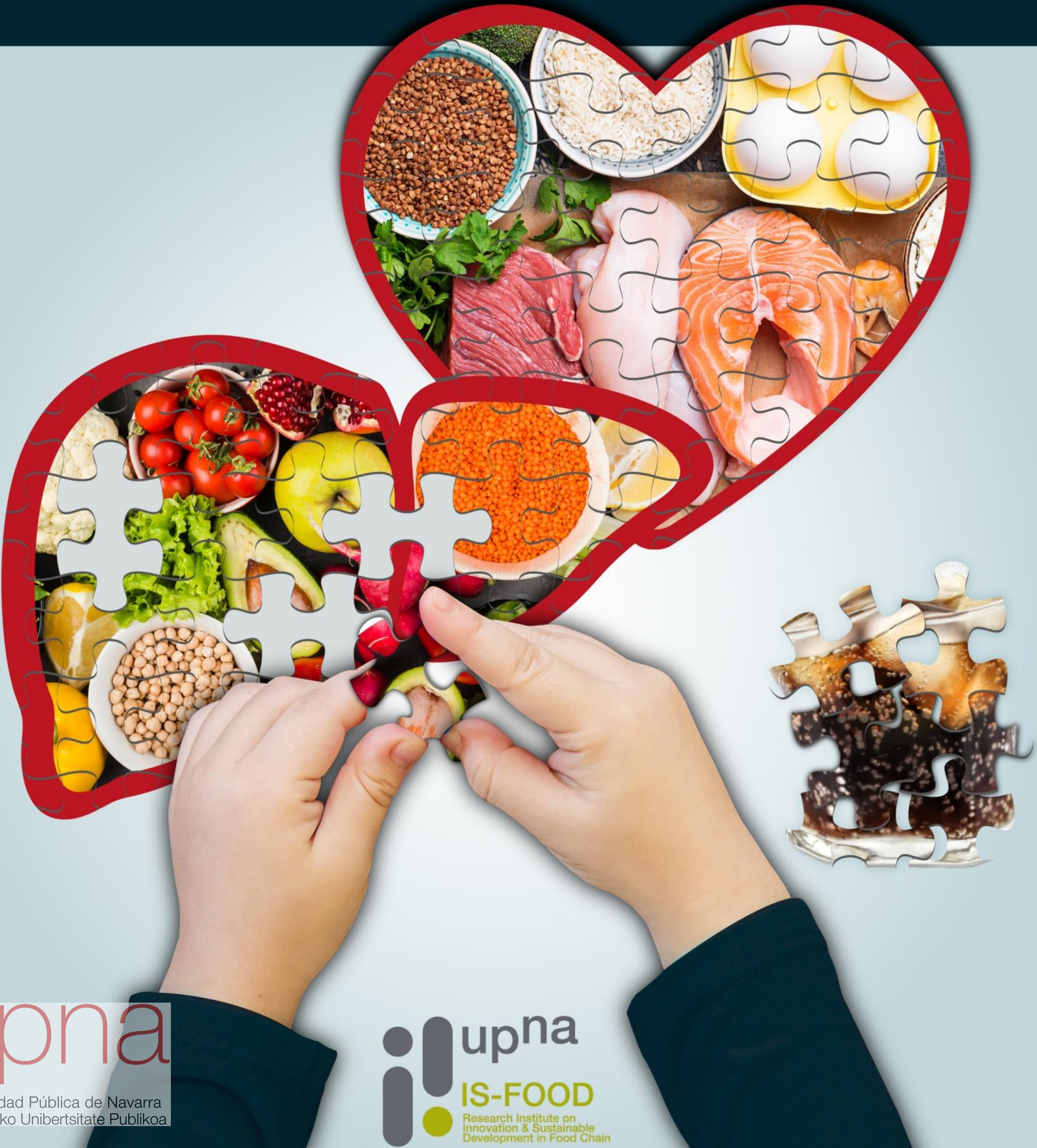
upna
Universidad Pública de Navarra
Nafarroako Unibertsitate Publikoa

DOCTORAL PROGRAMME IN HEALTH SCIENCES

THE INFLUENCE OF DIETARY HABITS ON HEPATIC STEATOSIS AND CARDIOVASCULAR HEALTH IN CHILDREN

Author ***Lide Arenaza Etxeberria*** | Director ***Idoia Labayen Goñi***



upna
Universidad Pública de Navarra
Nafarroako Unibertsitate Publikoa

upna
IS-FOOD
Research Institute on Innovation & Sustainable Development in Food Chain

Universidad Pública de Navarra
Nafarroako Unibertsitate Publikoa

Universidad Pública de Navarra
Nafarroako Unibertsitate Publikoa

upna

Universidad Pública de Navarra
Nafarroako Unibertsitate Publikoa

NAZIOARTEKO DOKTOREGO TESIA

HAURREN ELIKADURA-OHITUREN ERAGINA GIBEL-ESTEATOSIAN ETA OSASUN KARDIOBASKULARREAN

Tesiaren Egilea: Lide Arenaza Etxeberria

Tesiaren Zuzendaria: Idoia Labayen Goñi

NAFARROAKO UNIBERTSITATE PUBLIKOA

OSASUN ZIENTZIAK SAILA

Universidad Pública de Navarra
Nafarroako Unibertsitate Publikoa

upna

Universidad Pública de Navarra
Nafarroako Unibertsitate Publikoa

The current International Doctoral Thesis is presented as a *compendium* of five studies. The references of these five studies included in this work are the following:

Ondorengo Nazioarteko Doktorego Tesia bost ikerlanek osasutako ikerketen bilduma bat bezala aurkeztuta dago. Lan honetan aurkitzen diren argitalpenen erreferentziak ondorengoak dira:

Study I: Labayen I, Arenaza L, Medrano M, García N, Cadenas-Sanchez 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. Eur J Nutr. 2018;57(8):2975-2983. Factor de impacto 4.449. Área temática: Nutrition & Dietetics (Q1).

Study II: Arenaza L, Huybrechts I, Ortega FB, Ruiz JR, De Henauw S, Manios Y, Marcos A, Julián C, Widhalm K, Bueno G, Kersting M, Kafatos A, Breidenassel C, Pedrero-Chamizo R, Gottrand F, González-Gross M, Moreno LA, Labayen I. Adherence to the Mediterranean diet in metabolically healthy and unhealthy overweight and obese European adolescents: the HELENA study. Eur J Nutr. 2019;58(7):2615-2623. Factor de impacto: 4.664. Área temática: Nutrition & Dietetics (Q1)

Study III: Arenaza L, Muñoz-Hernández V, Medrano M, Oses M, Amasene M, Merchán-Ramírez E, Cadenas-Sanchez 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):1066. Factor de impacto 4.171. Área temática: Nutrition & Dietetics (Q1)

Study IV: Arenaza L, Medrano M, Oses M, Huybrechts I, Díez I, Henriksson H, Labayen I. Dietary determinants of hepatic fat content and insulin resistance in overweight/obese children: a cross-sectional analysis of the Prevention of Diabetes in Kids (PREDIKID) study. Br J Nutr. 2019; 121(10):1158-1165. Factor de impacto 3.334. Área temática: Nutrition & Dietetics (Q2)

Study V: Arenaza L, Medrano M, Oses M, Amasene M, Díez I, Rodríguez-Vigil B, Labayen I. The Effect of a Family-Based Lifestyle Education Program on Dietary Habits, Hepatic Fat and Adiposity Markers in 8-12-Year-Old Children with Overweight/Obesity. Nutrients. 2020;12(5):1443. Área temática: Nutrition & Dietetics

The author of the current International Doctoral Thesis Lide Arenaza Etxeberria was able to perform this project thanks to the predoctoral grant she obtained from the Department of Education Government of the Basque Country (PRE_2016_1_0057, PRE_2017_2_0224, PRE_2018_2_0057, PRE_2019_2_0004). This is an International Thesis as Lide Arenaza Etxeberria conducted a three-month international research stay at the Saban Research Institute, Children´s Hospital of Los Angeles-University of Southern California. She was beneficiary of a complementary grant (EP_2019_1_0013) from the Government of the Basque Country during the period of the stay (resolution of november 13th 2018, of the Vice-Department of Universities and Research, which resolves the renewed grants given through Order of May 2 2018, of the Ministry of Education, correspondig to the 2018-2019 academic year; Official Journal of the Basque Country, may 21st 2018).

Nazioarteko Doktorego Tesi honetako egilea den Lide Arenaza Etxeberriak Eusko Jaurlaritzako Hezkuntza Sailaren eskutik eskuratutako doktorego-aurreko laguntza bati esker (PRE_2016_1_0057, PRE_2017_2_0224, PRE_2018_2_0057, PRE_2019_2_0004) burutu ahal izan du honako ikerlana. Lide Arenaza Etxeberriak the Saban Research Institute, Children´s Hospital of Los Angeles-University of Southern California-n hiru hilabeteko iraupena duen nazioarteko ikerketa egonaldia egin zuenez, Nazioarteko Doktorego Tesia kontsideratzen da. Egonaldian zehar Eusko Jaurlaritzaren eskutik laguntza osagarri baten (EP_2019_1_0013) onuraduna izan zen (Unibertsitate eta Ikerketako sailburuordetzaren 2018ko azaroaren 13ko ebazpena; Hezkuntza Saila 2018ko maiatzaren 2ko Aginduan (Euskal Herriko Agintzaritzaren Aldizkaria, 2018ko maiatzaren 21ean egindako deialdiko laguntzak, 2018-2019eko ikasturtea).

RESEARCH PROJECTS/ IKERKETA PROIEKTUAK

This International Doctoral Thesis includes data from the following research projects:

Nazioarteko Doktorego Tesi honek hurrengo ikerketa proiektu hauetatik eratorritako datuak biltzen ditu:

The PREFIT Study "Assessing FITness in PREschoolers"

Funding support: Spanish Ministry of Economy (MINECO). Ayudas a investigadores Ramón y Cajal para incorporación en inicios de líneas de investigación

Principal investigator: Francisco Ortega, Ramón y Cajal Researcher

Center coordinator: Idoia Labayen

Date: 2013-2016

The HELENA Study "Healthy Lifestyle in Europe by Nutrition in Adolescence"

Funding support: European Community Sixth RTD Framework Program (Con-tract FOODCT-2005-007034)

Principal investigator: Luis Moreno

Date: 2005-2008

Funding: 4.994.194,90€

The effect of a multidisciplinary intervention program on hepatic adiposity in overweight-obese children (Efectos de un programa de ejercicio físico sobre la grasa hepática en niños y niñas con sobrepeso)

Funding support: Carlos III Health Institute, Fondos Feder "Una manera de hacer Europa"

Principal investigator: Idoia Labayen

Date: 09/10/2014-08/10/2015

Funding: 136.972€

Prevention of diabetes in overweight/obese children through a family based intervention program including supervised exercise (PREDIKID Project)

Funding support: Spanish Ministry of Economy, Competition and Competitiveness "Programa Estatal de Investigación, Desarrollo e Innovación Orientada a los Retos de la Sociedad" (DEP2016-78377-R).

Principal investigator: Idoia Labayen

Date: 2016-2019

Funding: 108.900€

Other fundings:

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

The author of the current Thesis was additionally granted by *Ayudas a la Movilidad Internacional de Doctorandos; Ayudas para Asistencia a Congresos*

ACKNOWLEDGEMENTS/ESKERRAK

Badirudi iritsi dela beste etapa baten amaiera ere. Doktorego tesirako zubia eraikiko zidan masterra ekiteaz bat murgildu nintzen ikerketan. Hasieran, master garaian, helduekin, eta geroago haurrekin eta hauen familiako senideekin. Baina beti ere pertsonekin; pertsonak baitira egiten dugun orori zentzua ematen diotenak. Dagoeneko bost urte pasa dira ordutik, eta urte hauetan zehar milaka bizipen bizitzeko, eta bizipen hauei lotutako ehunka pertsona ezagutzeko aukera izan dut. Ondorengo lerroetan pertsona hauei jositako oroitzapenak gogora ekarri eta hauei eskerrak eman nahi nizkieke.

Hasteko, nire tesiko zuzendariari eman nahi nizkioke eskerrak. Eskerrik asko Idoia, unibertsitatean nire irakasle izan zinen garaian zure ikerketa taldera batzeko egin zenidan proposamenagatik. Ikerketa arloko ezagutzaz gain, asko ikasi dut zure profesionaltasuna, lanerako gaitasuna eta oztopoen gainetik aurrera egiteko duzun kemenarengandik. Zuri esker niretzat berria izan den ikerketa mundua ezagutzeko aukera izan dut; eta batzuetan bidea aldapatsua eta malkartsua dela konturatu naizen arren, ez naiz damu denbora honetan zehar bide honetatik abiatu izanagatik. Mila esker gaitasun desberdinak garatzeko bultzada eman eta nire ibilbide zientifikoaren kalitatea bermatzeagatik.

Urte hauetako proiektuetan lankide eta kolaboratzaile izan zaretenok ere ibilbide honi zuen nortasuna eskaini diozue. Darte las gracias a ti María, por tantos momentos compartidos; desde los días más intensos que incluían jornadas de despacho, sesiones y evaluaciones, hasta aquellas noches que se alargaban en Granada. Mila esker Maddi, taldera sartu zinenetik bide honi zure bizitasuna eta ilusioa eskaintzeagatik, baita Iruñan zure ingurukoen artean lekutxo bat egiteagatik ere. Eskerrik asko urte hauetan neurri handi batean ikerketa proiektuek aurrera egitea ahalbidetu duzuen praktiketako ikasle eta kolaboratzaile guztioi; Leire, Eukene, Iker, Amaia, María G, María O, Iñaki, Ander, Andoni, Marta, Maitane, Unai, Jon, Maite, Amaia, Ane, Oihane, Olaia…

Bizi-ohituren aldaketa prozesua zuen laguntza eta profesionaltasun gabe askoz ere konplikatuagoa litzatekeelako, Iratxe, Naiara eta Edurne; familiei egoera desberdinen aurrean aurrera egiteko eskaini dizkiezuen tresna, baliabide eta babesagatik eskertu nahi dizuet. Baita zuei ere, Ilargi, Pablo, Aitor, Natalia, Maitane, Sara eta Alicia, etapa honen hasieran elkarrekin konpartitutako momentuengatik. Eskerrik asko Diego, Farmazia Fakultatean bertan, eta handik haratago elkarrekin konpartitu ditugun momentuei zure xarma eta bizi-grina emateagatik.

Eskerrak eman nahi dizkiet EFIGRO ikerketan parte hartu duten familia guztiei, oso esperientzia aberasgarria izan baita zuekin lan egitea eta zuen bizitzetan lagun izan dezakegula pentsatzea. Baita froga kliniko guztietan parte hartu duten mediku, erizain eta langileei ere. Eskerrik asko Euskal Herriko Unibertsitatea eta Nafarroako Unibertsitate Publikoari, eta hauetan ezagututako guztioi, nire ikasketa eta ikerketa sendotzearen parte izanagatik. San Fermin Ikastolari eta bereziki bertako Arantxa erizaina eta Inge nutrizionistari; Noaingo San Miguel eskolari, eta proiektuetan parte hartu duten beste ikastetxe guztiei. Mila esker tesia gauzatu ahal izateko Euskal Jaurlaritzako Hezkuntza Sailari eskuratutako beka predoktoralarengatik eta nazioarteko egonaldirako laguntzagatik, baita Nafarroako Unibertsitate Publikoari Bostoneko American Society for Nutrition-eko kongresura joateko mugikortasun beka esleitzeagatik ere.

Agradecer al equipo de Granada por vuestro compañerismo y calidad investigadora que habéis aportado durante estos años. Muchas gracias Victoria, por tu alegría, generosidad y por todos los momentos que hemos compartido. Gracias también a ti Cristina, por tu dedicación e implicación en los trabajos y por esa actitud tan positiva frente a todo. Gracias a Guille, Borja, Pepe, Eli, Daniela, Juanma, Fran y Pedro, Mireia de Castellón, José Juan, Fer, María, María José, Jairo, Irene, Jonatan y Fran…por aportar vuestro intelecto a los proyectos y vuestro salero a los momentos que hemos vivido juntos. Agradecer también al equipo de EFIBAR de Almería y GENUD de Zaragoza, por vuestro recibimiento y buen trato.

Etapa hau Gasteizen hasi arren, bideak beti gogoko izan dudan Iruñara eraman ninduen. Bertako oroitzapen askorekin geratzen naiz, baita bertan ezagututako lagunekin ere. Mila esker Mikel zure eskuzabaltasuna, poztasuna eta ilusioa nirekin konpartitzearren, eta Iruñako kuadrilakoengana hurbiltzearren. Eskerrik asko Yeray, Fabri, Gari…eta beste guztioi Caldererian, eta bereziki Suberrin, murgildutako ordu luze zoragarriengatik. Eskerrik asko Arazeli, zure euskarako irakasle izaten hasi eta horren harreman polita eta berezia sortu dugulako. Tuve mucha suerte cuando elegí aquel piso en la Travesía Vuelta del Castillo. Fue ahí donde conocí a una inmejorable compañera de piso y amiga que desprendía una alegría canaria; muchas gracias Begoña por tantos momentos que he vivido contigo y por la familia tan maravillosa que tienes. María, gracias por ser tan simpática y agradable; y por todas tus historietas que nunca me aburría de escuchar. Mila esker Andrea eta Maddiri, ezagutu ginen unetik batzen gaituen konexio horri eustearren, zuek ezagutu izana plazer bat izan da. Eskerrik asko Uraitz, konpartitu ditugun momentu guztiei zure umorea eransteagatik. Mila esker Manu, zure adiskidetasun eta eskuzabaltasunagatik. Eskerrik asko Miren eta Ainhoa zuekin bizitako *une eta solasaldiengatik, mila esker Bigarren Hezkuntzako masterrean ezagututako besteoi ere.*

I would like to thank Inge Huybrechts for her collaboration during my brief stay in Lyon. Thanks to Michael Goran, Children´s Hospital of Los Angeles and The Saban Research Institute for giving me the opportunity to carry out my research stay with you. Thank you so much to all the members from the Goran´s Lab for sharing your knowledge and expertise with me. Thanks for doing your best for the baby´s and family´s health, you should be very proud of your job. My special thanks to Carla and Claudia for being so nice with me; also thanks to Yesica, Roshonda, Elizabeth, Emily, Briannie, Danielle, Sinthia… I would also like to acknowledge Jessica and Jacques for being my peculiar little family in LA. Thank you Louis, Mohan, Sudip and Lara as well for the adventures we lived in California.

Munduko beste puntatik herrira bueltauko naz, bertara. Eskerrik asko nirekin egon zarien danoi. Eskerrak kuadrilakuoi, urte honeitan ziher barriketaldixek eta momentu onek etenaldirik euki eztauielako; eta bereziki kuadrilako lagun gertukuenoi, bizitzako arlo danetan zuekin kontau neikela dakitxetelako. Eskerrak be Jacako lagunei; udan zein neguen, eguzkitxan zein edurretan, Pirineuen eta zuen konpainian gozamenagatik. Nire eskerrik berezixenetakuak Mariandako, gure adiskidetasuna unibersidadeko ikasketek sortu eta urtiek sendotu dauielako, beti gertu eta nirekin sentiruten zaitxutelako. Zelan zurekin ahaztu Jon, urte honeitako zure konpainia neguko eguzki errainu goxuena izen dalako; zure pazientziak eta nigan eta nire gaitxasunengan siniste horrek be esker berezixenak mereziru daue.

Bukatzeko, eta batez be, bihotzez eskerrik asko etxeko eta familixako danoi bizitzako momentu onak disfrutetan, eta ez hain onetan mundua bukatzen eztala erakustiarren. Beti be nigan sinistu eta zuen babesa emutiarren. Gaur egungo nire izetia zuetako danok ereindako maitxasun, balore eta nortasunan fruitu dalako.

CONTENTS/AURKIBIDEA

LIST OF TABLES

STUDY I

STUDY II

STUDY III

STUDY IV

STUDY V

LIST OF FIGURES

STUDY I

STUDY II

STUDY V

ABSTRACT

The prevalence of childhood overweight/obesity has dramatically increased worldwide, being Spain one of the European countries with the highest prevalence. The growing prevalence of overweight/obesity in children is of major public health concern due to its increased risk of morbidity and mortality. Excess of adiposity, and particularly visceral adiposity, is associated with cardiovascular disease (CVD) risk factors, such as insulin resistance, hypertension, dyslipidaemia and hepatic steatosis. Thereby, obesity increases the risk of developing CVD, type 2 diabetes (T2D) and non-alcoholic fatty liver disease (NAFLD). Metabolic syndrome (MetS) encompasses the condition where the previously mentioned CVD risk factors appear together, and NAFLD is considered its hepatic manifestation. Interestingly, there also exists a subgroup of patients that despite having obesity, they do not present obesity-related comorbidities. This condition is known as metabolically healthy obesity (MHO) and little is known about the factors involved in this healthy metabolic phenotype in children.

Healthy lifestyle habits have been shown to protect cardiovascular health. Evidence suggests that while Mediterranean dietary pattern (MDP) seems to prevent CVD, sugar-rich foods, and particularly, sugar-sweetened beverages (SSB), might promote hepatic fat deposition by leading to hepatic steatosis and NAFLD progression. Breakfast consumption has been also considered as an important obesity-preventing strategy. Physical activity also protects cardiovascular health, as it is established that its regular practice improves cardiorespiratory fitness (CRP), a powerful marker of cardiovascular health. Therefore, lifestyle interventions focused on the promotion of both healthy diet and physical activity are crucial for the prevention and treatment of obesity-related comorbidities in children with overweight/obesity. Nonetheless, sugar-rich and fibre-and nutrient-poor diets, as well as skipping breakfast or having an unhealthy one, are common in paediatric population. Considering that children are still acquiring and establishing their habits, lifestyle changes for

the adoption of healthy habits are more likely to succeed in childhood. In this line, nutritional education can be an appropriate tool to encourage children to learn how to make healthier dietary choices in order to make an improvement in their energy balance and body composition.

Our hypothesis is that MDP and eating behaviours as having a healthy breakfast will prevent adiposity and its related comorbidities in children. In contrast, energy-dense diets based on the consumption of ultra-processed foods and SSB will negatively influence liver and cardiovascular health of overweight/obese children. Furthermore, healthy lifestyle programs based on nutritional education and physical activity promotion might improve dietary habits of children with overweight/obesity by increasing the awareness and knowledge between lifestyle and health of both children and parents.

However, most of studies examining the influence of dietary habits on hepatic steatosis and cardiovascular health are in adults, and the evidence in children with overweight/obesity is scarce. Therefore, the aims of the current International Doctoral Thesis were the following: 1) to examine the associations of the adherence to the MDP and CRP with total and central adiposity in preschool children; 2) to examine the adherence to the MDP in metabolically healthy and unhealthy overweight/obese European adolescents; 3) to analyze the associations of breakfast quality and energy density from both solids and beverages with CVD risk factors, and to explore whether physical activity levels may attenuate these relationships in children with overweight/obesity from the north and south of Spain; 4) to examine the association of food and food components, dairy desserts and substitutes (DDS), SSB, as well as total and added sugars, with hepatic fat and insulin resistance in children with overweight/obesity; and 5) to explore the effectiveness of a 22-week family-based lifestyle education program on dietary habits, and to analyze the associations of changes in dietary intake with the reductions of percent hepatic fat and adiposity in children with overweight/obesity.

Five works were carried out in the background of the HELENA and the PREFIT cross-sectional studies, together with EFIGRO, PREDIKID and ActiveBrains trials in order to address the established aims. The conclusions from the current Thesis are: I) high adherence to the MDP might prevent pre-school children from abdominal adiposity and later CVD, whereas high CRP seems to have benefits not only preventing abdominal adiposity, but also total adiposity in pre-school children. These findings highlight the importance of promoting a healthy lifestyle including a MDP and physical activity since early childhood; II) high adherence to the MDP and its components, such as the intake of fish, seem to have a protective role against adiposity-related cardiovascular risk factors development in adolescents with overweight/obesity, leading to present a MHO phenotype. Moreover, CRP may also play a key role in the healthy metabolic phenotype, III) breakfast with high quality and with low energy density from both solids and beverages might be beneficial to prevent CVD risk factors in children with overweight/obesity. Therefore, besides breakfast components and its quality, breakfast energy density should also be as an additional dimension in nutrition-based education programs for an early prevention of CVD risk factors; IV) A diet moderate in cereals intake and low in SSB and their sugars content seems to be related to lower hepatic fat content in children with overweight/obesity. A 22-week family-based healthy lifestyle program mostly focused on nutritional education is effective on improving dietary habits and reducing SSB consumption in 8-12 years-old children with overweight/obesity. In addition, the reduction of SSB consumption was shown to be effective on reducing hepatic fat content in these children, by supporting the detrimental effects of sugary drinks on hepatic steatosis. Interventions in children with overweight/obesity should include specific strategies focused on the reduction and avoidance of SSB for the prevention and treatment of hepatic steatosis and its consequent CVD comorbidities.

The findings from the present Thesis might have clinical implications in the design and development of future healthy lifestyle education programs focused on the prevention of obesity and its related comorbidities. Nutritional education programs should include contents targeting the promotion of MDP, the consumption of a healthy breakfast, a diet low in total and added sugars and, particularly, SSB avoidance, for the prevention and treatment of obesity, hepatic steatosis and CVD risk factors in children. Altogether, health policies targeting healthy lifestyle promotion and sugar-rich food and beverages advertising restriction might additionally help to prevent and tackle obesity, hepatic steatosis and CVD, together with increasing the well-being of people and reducing the public health costs.

LABURPENA

Haurtzaroko gainpisua/obesitatearen prebalentzia nabarmenki handitu da munduan zehar, prebalentzia handiena duen Europako herrialdeetako bat Espainia delarik. Haur gainpisua/obesitateak erikotarsun eta hilkortasun arriskua areagotzen duenez, honen prebalentziaren gorakadak kezka nagusia suposatzen du osasun publikoarentzat.

Gehiegizko adipositatea, eta bereziki erraietako adipositatea, insulinarekiko erresistentzia, hipertentsioa, dislipemiak eta esteatosi hepatikoa bezalako gaixotasun kardiobaskularraren (GKB) arrisku faktoreekin lotuta dago. Horrela, obesitateak GKB, 2 motako diabetea eta gibel koipetsu ez-alkoholikoa garatzeko arriskua areagotzen du. Aurrez aipatutako GKB arrisku faktoreen agerpen bateratua ematen den baldintzari sindrome metabolikoa deritzo, eta gibel koipetsu ez-alkoholikoa (ingelesez NAFLD; *non-alcoholic fatty liver disease*) honakoaren gibeleko adierazpena kontsideratzen da.

Interesgarriki, badago obesitatea izan arren, obesitateari lotzen zaizkion komorbilitateak pairatzen ez dituen pertsonen azpitalde bat. Honako baldintza hau metabolikoki osasuntsua den obesitatea izendatzen da, eta haurrei dagokienez, metabolikoki osasuntsua den fenotipo honen eragileen inguruan ez da gehiegi ezagutzen.

Elikadura-ohitura osasuntsuek osasun kardiobaskularra babesten dutela ikusi da. Ebidentzian oinarrituz, patroi dietetiko Mediterranearrak (PDM) GKB prebenitzen duela dirudien bitartean, azukreetan aberatsak diren elikagaiek, eta bereziki, azukredun edariek gibeleko gantz pilaketa sustatzen dute, esteatosi hepatikoa eta NAFLD-en progresioa eraginez. Gosariaren kontsumoa ere obesitatea prebenitzeko estrategia garrantzitsua kontsideratu izan da. Bestalde, ariketa fisikoak osasun kardiobaskularra babesten du; izan ere, bere jarduera erregularrak osasun kardiobaskularraren markatzaile garrantzitsua den bihotz-arnas gaitasuna hobetzen du. Hori dela eta, gainpisua/obesitatea duten haurrei bideratutako elikadura osasuntsuan eta ariketa fisikoaren sustapenean oinarritzen diren bizi-ohituren esku-hartze programak ezinbestekoak dira obesitateari loturiko erikortasunaren prebentzio eta tratamendurako. Hala ere, azukreetan aberatsak eta zuntz eta mantenugaietan urriak diren dietak, ez gosaltzea edota gosaria ez-osasungarria izatea ohikoak dira haurretan. Haurren kasuan, oraindik beraien ohiturak bereganatzen eta finkatzen ari direla aintzat hartuta, haurtzaroan kokatzen diren bizi-ohitura osasungarriak barneratzeko bizi-ohituren aldaketek arrakasta gehiago izateko aukera handiagoa izaten dute. Honen harira, hezkuntza nutrizionala tresna egokia izan liteke haurrek elikagai-aukera osasungarriagoak egiten ikas dezaten, eta oreka energetikoa eta gorputz-konposizioa hobetzeko helburua oinarri izanik.

Gure hipotesia PDM jarraitzeak eta modu osasungarrian gosaltzeak haurren gehiegizko gantz-pilaketa eta honi loturiko konplikazioak prebenituko lituzkeela da. Elikagai ultra-prozesatu eta azukredun edarien kontsumoan oinarritutako energetikoki dentsoak diren dietek, ordea, gainpisua/obesitatea duten haurren gibel osasuna eta osasun kardiobaskularrean modu negatiboan eragingo lukete. Gainera, hezkuntza nutrizionalean eta ariketa fisikoaren sustapenean oinarritutako bizi-ohituren esku-hartze programek bizi-ohiturak eta osasunaren arteko harremanaren inguruko kontzientzia eta ezagutza handituko luke haur zein gurasoetan. Ildo honetatik abiatuz, horrelako esku-hartze programek gainpisua/obesitatea duten haurren elikadura-ohiturak hobe litzakete.

Dena den, elikadura-ohiturek esteatosi hepatikoan eta osasun kardiobaskularrean duten eragina aztertzen duten ikerlan gehienak helduetan izaten dira, haurrei dagokien ebidentzia urria delarik. Horregatik, honako Nazioarteko Doktorego Tesiaren helburuak ondorengoak dira: 1) PDM-ren atxikidura eta bihotz-hodiko gaitasuna eta eskolaurreko haurren adipositate totala eta zentralaren arteko erlazioak aztertzea; 2) PDM-ren atxikidura aztertzea metabolikoki osasuntsua eta metabolikoki ez-osasuntsua den gainpisua/obesitatea pairatzen duten Europako nerabeetan; 3) gosariaren kalitatea eta solido zein likidoen dentsitate energetikoa arrisku faktore kardiobaskularrekin erlazionatzea; eta ariketa fisiko mailak erlazio hauek arindu ditzakeen aztertzea gainpisua/obesitatea pairatzen duten Espainiako iparralde eta hegoaldeko haurretan; 4) elikagai eta elikagaien konposatuak, esneki-postre eta ordezkoak, azukredun edariak eta azukre total zein gehitutakoek gibeleko gantza eta intsulinarekiko erresistentziarekin duten erlazioa aztertzea gainpisua/obesitatea duten haurretan; eta 5) bizi-ohituren hezkuntza programa familiar batek 22 astetan elikadura ohituretan duen eragina ikertzea, eta elikadura ohituretan emandako aldaketak gibeleko gantza eta adipositatean murrizketarekin erlazionatzea gainpisua/obesitatea duten haurretan.

Ezarritako helburu hauek jorratzeko HELENA eta PREFIT izeneko zeharkako ikerketak, eta EFIGRO, PREDIKID eta ActiveBrains esku-hartze ikerketak batzen dituen testuingurua erabili da. Tesi honen ondorioak ondorengoak dira: I) PDM-kiko atxikidurak eskolaurreko haurrak gantz-pilaketa abdominala eta etorkizuneko GKB izatea prebeni lezakeen bitartean, bihotz-arnas gaitasunak gantz-pilaketa abdominala zein totala prebenitzeko onurak izan litzake. Aurkikuntza hauek PDM eta ariketa fisikoa barnebiltzen dituen txikitatik bizi-ohitura osasungarriak sustatzearen garrantzia azpimarratzen dute; II) PDM-kiko atxikidurak eta arraina bezalako dietako elikagaiek adipositateari loturiko GKB-ren arrisku faktoreen garapenaren aurrean funtzio babesgarria izan dezateke gainpisua/obesitatea pairatzen duten nerabeetan, metabolikoki osasuntsua den obesitatea izatera daramalarik. Era berean, bihotz-arnas gaitasunak ere rol garrantzitsua bete lezake metabolikoki osasuntsua den fenotipoan; III) Kalitate altuko eta solido zein edari jatorriko dentsitate energetiko baxuko gosaria GKB-ren arrisku faktoreak prebenitzeko onuragarria izan liteke gainpisua/obesitatea pairatzen duten haurretan. Ondorioz, gosariaren osagaiak eta honakoaren kalitateaz haratago, gosariaren dentsitate energetikoa ere oinarri nutrizionaleko hezkuntza programetan dimentsio gehigarritzat hartu beharko litzateke GKB-ren arrisku faktoreen prebentzio goiztiarra bermatzeko; IV) Zerealen neurrizko kontsumoa duen eta azukredun edarietan eta hauen azukre edukian urria den dieta bat gibeleko gantz-pilaketa txikiago batekin erlazionatzen da gainpisua/obesitatea duten haurretan. Hezkuntza nutrizionalean oinarritutako 22 astetako bizi-ohituren hezkuntza programa familiar bat elikadura-ohiturak hobetzeko eta azukredun edarien kontsumoa murrizteko eraginkorra izan daiteke gainpisua/obesitatea pairatzen duten 8-12 urte bitarteko haurretan. Horrez gain, azukredun edarien kontsumoaren murrizketa haur hauen gibeleko gantz edukia murrizteko eraginkorra dela behatu da, azukreetan aberatsak diren edariek esteatosi hepatikoan duten eragin kaltegarria azpimarratuz. Gainpisua/obesitatea pairatzen duten haurrei bideratutako esteatosi hepatikoaren eta GKB-ren prebentzioa helburu duten eta esku-hartze programek azukredun edarien kontsumoaren murrizketa eta ekiditean oinarritutako berariazko estrategiak barnebildu beharko lituzkete.

Tesi honen aurkikuntzek obesitatea eta ondoriozko konplikazioen prebentzioa helburu duten etorkizuneko bizi-ohitura osasungarrien esku-hartze programen diseinua eta garapenerako inplikazio klinikoak izan ditzake. Haurrei zuzendutako hezkuntza nutrizionaleko programek PDM, gosari osasungarria, azukre eta gehitutako azukreetan urria den dieta, eta bereziki, azukredun edarien saihestea sustatzen duten edukiak barnebildu beharko lituzkete obesitatea, esteatosi hepatikoa eta GKB-ren arrisku faktoreen haurren prebentzio eta tratamendurako.

Bizi-ohitura osasungarriak eta azukreetan aberatsak diren elikagaien iragarkien murrizketak helburu dituzten osasun politikek obesitatea, esteatosi hepatikoa eta GKB prebenitu eta hauei aurre egiten lagun lezakete. Ekimen hauek pertsonen ongizatea areagotu ez ezik, osasun publikoaren gastua ere murriztuko lukete.

LIST OF ABBREVIATIONS

ALT, Alanine aminotransferase

AST, Aspartate aminotransferase

BEDb, Energy density from beverages

BEDs, Energy density from solids

BMI, Body Mass Index

BQI, Breakfast Quality Index

CRF, Cardiorespiratory fitness

CVD, Cardiovascular disease

DASH, Dietary Approaches to Stop Hypertension

DBP, Diastolic blood pressure

DDS, Dairy products desserts and substitutes

DXA, Dual energy X-ray absorptiometry

EFIGRO, The effect of exercise on hepatic fat in children

EME_{ratio}, Evening/morning energy ratio

FMI, Fat Mass Index

F+V, Fruits and vegetables

GGT, Gamma-glutamyl transferase

GKB, Gaixotasun kardiobaskularrak

HDI, Healthy diet indicator

HDL-c, High density lipoprotein cholesterol

HELENA, Healthy Lifestyle in Europe by Nutrition in Adolescence

HOMA-IR, Homeostasis model assessment for insulin resistance

IR, Insulin resistance

KIDMED, Mediterranean diet quality index for children and teenagers

LDL-c, Low density lipoprotein cholesterol

MDP, Mediterranean dietary pattern

MetS, Metabolic syndrome

MHO, Metabolically healthy obesity

MRI, Magnetic resonance imaging

MRS, Magnetic resonance spectroscopy

MUFA, Monounsaturated fatty acids

MUO, Metabolically unhealthy obesity

MVPA, Moderate-to-vigorous physical activity

NAFLD, Non-alcoholic fatty liver disease

NASH, Non-alcoholic steatohepatitis

PA, Physical activity

PDM, Patroi dietetiko mediterranearra

PREDIKID, Prevention of diabetes in kids

PREFIT, Assessing fitness in preschoolers

SAT, Subcutaneous adipose tissue

SBP, Systolic blood pressure

SFA, Saturated fatty acids

SSB, Sugar-sweetened beverages

TG, Triglycerides

T2D, Type 2 diabetes

VAT, Visceral adipose tissue

VLDL, Very low density lipoprotein

WHO, World Health Organization

WOF, World Obesity Federation

1. GENERAL INTRODUCTION

1.1. Childhood overweight and obesity

1.1.1. Prevalence of childhood overweight and obesity

The worldwide prevalence of overweight/obesity in children and adolescents has dramatically increased in the last few decades, becoming a public health concern (1). Thus, in 2019, 38.2 million children under the age of 5 years were overweight/obese all over the world (2). In 2016, 18% of children and adolescents aged 5-19 years-old (340 million) were overweight/obese, far away from the 4% of overweight/obesity prevalence reported in 1975 (2).

Europe shows no better picture. Although the overall prevalence of overweight/obesity in children below the age of 10 years is around 20% according to the IDEFICS study, it ranges from 10% in northern European countries to up to 40% in the southern ones (3). These data collected between 2007 and 2010, placed Spain in the third position of highest obesity rates in Europe after Italy and Cyprus, being all of them Mediterranean-region countries. Few years later, the prevalence of overweight/obesity in Spain was reported to be close to 40% (26% and 12.6% for overweight and obesity, respectively), with an alarming prevalence of 45% in children aged 8-13 years (4).

The World Obesity Federation Obesity states that obesity is a chronic relapsing disease process (5). In addition, according to a systematic review and meta-analysis, children and adolescents with obesity are around five times more likely to be obese in their adulthood compared to non-obese ones (6).

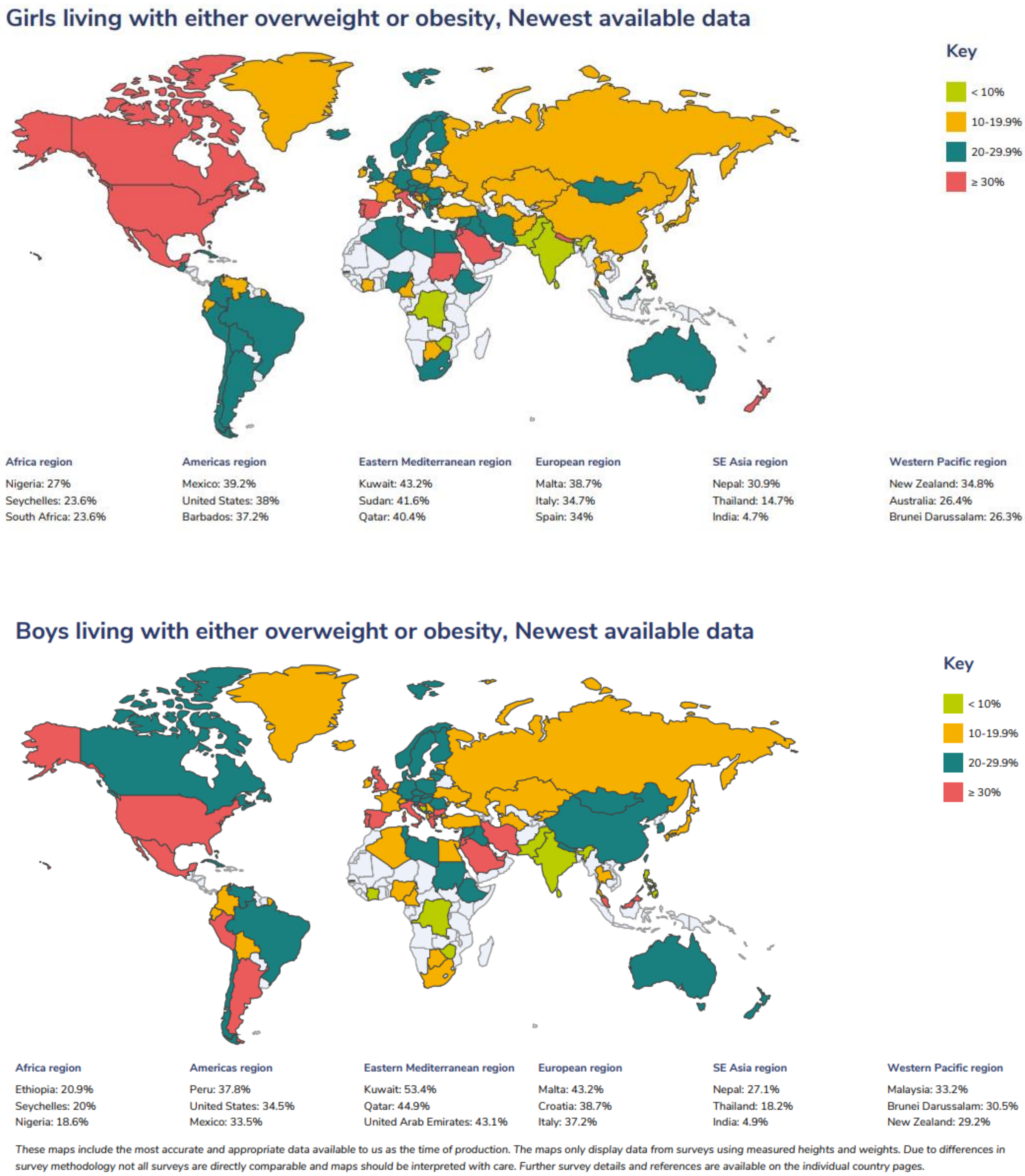


Illustration 1. The worldwide prevalence of overweight/obesity in girls (above) and boys (below) across countries. Presentation map obtained from the Global Obesity Observator, World Obesity Federation (7).

1.2. Adiposity-related comorbidities

The public health concern regarding the increasing prevalence of obesity is based on its increased risk of morbidity and mortality. Obesity leads to a wide range of physical and psychological consequences including cardiovascular diseases (CVD), type 2 diabetes (T2D), musculoskeletal disorders, certain types of cancer as well as low self-esteem and psychological problems (8,9). The risk of developing obesity-related non-communicable diseases depends partially on the age of the onset of obesity (10). The duration of obesity also matters, since the number of years lived with obesity has been positively associated with CVD (11). According to the Global Burden of Disease, it is estimated that by 2025, 268 and 91 million of children aged 5-17 years will present overweight and obesity, respectively; 38 million will suffer hepatic steatosis; 27 million hypertension; 12 million impaired glucose tolerance; as well as 4 million of children will have T2D (12).

1.3. Metabolic syndrome

Metabolic syndrome (MetS) refers to the condition where a cluster of CVD risk factors such as obesity, insulin resistance, hypertension and dyslipidaemia appear together. Moreover, as obesity is involved in the development of the majority of the following CVD risk factors, these health consequences are also known as obesity-related comorbidities.

There are several criteria to classify MetS in children, but all of them include some estimate of the glucose metabolism (such as having diabetes mellitus, impaired fasting glucose, impaired glucose tolerance or insulin resistance), lipid metabolism (such as total cholesterol, high density lipoprotein cholesterol (HDL-c), low lipoprotein cholesterol (LDL-c), triglycerides (TG)), abdominal adiposity (waist circumference, waist-to-hip ratio) and blood pressure (systolic blood pressure (SBP) and/or diastolic blood pressure (DBP), mean arterial pressure).

Furthermore, the inclusion of hepatic steatosis has recently been proposed as an additional component in the definition of the MetS (13), as usually has been shown to present together with the aforementioned CVD risk factors. The description and prevalence of each CVD or MetS components is detailed in the following lines.

1.3.1. Visceral adiposity

There is strong evidence that excess adiposity negatively influences cardiovascular health (14). However, adiposity-related comorbidities mostly depend on body fat distribution (15). Precisely, fat accumulation in the abdominal region, which is known as abdominal or central adiposity, is associated with a greater CVD risk already in childhood (16,17). Waist circumference has been shown to be a good indicator of abdominal fat content, and consequently, it is also an independent indicator of insulin resistance, dyslipidaemia and hypertension in both children and adults (18). Therefore, waist circumference is included in the screening criteria for metabolic syndrome in children (19).

In addition, body fat can be stored in different ways within the abdominal adipose tissue and this leads to a distinction in the metabolic activity and disease risk. Visceral adipose tissue (VAT) refers to the body fat located within the abdominal cavity around the visceral organs. VAT is related to a worse metabolic profile compared to the adipose tissue beneath the skin, which is known as subcutaneous adipose tissue (SAT) (20). Actually, SAT might prejudicially expand beyond principal fat depots causing ectopic fat deposition in other organs, such as the liver, by affecting negatively metabolic homeostasis (15,21).

1.3.2. Insulin resistance

The decrease on the response to insulin or low insulin sensitivity is known as insulin resistance (IR), which is a complex metabolic disorder involved in different diseases (22). Despite other causes may exist, obesity has been shown to be the main driver of IR in youth (23). Thereby, IR represents a link between obesity and other metabolic and cardiovascular complications (23). It has been shown that body composition, as well as VAT, predicts IR beyond the amount of total fat or obesity degree (22). Indeed, fat accumulation in key insulin-sensitive organs or tissues, might be the determinant of IR (24). Precisely, VAT is related to a worse adipokyne and inflammatory profile and, therefore, reduces whole body insulin sensitivity (22,25) by linking VAT with CVD risk. On the other hand, adipocyte hypertrophy has been associated with systemic inflammation and IR in children with obesity, regardless of BMI and overall adiposity (26).

IR can be determined by calculating homeostatic model assessment for insulin resistance (HOMA-IR). Fasting blood glucose and insulin values are needed to determine HOMA-IR, which can be calculated with the following formula: (insulin (µU/ml × glucose (mmol/l))/22.5) (27).

1.3.3. Hypertension

The prevalence of hypertension in children is increasing together with the prevalence of obesity (28). This CVD risk factor is defined as values >140 mmHg of SBP and/or >90 mmHg of DBP (29). Notwithstanding, in youth below the age of 16 years, hypertension is considered as SBP and/or DBP ≥95th percentile (30). The prevalence of paediatric hypertension differs in terms of the region. For instance, according to a survey carried out in China, 8.9% and 10.2% of 6-17 years-old girls and boys, respectively, had high blood pressure (31). In children and adolescents of the United States of America (USA), the data of the NHANES study revealed even higher figures, where over 14.2% of youth suffer from elevated blood pressure (32). Likewise, while adolescents from the Central and Eastern European countries show a prevalence of hypertension around 2-5%, the prevalence in the Southern European adolescents reaches up to 13% (30). Blood pressure is also associated with adiposity. In this line, it has been reported that per 10 kg of body weight gain 3.0 mmHg SBP and 2.3 mmHg DBP are increased (33).

1.3.4. Dyslipidaemia

Another typical condition promoting CVD is dyslipidaemia, which is characterized by elevated blood lipids concentrations that can already appear in childhood. This disorder is commonly presented as high levels of blood TG, total and low-density lipoprotein (LDL) cholesterol as well as low HDL-c (34). Just as happens with other CVD risk factors, childhood obesity is associated with dyslipidaemia (35–37). In this line, it is estimated that 27-43% of children and adolescents with overweight/obesity present dyslipidaemia (38). This is of great health concern since abnormal lipid concentrations in early life track into adulthood by increasing CVD risk (39).

1.3.5. Hepatic steatosis

Hepatic steatosis is the first step of non-alcoholic fatty liver disease (NAFLD)(40), and it is considered the hepatic manifestation of MetS (13). Hepatic steatosis is characterized by an excessive fat deposition in the liver not related to excessive alcohol consumption or other liver pathologies.

Hepatic steatosis is developed as a result of an imbalance between complex pathways of lipid metabolism. The main metabolic pathways involved in hepatic steatosis are the following: hepatic uptake of plasma free fatty acids, hepatic *de novo* lipogenesis, hepatic fatty acid oxidation and fatty acid secretion in very low density lipoprotein (VLDL)-TG (41). Briefly, the increase of free fatty acids uptake and *de novo* lipogenesis results in intrahepatic TG accumulation together with an oversecretion of VLDL-TG (41). In addition, hyperglycaemia and hyperinsulinemia occur as consequences of the increased production and decreased uptake of glucose. This pathways cause a vicious circle as both hyperglycaemia and hyperinsulinemia further promote *de novo* lipogenesis.

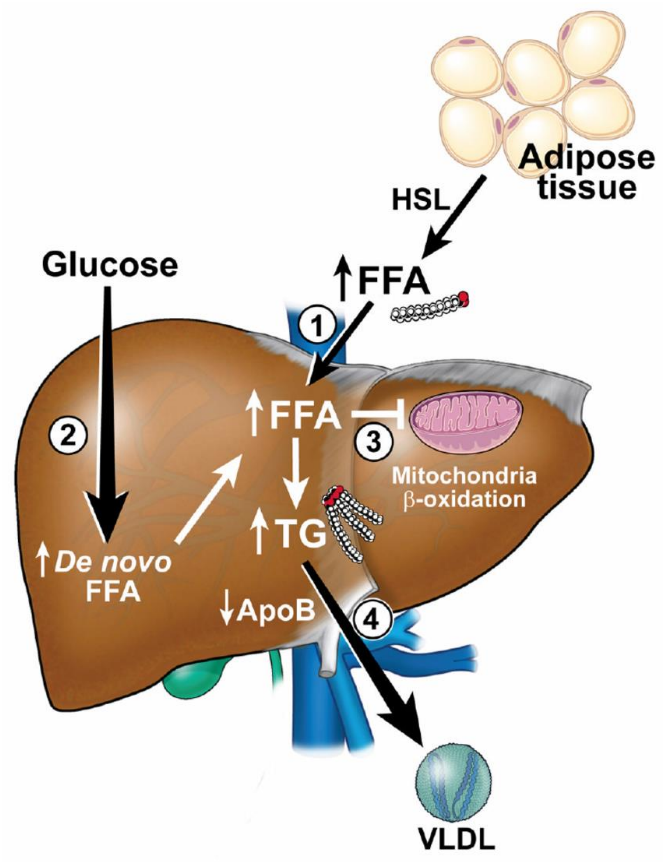


Illustration 2. Involved mechanisms in hepatic steatosis (41)

There are several criteria for the definition of hepatic steatosis in the scientific literature. These criteria depend on the threshold established in relation to the fat fraction within the hepatocytes. While some authors proposed histologically the presence of lipids above 4.85% (42), others suggest 5% (43), 5.5% (44–46) or even 6% (47) to define hepatic steatosis. In addition, hepatic steatosis can be classified as mild (5-33%), moderate (33-66%) or severe (>66%) according to the amount of fat present within the hepatocytes (48).

1.4. Comorbidities related to cardiovascular risk factors

1.4.1. Type 2 diabetes

The prevalence of T2D in childhood is increasing worldwide and it is of health concern due to its association with microvascular (diabetic retinopathy, nephropathy and neuropathy) and macrovascular (development of CVD) complications later in life (49). IR is crucial in the pathogenesis of T2D (50) and, based on prospective studies, also its best predictor (51). Moreover, when the onset of the disease is placed on childhood or adolescence, the time-risk for the development of micro- and macro-vascular complications as well as mortality in adulthood seems to be greater compared to the onset in adulthood, as a result of a longer duration of exposure to glycaemia and other metabolic and CVD risk factors (52–54). Based on the well-established relationship between T2D and CVD risk, CVD is the main cause of mortality among patients with T2D (55).

In addition, research studies have revealed the strong relationship between IR and NAFLD (45,56). It is estimated that over 70-80% of people with obesity and diabetes additionally have NAFLD (57). Similarly, according to a large multi-centre study from the USA, around one third of children and adolescents with NAFLD present prediabetes or T2D (58).

1.4.2. Cardiovascular disease (CVD)

CVD represent a group of heart and blood vessels disorders, including coronary heart disease, cerebrovascular disease as well as rheumatic heart disease (59). CVD is the main cause of mortality in all over the world (59). It is estimated that almost 18 million of people annually die due to CVD, supposing over 31% of deaths worldwide, being 85% of them as a consequence of strokes and heart attacks (55).

There is convincing evidence of the adverse effects of childhood obesity on cardiovascular health; where endothelial dysfunction and arterial stiffness, arterial wall thickness, as well as cardiac structure, can be affected due to excess weight in early life (14). Therefore, it is expected an earlier onset of coronary artery disease due to the elevated prevalence of childhood obesity (60). According to a 55-year longitudinal study, overweight in adolescence entails 2-fold higher risk of coronary heart disease mortality independently of adult body weight (14). In addition, it is well established that VAT has a negative impact on cardiovascular health, as it seems to be a key predictor of arterial wall thickening (61) .

On the other hand, atherosclerosis is a cardiovascular process characterized by artery wall thickening as a result of progressive vascular smooth muscle cellular proliferation and ground matrix deposition (62). This process can begin in childhood (63) and may be stimulated by high serum uric acid concentration, which promotes vascular inflammation and artery damage (62,64). It is well known that uric acid plays a key role in the development of CVD (65). However, the prevalence of paediatric hyperuricemia (elevated serum uric acid levels) is increasing worldwide (66).

1.4.3. Non-alcoholic fatty liver disease (NAFLD)

NAFLD encompasses a spectrum of disease which ranges from simple steatosis, through non-alcoholic steatohepatitis (NASH) to liver fibrosis, cirrhosis and hepatocellular carcinoma (67,68). It is estimated that up to a third part of patients with hepatic steatosis develop NASH (69,70) worsening and complicating the stage and the reversibility of the disease.

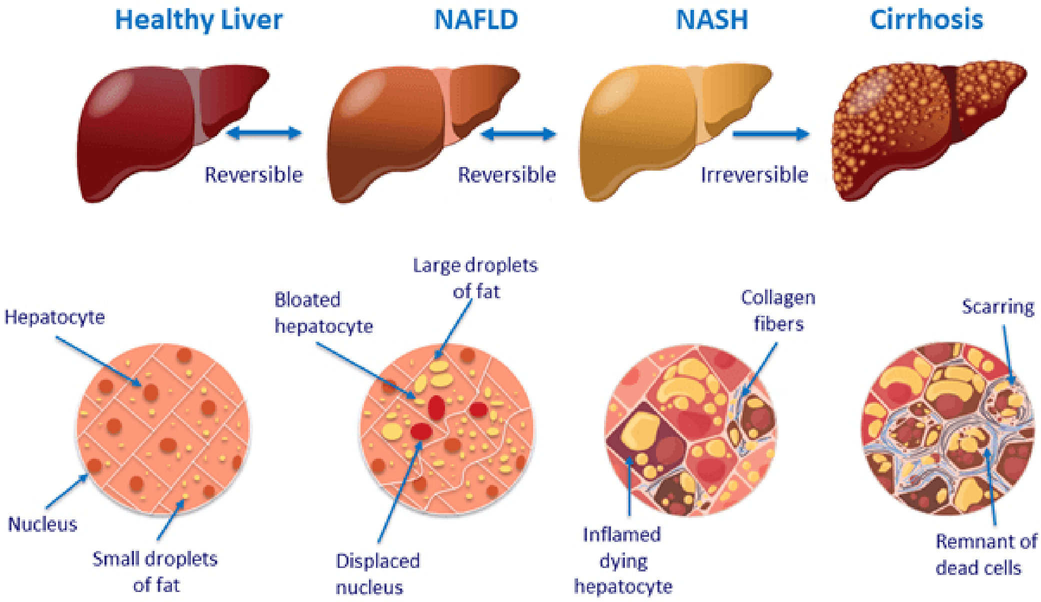


Illustration 3. The progression of NAFLD and histological features.

Together with the growing prevalence of childhood obesity, NAFLD is one of the most common causes of liver disease among both children and adolescents (71). It has been reported that whereas the prevalence of NALFD in the overall paediatric population is 7.6%, when it comes to children with obesity it reaches 34.2% (72). Other study reported even greater difference on the prevalence of NAFLD, where the prevalence among general children was 2-7% and 50-80% among children with obesity (73). The prevalence of NAFLD in children, as well as in adults, can vary significantly due to the wide range of criteria used, demographics of the region being sampled and diagnostic method applied to define and detect it (74). Besides the weight status, increasing age is also associated with NAFLD (75); thus, while less than 1% of children aged 2-4 years present fatty liver, over 17% 15-19 years-old adolescents present it (76). Another factor which seems to be related with increased risk of having NAFLD is the sex; according to the literature; the prevalence of NAFLD in boys is higher than in girls (74,76–79). Moreover, ethnicity and genetics play a key role in liver fat deposition. Actually, it has been shown that Hispanic population is more likely to develop NAFLD (44,77,80–82). A study conducted in 2-9 years old children reported a prevalence of 11.8% among Hispanic children, above 10.2% in Asian, 8.6% in white and 1.5% in black population (76). The higher prevalence of NAFLD in Hispanic population might be explained by genetics. Precisely, the C>G (Ile148Met) polymorphism in the patatin-like phospholipase 3 (*PNPLA3*), a gene which has been associated with greater susceptibility to storage fat in the liver, is more prevalent in Hispanic population than in other populations, revealing an ethnic predisposition for hepatic steatosis (44,80,81,83).

As far as of my awareness, there is no available data on the prevalence of paediatric NAFLD in Europe or Spain. Nonetheless, there exists data on the prevalence of NAFLD in the general European population measured by ultrasonography, which has been reported to range from 2 to 44% of inhabitants (84). Likewise, another study carried out in Spain with 15-85 years-old participants reported a prevalence of 33% and 20% of NAFLD for men and women, respectively (85). Furthermore, by 2030, Spain is predicted to be one of the countries with the highest prevalence of NASH-related advanced disease all over the world (86).

Early diagnosis of hepatic steatosis is of high interest to avoid NAFLD-related health consequences. Unfortunately, NAFLD is often asymptomatic (68) and frequently it is not detected until major damage to the liver occurs or other related metabolic disorder is developed (73). Opportunely, a wide range of diagnostic tools are being employed to diagnose NALFD in the last decades, which briefly can be classified into three groups; liver biopsy, imaging techniques and biochemical analyses. From all of these methods used, it should be pointed out liver biopsy due to its consideration as the *gold standard* for NAFLD diagnosis (68,73). Liver biopsy has been shown to be the most accurate modality detecting hepatic steatosis and non-alcoholic steatohepatitis (NASH), as well as determining the severity of liver damage, inflammation and fibrosis (73,87,88). However, liver biopsy has some limitations because it is an invasive and costly technique and, therefore, it is not feasible in population-based studies (87,89). Concerning imaging-based diagnostic methods, ultrasonography is a non-invasive tool; nevertheless, its usefulness has been questioned because of its limited sensitivity and specificity, and its low utility for grading fatty liver in children (47,90). Likewise, although computed tomography can detect fat deposition in the liver based on the analysis of the attenuation of its parenchyma, it is not suitable specially for children due to its ionizing radiation (47,89). Magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) can both considered as non-invasive methods with similar accuracy potential for hepatic steatosis diagnosis (68,91). In spite of being appropriate to determine small amounts of fat due to its quantification of proton density fat fraction, MRS involves a complex post-processing techniques and it is not usually available on routine scanners (47,88). MRI is a more feasible method, which has been validated in both children and adults (92,93), and has been demonstrated to be an accurate technique for liver fat quantification in paediatric NAFLD (42). On the other hand, liver biochemistry by using serum liver enzymes profile is another more inexpensive and less invasive alternative which have been proposed as surrogate marker of NAFLD in adolescents. Indeed, higher levels of alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT), as well as lower levels of the aspartate aminotransferase to alanine aminotransferase (AST/ALT) ratio have been associated with adiposity and CVD risk factors in adolescents (94). ALT could be a good screening tool for NALFD in children as long as it is interpreted based on sex-specific limits of healthy children (22U/L and 26 U/L for girls and boys, respectively). In any case, liver injury cannot be dismissed even having normal levels of ALT in children with NAFLD (95,96), and this liver enzyme is neither able to distinguish disease severity (74). Due to its low accuracy, ALT has been considered as a screening tool rather than a diagnostic tool by itself (74); and neither AST nor GGT have been independently tested as screening tests for paediatric NAFLD yet (68).

As beforehand mentioned, paediatric NAFLD is part of the metabolic syndrome since it is belief to be its hepatic manifestation. This is based on the fact that paediatric NAFLD is strongly associated with different components of metabolic syndrome such as abdominal adiposity (97), IR (56), dyslipidaemia (98) and CVD (99,100). Thereby, several metabolic disorders including lipid accumulation, insulin resistance and inflammation, are implicated in the pathogenesis of NAFLD.

Early theories proposed the "two hits theories" to explain the pathogenesis of NAFLD; where the *first hit* was based on IR and excessive circulating fatty acids, leading to TG accumulation on liver (hepatic steatosis); whereas the *second hit* involves inflammatory cytokines, oxidative stress, lipid peroxidation, mitochondrial dysfunction and apoptosis, promoting the progression of the disease towards NASH (57,101). From then on, the proposed "multiple hit theory"(102) has been widely accepted, which includes the interaction of both genetic and environmental factors, together with changes in cross-talk between adipose tissue, pancreas, gut and liver (40).

1.5. Metabolic phenotype

It is recognized that not all the patients who suffer from obesity develop metabolic disorders. Interestingly, there is a subgroup of people with obesity who seem to be protected from these complications, and therefore, have a healthier metabolic profile. This condition is known as metabolically healthy obesity/obese phenotype (MHO) and may be at lower risk of all-cause mortality (103). Several criteria have been proposed to classify patients with obesity as MHO or metabolically unhealthy obesity/obese (MUO) according to their metabolic status, as has been compiled in a recent review (104). Given that metabolic abnormalities in youth are not often as apparent as in adulthood, it should be highlighted that there is less consensus about the definition of metabolically healthy phenotype in this population (104). Interestingly, the MetS cut-off criteria published by Jolliffe and Janssen (105), which are equivalent to the ones proposed for adults by IDF and ATP-III, include the growth curves of youth population with age and sex adjustments, and therefore, seems to be appropriate. Although other definitions have been proposed for youth, they are not sex- and age-specific, and thus, do not consider growth- and puberty-related physiological changes (104).

The components taken into consideration for the classification of MetS status are the following: having elevated TG, reduced HDL-c, elevated blood pressure and elevated fasting glucose, whereas waist circumference is excluded from the definition. By following this *criterium*, a young patient is classified as having a MHO phenotype if he/she presents obesity, but none of the previously mentioned criteria. On the contrary, a children is considered as presenting a MUO phenotype when he/she has obesity plus at least one of any of the above mentioned metabolic abnormalities (104).

1.6. Prevention and treatment of cardiovascular risk factors

The prevention and treatment of obesity, NAFLD as well as of CVD, in childhood is a challenge of the 21st century. Thereby, non-communicable diseases such as the previously mentioned disorders cause over 41 million of deaths each year, which is the equivalent to 71% of all worldwide deaths (106). However, most of these kinds of diseases are preventable since lifestyle is the basis of the prevention and development of CVD disorders. The aetiology of childhood obesity, as well as of the obesity-related health consequences, is the results of an interaction between genetic and environmental factors, caused because of the energy imbalance between calories consumed and expended. More specifically, a diet composed of high fat and sugar energy-dense foods, and low in fibre, vitamins and minerals, together with a sedentary lifestyle and low levels of physical activity contribute to the pathogenesis of obesity and related comorbidities (107).

Therefore, and taking into consideration that these comorbidities are usually presented together with obesity, lifestyle interventions focused on healthy dietary habits and physical activity promotion are the cornerstone in the prevention and treatment of the liver and cardiovascular health-related complications (14,68). Moreover, as children are still acquiring and establishing their habits, lifestyle changes and adoption of healthy habits is more likely to succeed in childhood and adolescence. Nonetheless, the success and effectiveness of these interventions is not always guaranteed, since lifestyle changes are far from being an easy task (108).

Physical activity is an important dimension of lifestyle. Physical activity, defined as any body movement produced by skeletal muscles resulting in energy expenditure, has been shown to have remarkable benefits on liver and cardiovascular health (109,110). The WHO guidelines recommends at least 60 minutes of daily moderate-to-vigorous physical activity in addition to strength activities three times per week for children aged 5-17 years;

notwithstanding, more than 80% worldwide children is insufficiently physically active, which is of public health concern (111). Albeit the existing questionnaires for measuring physical activity levels, these have not shown a good validity and reliability in children (112). In contrast, accelerometry is a feasible method to objectively measure physical activity levels in children, where participants have just to wear a kind of bracelet (accelerometer) in their wrist (113).

Another important component when focusing on physical activity is cardiorespiratory fitness (CRF); the capacity of both cardiovascular and respiratory systems to transport and consume oxygen as well as the ability to perform prolonged strenuous exercise (114). CRF not only is a powerful health marker, but it is also related with lower cardiovascular mortality (114,115). According to a longitudinal study carried out in adolescents, baseline CRF was inversely associated with SBP and rate pressure product two years later (116). Higher CRF has been also shown to be associated with lower hepatic fat content and healthier profile of liver enzymes (117). Moreover, it has been reported that high levels of CRF among children might attenuate the influence of parental obesity on the weight status of their children (118). Despite the fact that CRF has a genetic component, it is enhanced with regular physical activity (119), and therefore, physically active lifestyle should be promoted to prevent CVD disorders.

The direct cardiopulmonary progressive incremental test with metabolic gas exchange to exhaustion is considered the gold standard for CRF assessment; nevertheless, due to its cost is not feasible to use with large samples of participants (120). As an alternative, the 20 meters shuttle run test is a widely used method for measuring CRF, and its use in children has been shown to be reliable and feasible (121,122).

1.7. Dietary habits and hepatic and cardiovascular health

Dietary habits are closely related to body composition, as well as to liver and cardiovascular health (123–125). Thereby, an energy-dense, low-fibre and high-fat diet has been associated with greater odds of excess adiposity in childhood (123). Likewise, it has been shown that westernized dietary patterns, which are rich in red and processed meat, refined grains as well as in sugar, are associated with increased inflammation and cardiovascular markers (126,127).

While several studies examine the influence of the consumption of certain foods on specific health biomarkers, others focus on examining the effect of dietary patterns on different outcomes. Studying dietary patterns has increased popularity in nutrition research field in the last few years, since besides they allow for a better description of dietary habits of the population, possible interactions between nutrients or foods are also taken into account (127,128). One of the most well-known dietary patterns is the Mediterranean dietary pattern (MDP). Few decades ago, research focused attention on the Mediterranean region given that this was the area with the highest life expectancy and lower rates of coronary heart disease worldwide. Interestingly, they realized that population living in this region traditionally had similar dietary habits, characterized by high intakes of plant-based foods (fruits and vegetables, nuts, legumes, cereals) and fish, by emphasizing olive oil as principal use of fat, moderate consumption of dairy products and wine as well as low consumption of meat and poultry (129). Since then, several studies have reported the beneficial effects of MDP on blood pressure and overall on CVD risk, liver health, T2D, as well as on obesity in both children and adults (124,130–132). On the other hand, Dietary Approaches to Stop Hypertension (DASH) diet is a widely studied dietary pattern in relation to cardiovascular health. Briefly, the DASH diet is mainly composed of magnesium-, calcium- and potassium- and fibre-rich foods and in those low in sodium, saturated fat and cholesterol, and therefore, is basically a plant-based and whole-grain diet, albeit it does not exclude meat or fish (133). Besides its beneficial effects on cardiovascular and hepatic health in adults (134,135), the DASH diet has been shown to be effective in reducing blood pressure as well as improving MetS and BMI status in children and adolescents (133,136,137).

Energy intake during childhood and early adolescence has also been associated with greater risk of having NAFLD (138). Similarly, a systematic review and meta-analysis of observational studies including children and adults, concluded that there was a positive association between dietary energy density and adiposity (139).

Sugars require special attention in this topic given that they are considered as one of the major contributors of an energy-dense and nutrient-poor diet (140). Moreover, sugar consumption may additionally reduce the intake of nutritionally more adequate foods, leading to an unhealthy diet and increased risk of non-communicable diseases (141). Accumulating evidence implicates sugars, and particularly added sugars (those which are not naturally present and are artificially added, such as in processed foods), in the increment of CVD risk through increased energy intake, adiposity and dyslipidaemia (142). However, due to the consumption of nutritionally poor and ultra-processed foods, sugar intake in the general population remains high, which is of public health concern. In order to reduce the amount of sugar consumed, WHO recommends reducing the intake of free sugars (sugars added to foods/ beverages by manufacturer, cook or consumer and those naturally present in honey, syrups, and fruits juices/concentrates) to less than 10% of total daily energy intake for both children and adults, suggesting a further reduction to below 5% as conditional recommendation (141). According to a review of sugars dietary sources using data from eleven European countries surveys, children had an intake of 16-26% and 11-17% of total and added sugar, respectively, from total energy intake (140). Added sugars intake of children in these studies was provided by sweet products, such as confectionary, chocolates, biscuits or cakes (40-50%), beverages (20-34%) and dairy products (6-18%). However, other studies place sugar-sweetened beverages (SSB) as the primary source of dietary added sugar in children and adolescents

(143,144). In this line, there is strong evidence that SSB and fructose have detrimental effects on cardiovascular health, and particularly, on liver health in both children and adults (145–148).

Regarding meals, skipping breakfast is a frequent behaviour in youths which has been related with total and visceral adiposity (149,150). Actually, studies suggest that breakfast consumption might protect from obesity during childhood and adolescence (151). Furthermore, skipping meals and mainly breakfast, has also been associated with glucose and levels of serum lipids in children and adolescents with obesity (152).

2. HYPOTHESIS AND OBJECTIVES/ HIPOTESIA ETA HELBURUAK

2.1. Hypothesis

The hypothesis of the current International Doctoral Thesis is that healthy dietary patterns such as the Mediterranean or DASH diet, as well as behaviours like eating breakfast will prevent adiposity and related comorbidities. In contrast, energy-dense diets based on sugar- and fat-rich ultra-processed foods, including sugary drinks, will negatively affect hepatic and cardiovascular health. Healthy lifestyle programs based on nutritional education and physical activity promotion will increase the knowledge of both children and parents on health and lifestyle by leading to improve dietary habits and increasing physical activity levels. Thus, healthy dietary habits together with other healthy behaviours, such as having a physically active lifestyle, will help improving body composition and preventing adiposity-related CVD risk factors in both childhood and later in life.

2.2. Objectives

The objectives of the present Thesis are the following:

1) To examine the associations of the adherence to the MDP and CRF with total and central adiposity in preschool children.

2) To examine the adherence to the MDP in MHO and MUO European adolescents.

3) To analyze the associations of breakfast quality and energy density from both solids and beverages with CVD risk factors; and to explore whether physical activity levels may attenuate these relationships in children with overweight/obesity from the north and south of Spain.

4) To examine the association of food and food components, DDS, SSB, as well as total and added sugars, with hepatic fat and insulin resistance in children with overweight/obesity.

5) To explore the effectiveness of a 22-week family-based lifestyle education program on dietary habits, and to analyze the associations of changes in dietary intake with the reductions of percent hepatic fat and adiposity in children with overweight/obesity.

2.1. Hipotesia

Honako Nazioarteko Doktorego Tesiaren hipotesia dieta Mediterranearra edo DASH bezalako patroi dietetiko osasuntsuek eta gosariaren kontsumoa gisako elikadura portaerek adipositatea eta honi loturiko komorbilitateak prebenitu litzaketela da. Azukre eta gantzetan aberatsak diren elikagai prozesatuetan oinarritutakoak diren dieta energetikoki dentsoek, ordea, eragin kaltegarria izango lukete osasun hepatiko eta kardiobaskularrean. Nutrizio-hezkuntza eta ariketa fisikoaren sustapenean oinarritutako bizi-ohitura osasuntsuen programek haur eta horien gurasoen bizi-ohitura eta osasunaren inguruko ezagutza handituko luke, elikadura-ohiturak eta ariketa fisikoaren mailak hobetuz. Horrela, elikadura-ohitura osasuntsuak barneratzearekin batera, bizimodu fisikoki aktiboa izatea bezalako ohitura osasuntsuak izateak gorputz-konposizioa hobetzen, eta haurtzaroan zein etorkizunean adipositatearekin loturiko gaixotasun kardiobaskularraren arrisku faktoreak prebenitzen lagunduko luke.

2.2. Helburuak

Tesi honen helburuak ondorengoak dira:

1) Patroi dietetiko Mediterranearraren atxikidura eta bihotz-hodiko gaitasuna eta eskolaurreko haurren adipositate totala eta zentralaren arteko erlazioak aztertzea.

2) Patroi dietetiko Mediterranearraren atxikidura aztertzea metabolikoki osasuntsua eta metabolikoki ez-osasuntsua den gainpisua/obesitatea pairatzen duten Europako nerabeetan.

3) Gosariaren kalitatea eta solido zein likidoen dentsitate energetikoa arrisku faktore kardiobaskularrekin erlazionatzea; eta ariketa fisiko mailak erlazio hauek arindu ditzakeen aztertzea gainpisua/obesitatea pairatzen duten Espainiako iparralde eta hegoaldeko haurretan.

4) Elikagai eta elikagaien konposatuak, esneki-postre eta ordezkoak, azukredun edariak eta azukre total zein gehitutakoek gibeleko gantza eta intsulinarekiko erresistentziarekin duten erlazioa aztertzea gainpisua/obesitatea duten haurretan.

5) Bizi-ohituren hezkuntza programa familiar batek 22 astetan elikadura ohituretan duen eragina ikertzea, eta elikadura ohituretan emandako aldaketak gibeleko gantza eta adipositatean murrizketarekin erlazionatzea gainpisua/obesitatea duten haurretan.

Universidad Pública de Navarra
Nafarroako Unibertsitate Publikoa

3. RESULTS

3.1. Study I

Published in: European Journal of Nutrition.

Eur J Nutr
DOI 10.1007/s00394-017-1571-3

ORIGINAL CONTRIBUTION

Associations between the adherence to the Mediterranean diet and cardiorespiratory fitness with total and central obesity in preschool children: the PREFIT project

Idoia Labayen[1,2], Lide Arenaza[2], María Medrano[2], Natalia García[2], Cristina Cadenas-Sánchez[3], Francisco B Ortega[3]

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

[2]Nutrition, Exercise and Health Research group, ELikadura, arIKeta Fisikoa eta OSasuna, ELIKOS group, Department of Nutrition and Food Science, Faculty of Pharmacy, University of the Basque Country, UPV/EHU, Vitoria, Spain

[2]Department of Curricular Development and Teaching, Faculty of Psychology and Education, Deusto University, Bilbao, Spain

[3]PROmoting FITness and Health through physical activity research group (PROFITH), Department of Physical Education and Sports, Faculty of Sport Sciences, University of Granada

Running title: Mediterranean diet, fitness and overweight in preschoolers

Corresponding author: Idoia Labayen Goñi, idoia.labayen@ehu.es

ABSTRACT

Purpose: Early recognition of risk factors associated with overweight/obesity is an important step towards preventing long-term health consequences. The aim of the current study was to examine the associations of the adherence to the Mediterranean dietary pattern (MDP) and cardiorespiratory fitness (CRF) with adiposity in preschool children from the north of Spain.

Methods: The adherence to the MDP (KIDMED), CRF (20-m shuttle run test), total (BMI) and central (waist circumference) adiposity and socio-demographic factors were assessed in 619 children (48.6% girls) who were on average 4.7 years old.

Results: Higher MDP index ($P < 0.05$) and CRF levels ($P < 0.01$) were significantly related to lower waist circumference. CRF was inversely associated with BMI ($P \leq 0.001$), yet no significant association was observed between MDP and BMI. Children not having high CRF levels and high MDP (i.e., non-upper sex-specific tertile of CRF or MDP, respectively) had the highest waist circumference.

Conclusions: Our findings support that higher adherence to the MDP and higher CRF are associated with lower waist circumference in preschool children, pointing them as relevant modifiable factors to be targeted by educational strategies aiming to prevent central obesity and later obesity-related comorbidities.

Keywords Preschool children, Obesity, Adherence to the Mediterranean diet, Cardiorespiratory fitness, Dietary habits

INTRODUCTION

The manifestation of obesity in children is occurring at progressively younger ages worldwide [1]. This is of great concern from a public health perspective due to the tracking of childhood obesity into the adulthood. Moreover, there is strong relationship between the number of years lived with overall [2] or abdominal obesity [3] and the risk of cardiovascular disease (CVD) mortality and all-cause and mortality. Hence, the results of the Framingham Cohort Study showed that the risk of CVD mortality increased 7% for every 2 years additional lived with obesity [2].

The rise in the prevalence of overweight/obesity in preschool children has been particularly dramatic in the last three decades [4]. Likewise, the increase of early childhood overweight/obesity worldwide was 21% from 1990 to 2000, and 31% from 2000 to 2010[5]. In 2010, the Early Childhood Longitudinal Study reported that almost 15% of kindergarten-age children were overweight and 12.4% obese in the United States [6]. The latest systematic review in Europe reported prevalence rates of overweight or obesity between 8.4% and 31% in 2-5 years children, and observed the highest rates in countries in the Mediterranean region [7]. Spain, together with Malta and Sicily is leading the ranking of overweight and obesity rates in children aged 5-10 years [8].

Childhood is a crucial period in life in which individuals adopt lifestyle patterns that are likely to track into their adulthood. Healthy dietary habits and adequate physical activity levels during early life reduce the risk of major chronic diseases later in life [9]. Physical activity level, particularly moderate to vigorous physical activity, is strongly associated with cardiorespiratory fitness (CRF), a powerful health marker. However, in the last decades, the consumption of unhealthy diets has become average dietary patterns [10] and physical activity levels, and consequently CRF, have decreased among children [11]. Low level of CRF is engaged in the etiology and occurrence of many chronic non-communicable diseases, such as cardiovascular diseases, diabetes or obesity. For instance, higher total and central adiposity have been associated with low levels of CRF in children and adolescents [12]. This is potentially of great interest and concern because CRF is an important marker of health already in childhood.

The Mediterranean dietary pattern (MDP), based on the typical dietary habits followed by people from countries around the Mediterranean Sea, has been extensively studied due to its health benefits [13]. High adherence to the MDP may reduce major chronic disease morbidity and mortality [13], and with lower adiposity and overweight prevalence in youths [14,15], while other found no significant associations between the adherence to the MDP and obesity prevalence[16]. Interestingly, epidemiological evidence suggests that dietary patterns in the South of Europe are changing especially among youths, and that children living in countries in the South of Europe can even have lower adherence to the MDP than their peers living in the North of Europe [14].

The majority of the studies examining the influence of CRF level and the adherence to the MDP in overweight/obesity risk have been conducted in school children or adolescents. There are no previous epidemiologic studies examining the influence of both the adherence to the MDP and CRF levels on adiposity measures in preschool children. Early recognition of risk factors associated with overweight and obesity and monitoring overweight/obesity rates are important steps towards preventing long term health consequences. Therefore, the aims of the current study were: 1) To examine the adherence to the MDP in preschool children; and 2) To examine the associations of the adherence to the MDP and CRF with body mass index (BMI) and waist circumference in a sample of preschool children from the North of Spain.

METHODS

Design and participants

The PREFIT study (Assessing FITness in PREschoolers) is a multicentre cross-sectional study performed in 11 towns/cities of Spain (Almería, Cádiz, Castellón, Cuenca, Granada, Las Palmas de Gran Canaria, Madrid, Palma de Mallorca, Zaragoza, and Vitoria-Gasteiz) that aimed to evaluate nutritional status and fitness in preschool children **(Supplemental Figure 1**). The present study focused on the sample from Vitoria-Gasteiz (North of Spain), the only center in which the adherence to the MDP was studied, and it is not representative of the whole Spanish population of preschoolers. This study therefore comprised 619 children (8% of the children aged 3-5 years living in the city, 48.6% girls) aged 4.7±0.8 years recruited in six schools, three public (N=328) and three private (N=291). Participation rate was lower in children aged 3 years (55.4%), than in 4 (77.8%) or 5 (77.4%) years old children (**Supplemental Figure 2**). The reason for non-participation was not registered. Data collection took place from March to November 2015.

The protocol of the study was approved by the Human Research Ethics Committee of the University of the Basque Country. Detailed information about the aims of the study and tests, as well as questionnaires and informed consent sheets, were delivered to the families by the schools participating in the study. All the parents sent the informed consent for their children's participation in the study to the schools.

Anthropometric measurements

Waist circumference, body mass (kg) and height were measured following standard protocols and body mass index (BMI) was calculated. BMI was categorized into underweight, normalweight, overweight and obesity according to the World Obesity Federation (WOF) criteria [8]. Sex-specific z-score of BMI and waist circumference were calculated as follows: (data-mean of the sample)/standard deviation of the sample.

Adherence to the Mediterranean diet

The adherence to the MDP was assessed by using the KIDMED questionnaire (Mediterranean Diet Quality Index for children and teenagers) [17]. This test has been previously validated and it is widely used in childhood [17]. Since the participants were too young to fill the questionnaire, their parents or legal guardians were asked to answer the questions on their children dietary habits. The test is composed of 16 nutritional items, 10 questions about the consumption of the different food groups and six more about healthy dietary habits not directly associated to the MDP [14]. For the purpose of the current study only those items related to the MDP were considered, while questions about breakfast habit, eating in fast food restaurants or taking sweets were not included in the main analyses (**Supplemental Table 1**). A value of 1 was given to the questions which have a positive connotation in accordance to the MDP. The total MDP score (MDP index) was computed by adding up all the values obtained in the 10 items. Thereby, the MDP index test ranged from 0 to 10 points. The MDP score was also expressed as percentage of adherence. Optimal adherence to the MDP when the MDP index was ≥8 or the percentage of adherence to the MDP was ≥80%)

Cardiorespiratory fitness

The level of CRF was estimated using the PREFIT 20-m shuttle run test as described elsewhere [18]. This test was adapted from the original protocol mainly reducing the initial speed from 8.5 km/h to 6.5 km/h. Briefly, children started the test at 6.5 km/h and the speed increased 0.5 km/h per minute. The test finished when the children failed to reach the end line concurrent with the audio signal on two consecutive occasions or when the child stop because of exhaustion.

Potential confounding factors

The following socio-demographic variables have been previously shown to be associated with obesity, dietary habits and/or CRF in childhood and were considered as potential confounders: maternal and paternal educational level ([1] non-university: lower education, lower secondary school and higher education and [2] university degree), and ethnicity that was defined as the country of origin of the mother and was categorized for statistical analyses as: 1=Spanish and 2=non-Spanish.

Statistical analyses

Differences in anthropometric, socio-demographic, dietary and CRF characteristics between boys and girls were examined using t-Student tests for independent samples (continuous variables) or Chi-square tests (categorical variables). The distribution of all the continuous variables was tested for normality.

To investigate associations between the MDP index and CRF levels with BMI, BMI z-score, waist and waist z-score linear regression analyses were conducted. Two regression models were created: (1) model adjusted with age and sex (Model 1), and (2) model adjusted with age, sex and socio-demographic variables (Model 2). Analyses examining the associations with waist circumference and waist circumference z-score were additionally controlled for height, while CRF was further controlled for body mass. We also examined interactions by sex including interaction terms into the models; as there were no significant interactions ($P>0.05$), the results for boys and girls are presented together.

Differences in adiposity between having high adherence to the MDP (within the upper sex-specific tertile of the MDP index) and non-having high adherence to the MDP (non-upper sex-specific tertile of the MDP index) were explored by ANCOVA adjusting with age, sex and socio-demographic variables (basic covariates). Similarly, having high CRF levels (within the upper sex-specific tertile of CRF) vs. Non-having high CRF levels (non-upper sex-specific tertile of CRF) were also analysed by ANCOVA controlling with basic covariates. The existence of an interaction effect between the MDP index and CRF on anthropometric variables was also tested by ANCOVA adjusting with the same covariates. Thereafter, the combined influence of having or non-having high adherence to the MDP and having or non-having high CRF on anthropometric variables was examined by ANCOVA adjusting with age, sex and socio-demographic variables. All analyses were performed using the Statistical Package for Social Sciences (SPSS, version 21.0 for WINDOWS; SPSS Inc, Chicago), and the level of significance was set at $\alpha = 0.05$.

RESULTS

Socio-demographic and anthropometric characteristics, as well as CRF, separated for boys and girls are shown in **Table 1**. It was observed that 4.7% (4.1% in boys and 5.3% in girls) of preschool children had insufficient body weight, 16% were overweight (15.2% in boys and 16.9% in girls), and 4.9% were obese (3.5% in boys and 6.3% in girls). Boys were taller (P<0.05), had lower waist circumference (P<0.05) and higher CRF level (P<0.001) than girls.

Dietary habits of study participants are shown in **Supplemental Table 1**. The mean adherence to the MDP was higher in girls than in boys (p<0.001, Table 1). It was observed that less than 25% of participants had at least 80% of adherence to the MDP (24.3%, 32.3% of girls and 17.2% of boys) and that 35% of preschool children did not exceed 50% of adherence to the MDP (29.7% of girls and 39.7% of boys, Supplemental Figure 2). Only 2.4% of preschoolers showed 100% of adherence to the MDP (3.9% of girls and 1.0% of boys).

Table 1. Descriptive characteristics of the preschool children.

	Boys		Girls			All	
	N	**Mean (SD)**	**N**	**Mean (SD)**	**P**	**N**	**Mean (SD)**
Age (years)	318	4.7 (0.8)	301	4.7 (0.9)	0.765	619	4.7 (0.8)
Maternal educational level (N, %)	313		298		0.867	611	
Non-University		197, 61.9		190, 63.1			387, 63.3
University		116, 37.1		108, 36.9			224, 36.7
Paternal educational level (N, %)	303		282		0.627	585	
Non-University		228, 75.2		218, 77.3			446, 76.2
University		75, 24.8		64, 22.7			139, 23.8
Ethnicity (N, %)	317		300		0.587	617	
Spanish		235, 74.1		232, 77.3			467, 75.4
Maghrebi		29, 9.1		27, 9.0			56, 9.0
Sub-Saharan		32, 10.1		21, 7.0			53, 8.6
Others		21, 6.6		20, 6.7			41, 7.0
Body mass (kg)	316	19.4 (3.4)	301	18.9 (3.3)	0.075	617	19.1 (3.3)
Height (cm)	316	108.5 (7.5)	301	107.1 (7.0)	**0.020**	617	107.8 (7.3)
BMI (kg/m^2)	316	17.3 (2.6)	301	17.3 (2.7)	0.798	617	17.3 (2.6)
BMI z-score	316	0.431 (1.010)	301	0.469 (0.991)	0.640	617	0.449 (1.000)
Weight status (N, %)[a]	316		301		0.331	617	
Under weight		13, 4.1		16, 5.3			29, 4.7
Normoweight		244, 77.2		215, 71.4			459, 74.4
Overweight		48, 15.2		51, 16.9			99, 16.0
Obese		11, 3.5		19, 6.3			30, 4.9
Waist (cm)	318	53.1 (5.0)	301	54.1 (4.6)	**0.016**	619	53.6 (4.8)
Waist z-score	318	0.04 (1.00)	301	1.76 (1.00)	0.093	619	0.107 (1.00)
Adherence to the MDP (%)	310	60.0 (16.0)	279	65.0 (17.0)	**<0.001**	589	62.0 (17.0)
PREFIT-20-m shuttle run (laps)	312	22.2 (12.8)	294	18.8 (9.9)	**<0.001**	606	20.6 (11.6)

BMI: body mass index. MDP: Mediterranean dietary pattern. SD: standard deviation.
[a]According to the World Obesity Federation criteria[24].

Adherence to the Mediterranean diet, cardiorespiratory fitness and adiposity

A higher adherence to the MDP was significantly related to lower waist and waist z-score regardless of sex, age and height (Model 1, Table 2) and age, sex, height, and sociodemographic factors (Model 2, Table 2). A higher CRF level was significantly associated with lower BMI, BMI z-score, waist and waist z-score regardless of sex and age (Model 1, Table 2) and age, sex, and sociodemographic factors (Model 2, Table 2).

Table 2. Associations of the adherence to the Mediterranean diet (MDP index) and cardiorespiratory fitness (PREFIT 20-m shuttle run) with overall (BMI and BMI z-score) and central adiposity (waist circumference and waist circumference z-score) in pre-school children.

	BMI		**BMI z-score**		**Waist[a]**		**Waist z-score[a]**	
	β	**P**	**β**	**P**	**β**	**P**	**β**	**P[a]**
MDP index								
Model 1	-0.053	0.409	-0.014	0.580	-0.222	**0.032**	-0.051	**0.024**
Model 2	-0.052	0.428	-0.011	0.676	-0.239	**0.026**	-0.055	**0.020**
PREFIT 20-m shuttle run (stages)								
Model 1	-0.145	**0.002**	-0.154	**<0.001**	-0.102	**0.023**	-0.150	**0.001**
Model 2	-0.136	**0.006**	-0.156	**0.001**	-0.105	**0.025**	-0.142	**0.004**

Model 1: adjusted with age and sex. Model 2: Model 1 was further adjusted with maternal and paternal educational level and ethnicity. [a]Additionally adjusted with height. β: standardized regression coefficient.

Likewise, waist and waist z-score were lower in preschoolers within the upper sex-specific tertile of the adherence to the MDP (P<0.05, Figure 2) and in those children within the upper sex-specific tertile of CRF than in their peers with lower CRF levels (P<0.01, Figure 1).

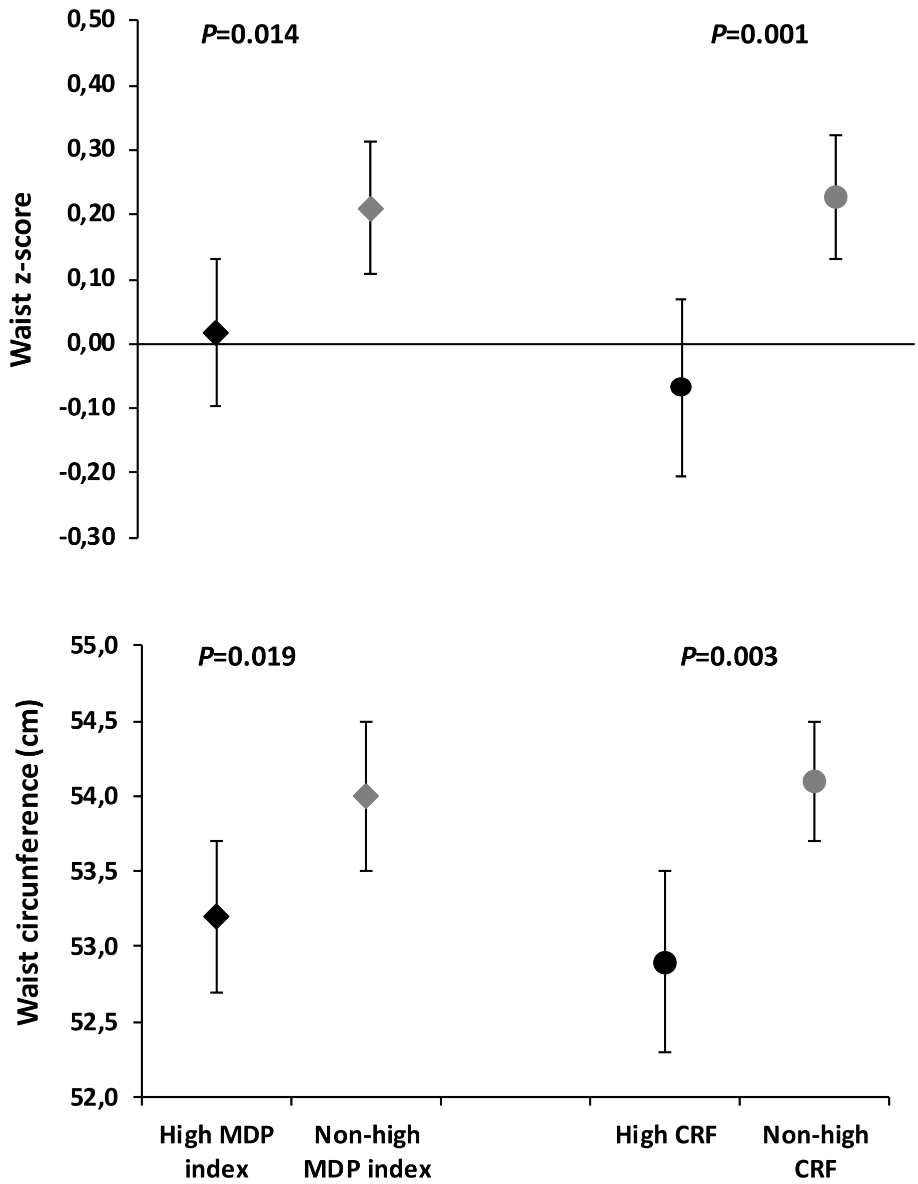


Figure 1. Influence of having high CRF (upper sex specific tertile of cardiorespiratory fitness) or non-high CRF (non-upper sex specific tertile of cardiorespiratory fitness) and having high MDP index (upper tertile of sex specific MDP index) or non-having high MDP index (non-upper sex-specific tertile of the MDP index) on waist circumference and waist circumference z-score in preschool children. Analyses were adjusted with age, sex, maternal and paternal education level, ethnicity and height squared (covariates). MDP index: adherence to the Mediterranean dietary pattern. Values are adjusted means ± 95% CI.

There were not found significant interaction effects between the adherence to the MDP and CRF level on BMI and BMI z-score (P>0.05). However, there were observed significant interaction effects between the adherence to the MDP and CRF level on waist (P=0.013) and waist z-score (P=0.008). Therefore, it was examined the combined influence of having or non-having a high adherence to the MDP (upper or non-upper sex-specific MDP index) and having or non-having high CRF levels (upper or non-upper sex-specific tertile of CRF) in waist and waist z-score (**Figure 2**).

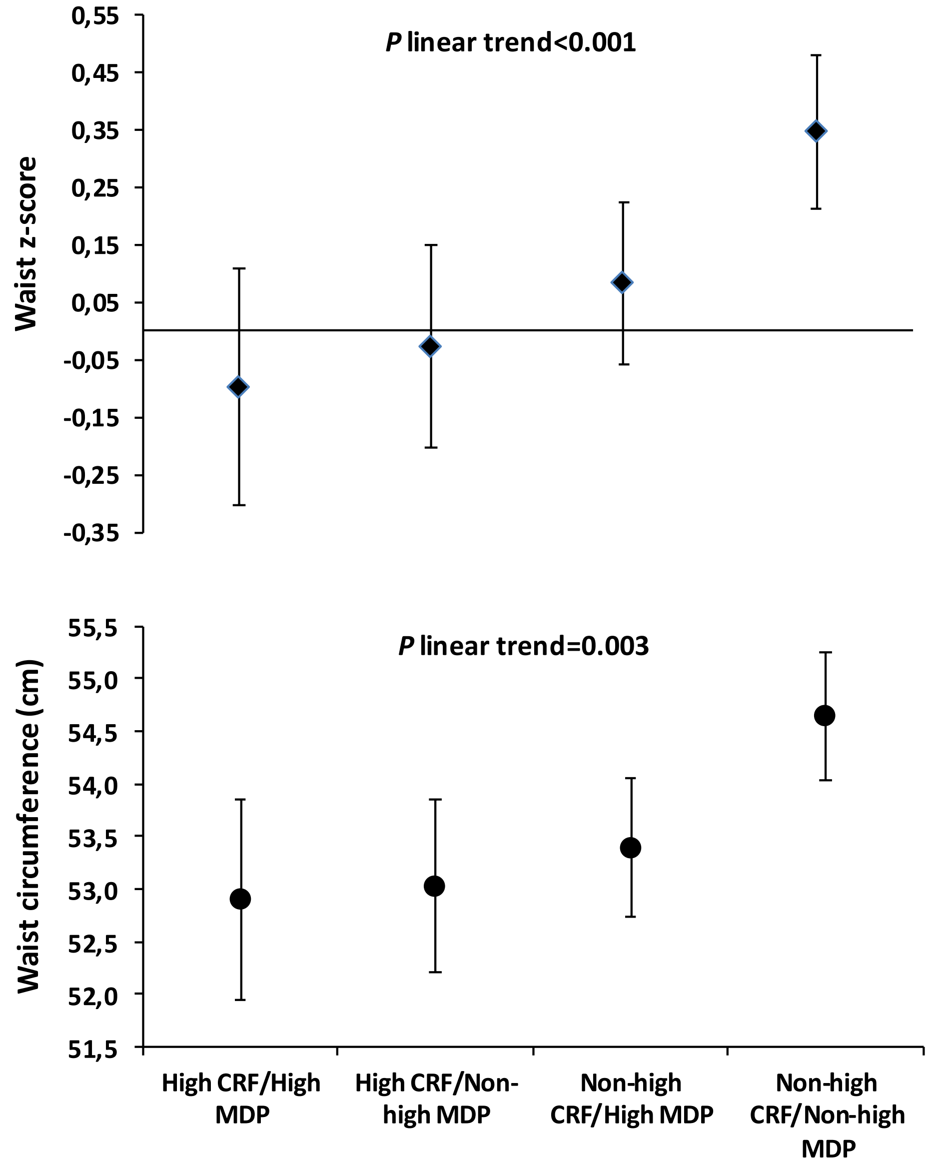


Figure 2. Combined influence of having high CRF (upper sex-specific tertile of cardiorespiratory fitness) or non-having high CRF (non-upper sex-specific tertile of cardiorespiratory fitness), and having high MDP index (upper sex-specific tertile of the MDP index) or non-having high MDP

index (non-upper of sex-specific tertile of the MDP index) on waist circumference and waist circumference z-score in preschool children. Analyses were adjusted with age, sex, maternal and paternal education level, ethnicity and height squared (covariates). MDP index: adherence to the Mediterranean dietary pattern. Values are adjusted means ± 95% CI.

The results showed that both waist and waist z-score were significantly higher in those children non-having both high CRF levels and high MDP index than in those non-having high CRF levels, but with high MDP index (P=0.034 and P=0.040, for waist and waist z-score, respectively) independently of age, sex, height squared, and sociodemographic factors. Moreover, children non-having high CRF levels and non-having high MDP index had higher waist and waist z-score than those having high CRF levels and high MDP index (P=0.020 and P=0.003, for waist and waist z-score, respectively), or non-high MDP index (P=0.016 and P=0.008, for waist and waist z-score, respectively), regardless of covariates. However, there were no significant differences in waist and waist z-score between children having high CRF and high MDP index and those having high CRF but non-high MDP index.

DISCUSSION

In the current study, BMI and waist circumference, the adherence to the MDP and CRF were examined in more than six hundred Spanish preschool children. The study aimed to explore the association of the adherence to the MDP and CRF levels on adiposity estimates. The main findings were (1) the adherence to the MDP was low to moderate; (2) higher adherence to the MDP was associated with lower waist circumference, while higher levels of CRF were associated with both BMI and waist circumference; (3) those children with low adherence to the MDP and low CRF had higher waist and waist z-score, regardless of age, sex and sociodemographic factors. To the best of our knowledge, our study is the first examining the combined associations of the adherence to the MDP and CRF with total and central adiposity estimates in preschool children.

In the current study, 16.0% of preschool met the criterium for overweight and 4.9% for obesity. These results reinforce findings from other studies demonstrating that Spain, together with other Mediterranean countries, showed the highest prevalence of overweight and obesity in early childhood compared with other European countries, particularly Scandinavian countries [19,20]. Likewise, overweight/obesity rates in our study sample were similar to those recently reported in a study of 128 preschoolers from the South of Spain (22% classified according to the WOF criteria)[20] and also to the percentage of overweight children aged 2 to 10 years identified as overweight or obese (21.2%) in the Spanish children participating in the IDEFICS study [21].

Findings of the current study support previous evidence for low to moderate adherence to the MDP among children [22]. Likewise, the average adherence of the preschoolers to the MDP was 62.7% and only 24.3% of children showed optimal adherence (≥80%). The low proportion of preschoolers with optimal adherence to the MDP observed in the present study is of concern and confirms that children living in Mediterranean countries are abandoning the traditional MDP [22]. Optimal adherence to the MDP in the current report was lower than in Spanish preschool children participating in the IDEFICS study (32.3%), whose data were obtained eight/nine years earlier in Zaragoza, one city located also in the North of Spain close to Vitoria-Gasteiz [14]. Although it cannot be interpreted as a secular trend of worsening dietary habits among preschool children, it seems that at least in the North of Spain the adherence to the MDP is decreasing in early childhood, in agreement with previous findings. Results from older children are even more alarming. In Italian 8-9 years old children, only 5.0% had optimal adherence to the MDP [23]. In older children (from 10 to 12 years old), several studies conducted in large samples of Greek [16], Cyprus [24] and Balearic Islands [25] reported optimal adherence to the MDP ranging from 4.3% to 7.1%. To note is that direct comparison among studies are difficult because of differences in the age of children and methods used to assess the adherence to the MDP. Indeed, three of the above mentioned studies assessed the MDP with the KIDMED questionnaire [16,23,24] and two with food frequency questionnaires [14,25].

We observed that lower adherence to the MDP was associated with higher waist circumference, which in turn is strongly related to cardiometabolic risk. The specific relationships between dietary factors and/or diet quality and abdominal and truncal fat mass, as well as with visceral and hepatic fat regardless of total body fat, have been previously reported in several studies[26-28]. The influence of the adherence to the MDP on waist circumference in older pediatric populations was previously reported in cross-sectional [29] and longitudinal studies [14], though no significant associations have also been shown [16,30]. The observed association of MDP with waist circumference in preschoolers may help to explain the previously reported relationship between the adherence to the MDP and metabolic syndrome in older children and adolescents [31], as well as with the preventive role of the MDP against cardiovascular disease later in life [13]. Given the influence of the MDP on the prevention of chronic diseases, and with the reduction in total mortality and improvement of longevity [13], health policies should focus on promoting this traditional dietary pattern from early childhood.

We observed that a worse performance in the 20m shuttle run test (i.e., CRF) was associated with higher BMI and waist circumference in preschoolers. Our results agree with previous studies conducted in school children and adolescents [11] . Furthermore, these findings concur with the few reports that have examined the association of CRF with central and overall adiposity in preschoolers [32,33]. Of note that CRF is a physical condition, while dietary pattern is a behavior. However, despite the strong genetic component of the CRF, regular physical activity (a lifestyle behavior) is one of its main determinants [34]. Therefore, our results reinforce the importance of promoting physical activity already in preschool children. Excess body fat gain is determined by a positive energy balance; thus, the increase of physical activity level may have benefits improving CRF, but also increasing energy expenditure.

The most novel observation of the current study is the combined influence of the adherence to the MDP and CRF on waist circumference in preschoolers. The results showed that the highest levels of waist circumference were found in those children who were unfit and had an unhealthy diet. It can be also observed that children non-having high CRF levels had higher waist and waist z-score than those with high CRF, regardless of their adherence to the MDP. However, it should be also noted that children non-having high CRF and with low adherence to the MDP had higher waist circumference than those non-having high CRF but with a high adherence to the MDP. Actually it means that those children with non-high CRF levels and non-having a high adherence to the MDP are at a higher risk of central obesity and cardiometabolic risk later in life. These findings expand the current knowledge since, as far as we are aware, this is the first study reporting both the separate and combined influence of the adherence to the MDP and CRF on adiposity estimates in preschool children.

The current study has several limitations. Findings from our study should be taken with caution due to its cross-sectional design. Therefore, longitudinal studies are needed to confirm the long term influence of the adherence to the MDP and CRF on obesity and adiposity gain. Collection of dietary data for more than two days or the use of a food frequency questionnaire would have been desirable to compensate for day-to-day variability. However, the KIDMED has been previously validated to use in children [17] and chosen as the dietary assessment method to estimate the adherence to the MDP in many previous studies [16,23,24]. Specifically in preschool children, it has the advantage of its short administration time and high response rate. The sample is not representative of the population of the North of Spain; however, it is a relatively large sample size that covers both private and public schools that should be acknowledged.

CONCLUSIONS

The current study observed poor adherence to the MDP among children from 3 to 5 years together with a high prevalence of overweight and obesity (~21%). Our results demonstrate that only CRF was negatively related to total adiposity; however, both non-high adherence to the MDP and non-high CRF levels, are associated with higher waist circumference already in preschoolers. Likewise, children non-having high CRF levels and with low adherence to the MDP had the highest levels of waist circumference. Overall, these findings suggest that educational strategies aimed to increase CRF and promote healthy dietary habits are necessary from the early childhood to prevent children from suffering excess total and abdominal adiposity and later cardiovascular disease.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist

REFERENCES

1. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, Mullany EC, Biryukov S, Abbafati C, Abera SF, Abraham JP, Abu-Rmeileh NM, Achoki T, AlBuhairan FS, Alemu ZA, Alfonso R, Ali MK, Ali R, Guzman NA, Ammar W, Anwari P, Banerjee A, Barquera S, Basu S, Bennett DA, Bhutta Z, Blore J, Cabral N, Nonato IC, Chang JC, Chowdhury R, Courville KJ, Criqui MH, Cundiff DK, Dabhadkar KC, Dandona L, Davis A, Dayama A, Dharmaratne SD, Ding EL, Durrani AM, Esteghamati A, Farzadfar F, Fay DF, Feigin VL, Flaxman A, Forouzanfar MH, Goto A, Green MA, Gupta R, Hafezi-Nejad N, Hankey GJ, Harewood HC, Havmoeller R, Hay S, Hernandez L, Husseini A, Idrisov BT, Ikeda N, Islami F, Jahangir E, Jassal SK, Jee SH, Jeffreys M, Jonas JB, Kabagambe EK, Khalifa SE, Kengne AP, Khader YS, Khang YH, Kim D, Kimokoti RW, Kinge JM, Kokubo Y, Kosen S, Kwan G, Lai T, Leinsalu M, Li Y, Liang X, Liu S, Logroscino G, Lotufo PA, Lu Y, Ma J, Mainoo NK, Mensah GA, Merriman TR, Mokdad AH, Moschandreas J, Naghavi M, Naheed A, Nand D, Narayan KM, Nelson EL, Neuhouser ML, Nisar MI, Ohkubo T, Oti SO, Pedroza A, Prabhakaran D, Roy N, Sampson U, Seo H, Sepanlou SG, Shibuya K, Shiri R, Shiue I, Singh GM, Singh JA, Skirbekk V, Stapelberg NJ, Sturua L, Sykes BL, Tobias M, Tran BX, Trasande L, Toyoshima H, van de Vijver S, Vasankari TJ, Veerman JL, Velasquez-Melendez G, Vlassov VV, Vollset SE, Vos T, Wang C, Wang X, Weiderpass E, Werdecker A, Wright JL, Yang YC, Yatsuya H, Yoon J, Yoon SJ, Zhao Y, Zhou M, Zhu S, Lopez AD, Murray CJ, Gakidou E (2014) Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 384 (9945):766-781. doi:10.1016/s0140-6736(14)60460-8

2. Abdullah A, Wolfe R, Stoelwinder JU, de Courten M, Stevenson C, Walls HL, Peeters A (2011) The number of years lived with obesity and the risk of all-cause and cause-specific mortality. Int J Epidemiol 40 (4):985-996. doi:10.1093/ije/dyr018

3. Reis JP, Loria CM, Lewis CE, Powell-Wiley TM, Wei GS, Carr JJ, Terry JG, Liu K (2013) Association between duration of overall and abdominal obesity beginning in young adulthood and coronary artery calcification in middle age. JAMA 310 (3):280-288. doi:10.1001/jama.2013.7833

4. Lo JC, Maring B, Chandra M, Daniels SR, Sinaiko A, Daley MF, Sherwood NE, Kharbanda EO, Parker ED, Adams KF, Prineas RJ, Magid DJ, O'Connor PJ, Greenspan LC (2014) Prevalence of obesity and extreme obesity in children aged 3-5 years. Pediatr Obes 9 (3):167-175. doi:10.1111/j.2047-6310.2013.00154.x

5. de Onis M, Blossner M, Borghi E (2010) Global prevalence and trends of overweight and obesity among preschool children. Am J Clin Nutr 92 (5):1257-1264. doi:10.3945/ajcn.2010.29786

6. Cunningham SA, Kramer MR, Narayan KM (2014) Incidence of childhood obesity in the United States. N Engl J Med 370 (5):403-411. doi:10.1056/NEJMoa1309753

7. Cattaneo A, Monasta L, Stamatakis E, Lioret S, Castetbon K, Frenken F, Manios Y, Moschonis G, Savva S, Zaborskis A, Rito AI, Nanu M, Vignerova J, Caroli M, Ludvigsson J, Koch FS, Serra-Majem L, Szponar L, van Lenthe F, Brug J (2010) Overweight and obesity in infants and pre-

school children in the European Union: a review of existing data. Obes Rev 11 (5):389-398. doi:10.1111/j.1467-789X.2009.00639.x

8. Federation WO (2014) Overweight and Obesity in Pre (aged 5-10 years) and Post (14-17 yrs) Adolescent children. http:/www.wordlobesity.org/site_media/library/resource_images/Pre_and_Post_adolescents_chart_June_14.pdf (accesed 29 September 2016).

9. Flynn MA, McNeil DA, Maloff B, Mutasingwa D, Wu M, Ford C, Tough SC (2006) Reducing obesity and related chronic disease risk in children and youth: a synthesis of evidence with 'best practice' recommendations. Obes Rev 7 Suppl 1:7-66. doi:10.1111/j.1467-789X.2006.00242.x

10. Nicklas TA, Demory-Luce D, Yang SJ, Baranowski T, Zakeri I, Berenson G (2004) Children's food consumption patterns have changed over two decades (1973-1994): The Bogalusa heart study. J Am Diet Assoc 104 (7):1127-1140. doi:10.1016/j.jada.2004.04.029

11. Ortega FB, Labayen I, Ruiz JR, Kurvinen E, Loit HM, Harro J, Veidebaum T, Sjostrom M (2011) Improvements in fitness reduce the risk of becoming overweight across puberty. Med Sci Sports Exerc 43 (10):1891-1897. doi:10.1249/MSS.0b013e3182190d71

12. Ortega FB, Labayen I, Ruiz JR, Kurvinen E, Loit H-M, Harro J, Veidebaum T, Sjostrom M (2011) Improvements in Fitness Reduce the Risk of Becoming Overweight across Puberty. Medicine and Science in Sports and Exercise 43 (10):1891-1897. doi:10.1249/MSS.0b013e3182190d71

13. Liyanage T, Ninomiya T, Wang A, Neal B, Jun M, Wong MG, Jardine M, Hillis GS, Perkovic V (2016) Effects of the Mediterranean Diet on Cardiovascular Outcomes-A Systematic Review and Meta-Analysis. PLoS One 11 (8):e0159252. doi:10.1371/journal.pone.0159252

14. Tognon G, Hebestreit A, Lanfer A, Moreno LA, Pala V, Siani A, Tornaritis M, De Henauw S, Veidebaum T, Molnar D, Ahrens W, Lissner L (2014) Mediterranean diet, overweight and body composition in children from eight European countries: cross-sectional and prospective results from the IDEFICS study. Nutr Metab Cardiovasc Dis 24 (2):205-213. doi:10.1016/j.numecd.2013.04.013

15. Lydakis C, Stefanaki E, Stefanaki S, Thalassinos E, Kavousanaki M, Lydaki D (2012) Correlation of blood pressure, obesity, and adherence to the Mediterranean diet with indices of arterial stiffness in children. Eur J Pediatr 171 (9):1373-1382. doi:10.1007/s00431-012-1735-3

16. Farajian P, Risvas G, Karasouli K, Pounis GD, Kastorini CM, Panagiotakos DB, Zampelas A (2011) Very high childhood obesity prevalence and low adherence rates to the Mediterranean diet in Greek children: the GRECO study. Atherosclerosis 217 (2):525-530. doi:10.1016/j.atherosclerosis.2011.04.003

17. Serra-Majem L, Ribas L, Ngo J, Ortega RM, Garcia A, Perez-Rodrigo C, Aranceta J (2004) Food, youth and the Mediterranean diet in Spain. Development of KIDMED, Mediterranean Diet Quality Index in children and adolescents. Public Health Nutr 7 (7):931-935

18. Cadenas-Sanchez C, Martinez-Tellez B, Sanchez-Delgado G, Mora-Gonzalez J, Castro-Pinero J, Lof M, Ruiz JR, Ortega FB (2016) Assessing physical fitness in preschool children: Feasibility, reliability and practical recommendations for the PREFIT battery. J Sci Med Sport 19(11):910-915. doi:10.1016/j.jsams.2016.02.003

19. Serra-Majem L, Aranceta Bartrina J, Perez-Rodrigo C, Ribas-Barba L, Delgado-Rubio A (2006) Prevalence and deteminants of obesity in Spanish children and young people. Br J Nutr 96 (Suppl 1):S67-S72

20. Cadenas-Sanchez C, Nystrom C, Sanchez-Delgado G, Martinez-Tellez B, Mora-Gonzalez J, Risinger AS, Ruiz JR, Ortega FB, Lof M (2016) Prevalence of overweight/obesity and fitness level in preschool children from the north compared with the south of Europe: an exploration with two countries. Pediatr Obes 11(5):403-410. doi:10.1111/ijpo.12079

21. Ahrens W, Pigeot I, Pohlabeln H, De Henauw S, Lissner L, Molnar D, Moreno LA, Tornaritis M, Veidebaum T, Siani A (2014) Prevalence of overweight and obesity in European children below the age of 10. Int J Obes (Lond) 38 Suppl 2:S99-107. doi:10.1038/ijo.2014.140

22. Pereira-da-Silva L, Rego C, Pietrobelli A (2016) The Diet of Preschool Children in the Mediterranean Countries of the European Union: A Systematic Review. Int J Environ Res Public Health 13 (6). doi:10.3390/ijerph13060572

23. Roccaldo R, Censi L, D'Addezio L, Toti E, Martone D, D'Addesa D, Cernigliaro A (2014) Adherence to the Mediterranean diet in Italian school children (The ZOOM8 Study). Int J Food Sci Nutr 65 (5):621-628. doi:10.3109/09637486.2013.873887

24. Lazarou C, Panagiotakos DB, Matalas AL (2009) Level of adherence to the Mediterranean diet among children from Cyprus: the CYKIDS study. Public Health Nutr 12 (7):991-1000. doi:10.1017/s1368980008003431

25. Martinez E, Llull R, Del Mar Bibiloni M, Pons A, Tur JA (2010) Adherence to the Mediterranean dietary pattern among Balearic Islands adolescents. Br J Nutr 103 (11):1657-1664. doi:10.1017/s0007114509993576

26. Labayen I, Ruiz JR, Ortega FB, Huybrechts I, Rodriguez G, Jimenez-Pavon D, Roccaldo R, Nova E, Widhalm K, Kafatos A, Molnar D, Androutsos O, Moreno LA (2014) High fat diets are associated with higher abdominal adiposity regardless of physical activity in adolescents; the HELENA study. Clinical Nutrition 33 (5):859-866. doi:10.1016/j.clnu.2013.10.008

27. Black MH, Watanabe RM, Trigo E, Takayanagi M, Lawrence JM, Buchanan TA, Xiang AH (2013) High-Fat Diet Is Associated with Obesity-Mediated Insulin Resistance and beta-Cell Dysfunction in Mexican Americans. J Nutr. 43(4):479-85. doi: 10.3945/jn.112.170449

28. Mollard RC, Senechal M, MacIntosh AC, Hay J, Wicklow BA, Wittmeier KD, Sellers EA, Dean HJ, Ryner L, Berard L, McGavock JM (2014) Dietary determinants of hepatic steatosis and

visceral adiposity in overweight and obese youth at risk of type 2 diabetes. Am J Clin Nutr 99(4):804-812. doi:10.3945/ajcn.113.079277

29. Schroder H, Mendez MA, Ribas-Barba L, Covas MI, Serra-Majem L (2010) Mediterranean diet and waist circumference in a representative national sample of young Spaniards. Int J Pediatr Obes 5 (6):516-519. doi:10.3109/17477161003777417

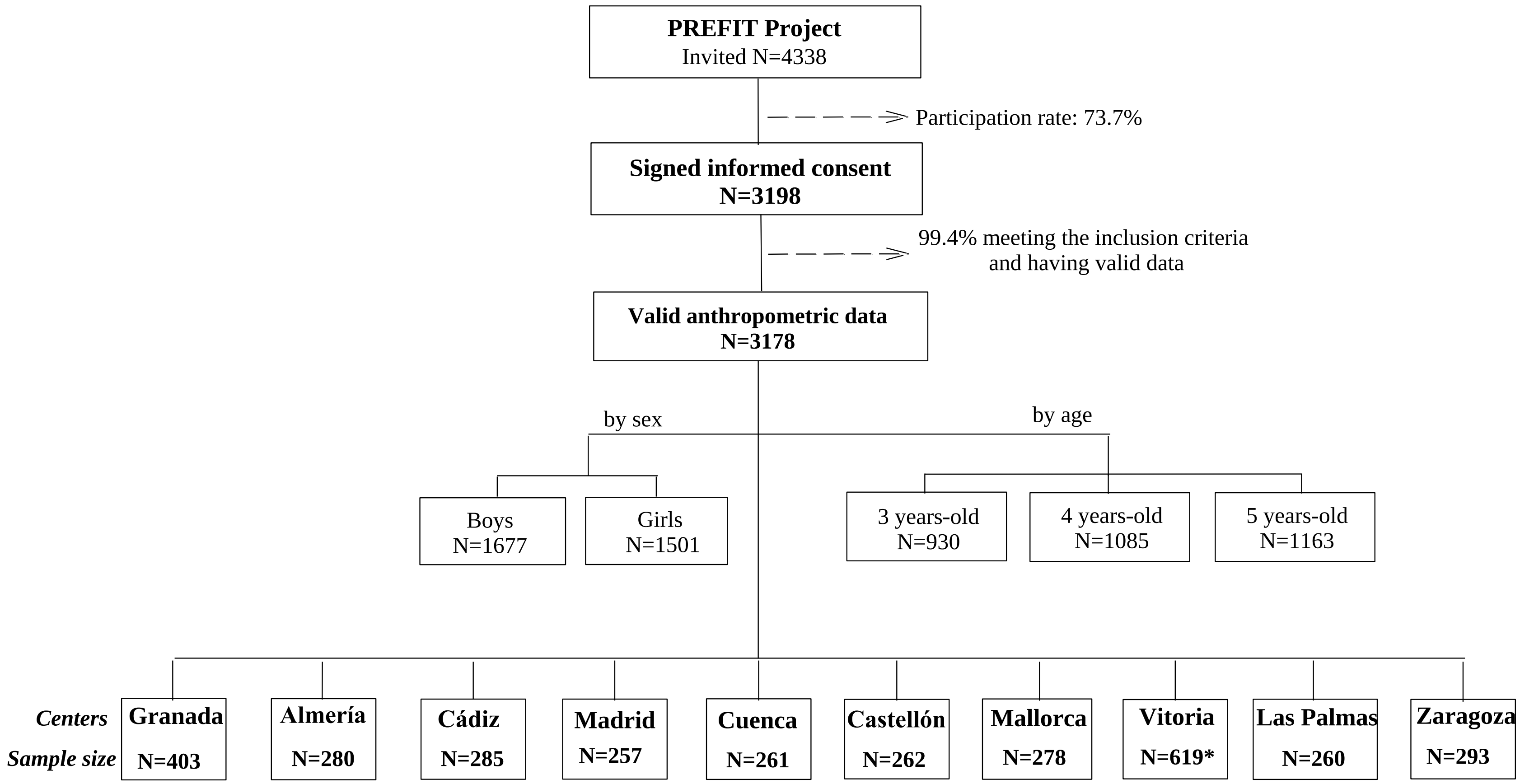
30. Jennings A, Welch A, van Sluijs EM, Griffin SJ, Cassidy A (2011) Diet quality is independently associated with weight status in children aged 9-10 years. J Nutr 141 (3):453-459. doi:10.3945/jn.110.131441

31. Martino F, Puddu PE, Lamacchia F, Colantoni C, Zanoni C, Barilla F, Martino E, Angelico F (2016) Mediterranean diet and physical activity impact on metabolic syndrome among children and adolescents from Southern Italy: Contribution from the Calabrian Sierras Community Study (CSCS). Int J Cardiol 225:284-288. doi:10.1016/j.ijcard.2016.10.008

32. Henriksson P, Cadenas-Sanchez C, Leppanen MH, Delisle Nystrom C, Ortega FB, Pomeroy J, Ruiz JR, Lof M (2016) Associations of Fat Mass and Fat-Free Mass with Physical Fitness in 4-Year-Old Children: Results from the MINISTOP Trial. Nutrients 8 (8). doi:10.3390/nu8080473

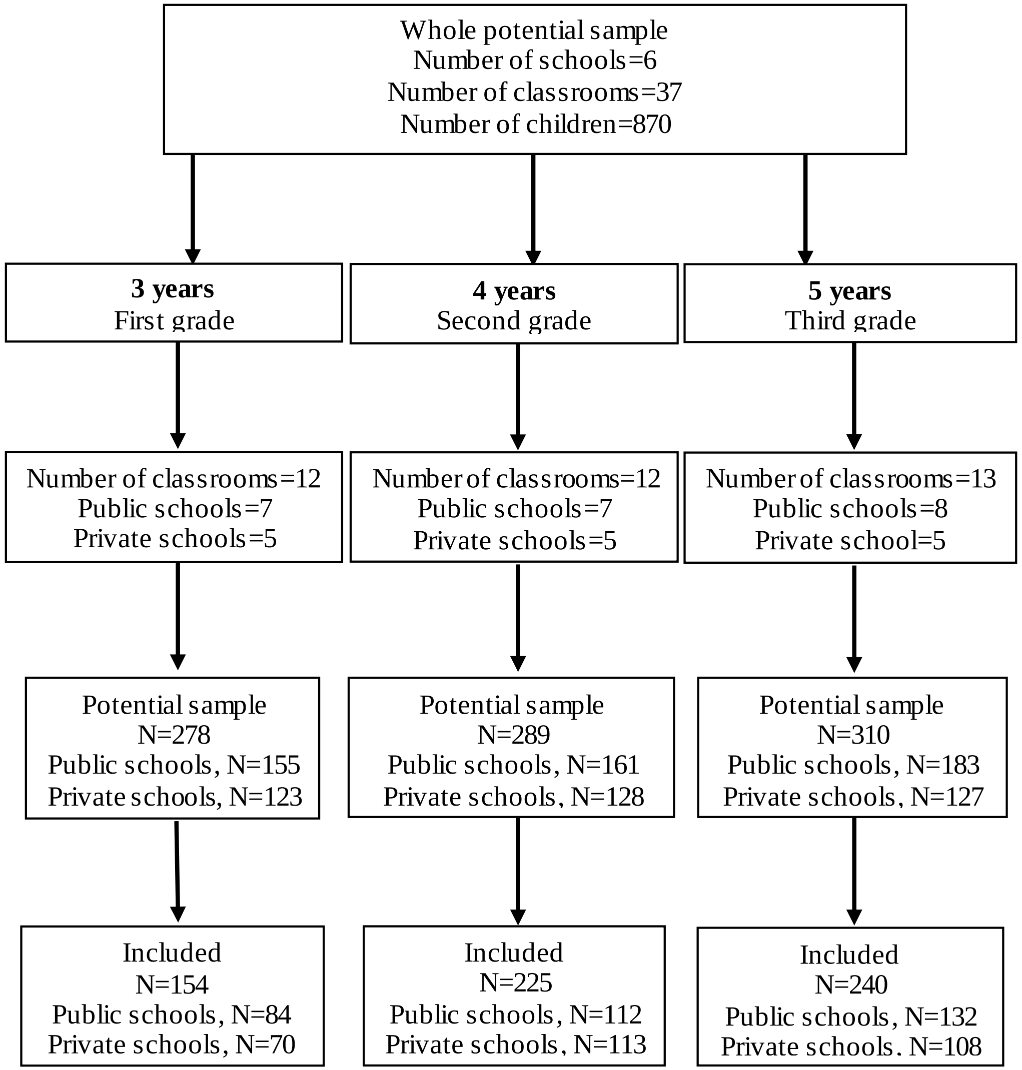
33. Martinez-Tellez B, Sanchez-Delgado G, Cadenas-Sanchez C, Mora-Gonzalez J, Martin-Matillas M, Lof M, Ortega FB, Ruiz JR (2015) Health-related physical fitness is associated with total and central body fat in preschool children aged 3 to 5 years. Pediatr Obes. doi:10.1111/ijpo.12088

34. Ruiz JR, Ortega FB, Martinez-Gomez D, Labayen I, Moreno LA, De Bourdeaudhuij I, Manios Y, Gonzalez-Gross M, Mauro B, Molnar D, Widhalm K, Marcos A, Beghin L, Castillo MJ, Sjostrom M, Grp HS (2011) Objectively Measured Physical Activity and Sedentary Time in European Adolescents The HELENA Study. American Journal of Epidemiology 174 (2):173-184. doi:10.1093/aje/kwr068

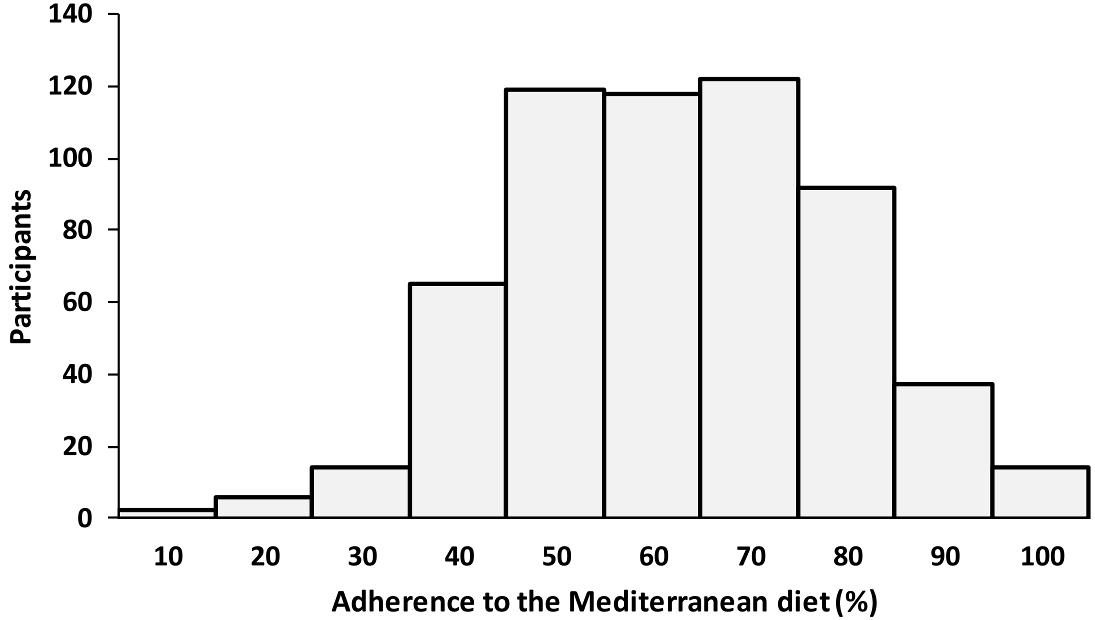


Supplemental Figure 1 Flow chart of the participants involved in the PREFIT Project.

* The sample size in this center in the North of Spain was over dimensioned in order to compensate the sample size in the South of Spain, Andalucía where 3 centers participated in the study



Supplemental Figure 2. Flow diagram of study participants.



Supplemental Figure 3. Distribution of percentage of adherence to the Mediterranean dietary pattern among preschool children participating in the PREFIT study.

Supplemental Table 1. Dietary habits and adherence to the Mediterranean dietary pattern (MDP) in pre-school children.

	Scoring	Boys	Girls	P
Takes a fruit or fruit juice every day (N, %)[a,b,c]	+1	260, 82.8	246, 87.9	0.105
Has a second fruit every day (N, %)[a,b,c]	+1	113, 36.0	127, 45.4	0.024
Has fresh or cooked vegetables regularly once a day (N, %)[a,b,c]	+1	201, 64.0	191, 68.2	0.298
Has fresh or cooked vegetables regularly more than once a day (N, %)[a,b,c]	+1	65, 20.7	79, 28.2	0.044
Consumes fish regularly (at least 2-3/week) (N, %)[a,b,c]	+1	266, 84.7	237, 84.6	0.981
Likes pulses and eats them >1 week (N, %)[a,b,c]	+1	286, 91.1	240, 85.7	0.037
Consumes pasta or rice almost every day (5 or more per week) (N, %)[a,b,c]	+1	46, 14.6	117, 41.8	<0.001
Has cereals/grains (bread, etc.) for breakfast (N, %)[a,b,c]	+1	251, 79.9	234, 83.6	0.285
Consumes nuts regularly (at least 2-3/week) (N, %)[a,b,c]	+1	81, 25.8	89, 31.8	0.122
Uses olive oil at home (N, %)[a,b,c]	+1	310, 98.7	268, 95.7	0.027
Goes ≥1/week to a fast food restaurant (hamburger) (N, %)[a,d]	-1	20, 7.5	36, 12.9	0.008
Skips breakfast (N, %)[a,d]	-1	12, 3.8	26, 9.3	0.007
Has a dairy product for breakfast (N, %)[a,c]	+1	304, 96.8	266, 95.0	0.282
Has commercially baked goods/pastries for breakfast (N, %)[a,d]	-1	129, 41.1	101, 36.0	0.237
Takes two yoghurts and/or some cheese (40g) daily (N, %)[a,c]	+1	259, 82.5	213, 76.3	0.067
Takes sweets and candy every day (N, %)[a,d]	-1	35, 11.1	44, 15.8	0.115
KIDMED score (mean, SD)		7.0 (1.9)	7.2 (1.9)	0.151
MDP index (mean, SD)		6.0 (1.6)	6.5 (1.7)	<0.001

[a] Included in the calculation of the KIDMED score. [b] Included in the calculation of the MDP index. SD: standard deviation.

[c] Scoring positive (+1). [d] Scoring negative (-1).

3.2. Study II

Published in: European Journal of Nutrition

European Journal of Nutrition
https://doi.org/10.1007/s00394-018-1809-8

ORIGINAL CONTRIBUTION

Adherence to the Mediterranean diet in metabolically healthy and unhealthy overweight and obese European adolescents: the HELENA study

Lide Arenaza [a], Inge Huybrechts [b], Francisco B. Ortega [c], Jonatan R. Ruiz [c], Stefaan De Henauw [d], Yannis Manios [e], Ascensión Marcos [f], Cristina Julián [g], Kurt Widhalm [h], Gloria Bueno [g], Mathilde Kersting [i], Anthony Kafatos [j], Christina Breidenassel [k], Raquel Pedrero-Chamizo [l], Frédéric Gottrand [m], Marcela González-Gross [l], Luis A. Moreno [g,n], Idoia Labayen [a]

[a] Institute for Innovation & Sustainable Development in Food Chain (IS-FOOD), Public University of Navarra, Pamplona, Navarra, Spain
[b] International Agency for Research on Cancer, Dietary Exposure Assessment Group, Lyon, France
[c] PROmoting FITness and Health through physical activity research group (PROFITH), Department of Physical Education and Sports, Faculty of Sport Sciences, University of Granada, Granada, Spain
[d] Department of Public Health, Ghent University, Ghent, Belgium
[e] Department of Nutrition and Dietetics, Harokopio University, Athens, Greece
[f] Immunonutrition Research Group, Department of Metabolism and Nutrition, Instituto del Frio, Institute of Food Science, Technology and Nutrition (ICTAN), Spanish National Research Council (CSIC), Madrid, Spain
[g] GENUD (Growth, Exercise, Nutrition and Development) Research Group, University of Zaragoza, Zaragoza, Spain
[h] Division of Nutrition and Metabolism, Department of Pediatrics, Medical University of Vienna, Vienna, Austria
[i] Research Institute of Child Nutrition, Dortmund, Germany
[j] School of Medicine, University of Crete, Crete, Greece
[k] Department of Nutrition and Food Science, University of Bonn, Bonn, Germany
[l] Faculty of Physical Activity and Sport Sciences (INEF), Universidad Politécnica de Madrid, Madrid, Spain
[m] Department of Pediatrics, Jeanne de Flandre Children's University Hospital, Lille, France
[n] Department of Preventive Medicine, Faculty of Medicine, University of Sao Paulo, Sao Paulo, Brazil
Corresponding author: Lide Arenaza Etxeberria, lide.arenaza@unavarra.es

ABSTRACT

Purpose: To examine the adherence to the Mediterranean dietary pattern (MDP) in metabolically healthy overweight or obese (MHO) and metabolically unhealthy obese (MUO) European adolescents.

Methods: In this cross-sectional study 137 overweight/obese adolescents aged 12-17 years old from the HELENA study were included. Height, weight, waist circumference and skinfolds thicknesses were measured and body mass index (BMI) and body fat percent were calculated. Systolic and diastolic blood pressure, glucose, HDL-cholesterol, triglycerides and cardiorespiratory fitness (20-m shuttle run test) were measured. MHO and MUO phenotypes were categorized following the Jolliffe and Janssen criteria. Two non-consecutive 24-h recalls were used for dietary intake assessment and the adherence to the MDP was calculated using the Mediterranean dietary pattern score (MDP score) (range 0-9).

Results: A total of 45 (22 girls) adolescents (32.8%) were categorized as MHO. The adherence to the MDP was significantly higher in MHO than in MUO adolescents regardless of age, sex, body fat percentage, energy intake and center (MDP score: 4.6±1.6 *vs*. 3.9±1.5, p=0.036), but this difference became non-significant after further adjustment for cardiorespiratory fitness. Participants who had a low adherence to the MDP (MDP score ≤4) had a higher likelihood of having MUO phenotype regardless of sex, age, energy intake, center and body fat percentage (OR 2.2; 95% CI, 1.01-4.81, p=0.048).

Conclusions: Adherence to the MDP might be beneficial to maintain metabolic health in overweight/obese adolescents, yet cardiorespiratory fitness seems to play a key role on the metabolic phenotype.

Keywords: Metabolic health, Obesity, Mediterranean diet, Adolescents, Cardiorespiratory fitness

INTRODUCTION

Childhood and adolescence obesity prevalence has increased over the last few decades [1]. This chronic disease is associated with cardiometabolic risk factors such as insulin resistance, dyslipidemia and hypertension [2]. However, several studies reported the existence of metabolically healthy phenotypes, referred to as metabolically healthy overweight or obesity (MHO), in whom no obesity associated comorbidities are found, whereas obesity associated with metabolic abnormalities is known as metabolically unhealthy overweight or obesity (MUO)[3].

Although there is no a standard definition of MHO, which in turn leads to vary the rates of MHO, the prevalence of the healthy phenotype seems to be close to 30% of population with obesity and it is also negatively associated with obesity degree [4,5,6].

Diet and physical activity are lifestyle determinants of health closely related to obesity and its complications [5]. Nowadays, nutrition research focuses more on examining the impact of dietary patterns such as the Mediterranean diet (MDP) on health outcomes, instead of exploring the effect of nutrients or individual food groups on health status. Thereby, dietary patterns offer a more holistic description of dietary habits considering also the possible interactions among nutrients and foods [6]. The MDP shows the typical dietary pattern followed by people from Creta, Greece and Southern Italy in the 1960s [7]. This Mediterranean region was the area with the highest life expectancy and with the lowest incidence of coronary heart disease in the world. Adherence to the MDP has been associated with a reduction in cardiovascular and all-cause mortality and type 2 diabetes incidence [8,9]. MDP is characterized by high intakes of vegetables, fruits, nuts, cereals, legumes and olive oil as the principal source of fat, moderate to-high- intake of fish, moderate consumption of dairy products and wine (during meals) and low consumptions of meat and poultry [7]. Several studies reported that adherence to the MDP improves health status in youths [9]. Accordingly, we hypothesized that MHO adolescents might have a higher adherence to this dietary pattern than their MUO peers. The current study aimed to examine the association between the adherence to MDP and metabolic health status in overweight and obese European adolescents from the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study.

MATERIAL AND METHODS

Study design

The HELENA study is a multicenter cross-sectional study which was designed to evaluate the nutritional status and lifestyle in adolescents (12.5-17.5 years) from 10 European cities between 2006 and 2007 [10]. Among the total sample of 3,528 participants, blood samples were randomly selected and collected in a subsample of 1,069 adolescents [10]. A detailed description of the HELENA study procedures and methodology has been published elsewhere [11,12] . All adolescents participating in the study and their parents or legal guardians had to sign an informed written consent so that they could be enrolled in the study. The study protocol was approved by the corresponding local Human Research Review Committees of the centers involved, which were the following ones: Athens in Greece, Dortmund in Germany, Gent in Belgium, Lille in France, Roma in Italy, Stockholm in Sweden, Vienna in Austria and Zaragoza in Spain. For the target of the current study, a total of 137 (14.8±1.3 years, 48.9 % girls) overweight or obese adolescents from whom dietary and biomarkers data were available were included (**Fig. 1**).

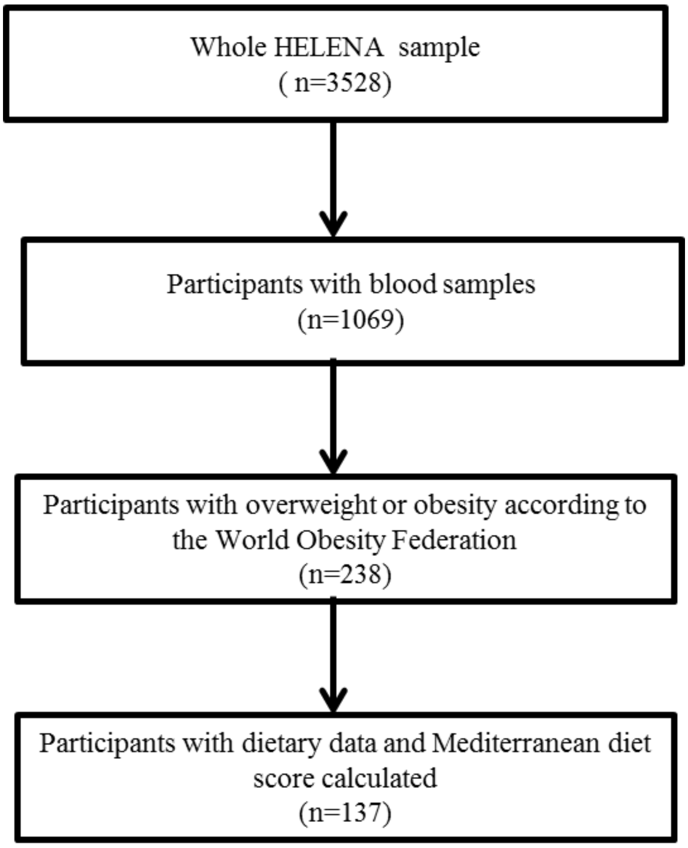


Fig 1. Flow diagram of study participants

Anthropometry and cardiometabolic risk factors

Anthropometric assessment was performed to categorize weight status, as well as to examine body composition. Weight (SECA 861, Hamburg, Germany) and height (SECA 225, Hamburg, Germany) were measured with a high-precision scale to the nearest 0.05 kg and with a telescopic height measuring instrument to the nearest 0.1cm, respectively [11]. BMI was determined as body weight divided by height squared (kg/m2) and overweight and obesity status were categorized according to the World Obesity Federation criteria [12]. A non-elastic tape (SECA 200) to the nearest 0.1 cm was used for waist circumference measurement. In addition, tricipital and subscapular skinfold thicknesses were measured (Holtain) and body fat percentage was computed using the Slaughter equation [13]. Blood pressure (systolic and diastolic) was measured twice with a 10 minutes time period between, using a clinical automated digital blood pressure device (OMRON) and the lowest value was recorded. Glucose, triglycerides and HDL-cholesterol, were obtained from blood samples collected after 10-h overnight fast by following an established blood collection and analysis protocol [14]. Cardiorespiratory fitness was determined using the 20-m shuttle run test as described elsewhere [15]. Briefly, adolescents had to run between two lines 20m apart following an audio signal from a recorded CD. The audio signal started with an 8.5km/h speed increasing by 0.5km/h per minute. When the participants did not reach the end line along with the audio signal on two consecutive times or when the adolescent stopped because of exhaustion the test was finished. Thereby, aerobic fitness was examined calculating the maximal oxygen consumption (ml/kg/min) using the equation suggested by Leger et al [16].

MHO and MUO classification criteria

Several criteria have been proposed for metabolic health classification in pediatric populations. Nevertheless, in the current study, in order to establish the criteria for classifying metabolic status we followed the definition of MHO proposed by Ortega et al, which are a detailed criteria based on a comprehensive review of the literature summarized into 7 scientific arguments (for more information, see tables 2 and 3 of the review) [17]. The criteria for the classification of overweight/obese individuals as MHO or MUO of this review is in accordance with previous large collaborative studies and international organizations agreements, and it is also appropriate for using with youths [17]. This definition recommends the use of the criterium proposed by Jolliffe and Janssen [18] for adolescent population, since they classify metabolically healthy and unhealthy status based on specific sex and age cut-off points. Using these criteria, adolescents were considered as MHO if they met zero of the criteria; whereas adolescents were considered as MUO if they presented one or more of the following cardiometabolic risk factors: high systolic and/or diastolic blood pressure, high blood glucose level, high triglycerides (TG) levels and low values of HDL-cholesterol. In accordance with previous studies, waist circumference was not included in the definition of metabolic health, considering that it is above the established thresholds in the majority of individuals with overweight or obesity [18,20].

Dietary intake assessment

Two non-consecutive computerized 24-h recalls were used to assess dietary intake in a time span of two weeks. These 24-h recalls were collected using the HELENA-Dietary Assessment Tool (HELENA-DIAT) 24-h dietary recall software, a nutrition assessment tool which is organized in six meal occasions (breakfast, mid-morning snack, midday meal, afternoon snack, evening meal and evening snack) referring to the day before the interview. This 24-h recalls were self-reported, but the adolescents were helped by trained dieticians [20].

Adherence to the MDP was determined as the sum of the score assigned to nine food groups and nutrients, including seven positive and two negative dietary components. The current scale was based on the MDP scale of Trichopoulou et al [7]. Dietary intake of vegetables, fruits and nuts, cereals roots, pulses, fish, dairy products and unsaturated to saturated fat ratio were scored positively, scoring 1 point when the intakes were above the sex-specific median (50th percentile) and scoring 0 when the intakes were below the sex-specific median (see Supplemental Table 1 to find information about food intake and adherence to the MDP in the whole HELENA sample including non-overweight adolescents). The intake of dairy products was considered as a positive dietary component because in adolescence dairy products are recommended due to growth and development processes. The intakes of fruits, nuts and olives were included in the fruit and nuts variable; bread, cereals, flour, rice cereals, pasta and potatoes were considered as cereal roots; and dairy products included milk, yoghurt and cheese. In contrast, meat and processed meat and alcohol consumption were scored inversely.

The value of 0 was given to a meat intake above the sex-specific median and a value of 1 when the intake was below the median. Alcohol intake was considered as an unhealthy product in adolescents and therefore a value of 1 was given if there was no consumption, while a value of 0 if there was any alcohol intake. Likewise, the range of the Mediterranean diet score (MDP score) ranged from 0 (minimal adherence) to 9 points (maximal adherence). Thereafter, the adherence to the MDP was classified into two groups, low (≤4 points) and high (>5 points) adherence to the MDP.

Statistical analysis

Independent t tests were used to identify differences between MHO and MUO groups in continuous variables, while to examine differences in categorical variables chi-square tests were used. Age and tanner stage were categorized to classify younger (age below median) from older adolescents (age equal or above median) and adolescents with lower puberty stage (below or equal puberty stage III) from those participants with high sexual maturation (equal or above IV). (Variables with a non-normal distribution (glucose, triglycerides, HDL-cholesterol, systolic and diastolic blood pressure, cardiorespiratory fitness and MDP score) were logarithmically transformed. As the adherence to the MDP should be higher in the Southern (Athens, Rome and Zaragoza) than in Central-Northern (Dortmund, Ghent, Lille, Stockholm and Vienna) European countries, this categorical variable thereafter called center (Central-Northern vs. Southern country) was used as covariate in the analyses. As there were no statistically significant differences in the prevalence of MHO and MUO phenotypes according to the maternal educational level, the socioeconomic status of the parents was not included as a confounder in the analyses. Differences in adherence to the MDP between MHO and MUO groups were analyzed using univariate linear models with sex, age, energy intake and center (model 1); sex, age, energy intake, center and body fat percentage (model 2) and sex, age, energy intake, center, body fat percentage and cardiorespiratory fitness (model 3) as covariates. Binary logistic regression models were developed to analyze the relationship between adherence to the MDP (low vs. high MDP score score) and metabolic phenotypes (MHO vs. MUO) adjusted for sex, age, energy intake and center (model 1); sex, age, energy intake, center and body fat percentage (model 2) and finally using sex, age, energy intake, center and cardiorespiratory fitness (model 3) as covariates. Sensitivity analyses were carried out with continuous odds ratio to analyze the relationship between the adherence to the MDP and metabolic phenotypes. Statistical analyses were carried out with the statistical software SPSS version 20.0 (SPSS Inc, Chicago) with a level of significance of α=0.05.

RESULTS

Anthropometric and biological characteristics in MHO and MUO adolescents are shown in **Table 1**. Weight, BMI, maternal educational level and the percentage of participants with obesity were significantly higher in MUO compared to MHO groups (p<0.05, Table 1). In contrast, sex, age, pubertal status, height, waist circumference, body fat percentage and maternal educational level did not differ between the two metabolic phenotypes. Overall, as expected due to the definition of the metabolic phenotype, MHO adolescents had a healthier cardiovascular profile, i.e., lower values of TG, systolic and diastolic blood pressure and higher levels of HDL (p<0.001, Table 1). There were no significant differences (p=0.915) in the distribution of MHO and MUO adolescents across the Center-North and South of Europe (**Supplemental Table 2**).

Table 1. Anthropometric and biological characteristics in metabolically healthy overweight or obese (MHO) and metabolically unhealthy overweight or obese (MUO) adolescents participating in the HELENA Study.

	MHO **n=45**	**MUO** **n=92**	**p**
	Mean (SD)	Mean (SD)	
Age (years)	14.7 (1.3)	14.7 (1.4)	0.812
Sex (males/females)	23/22	47/45	0.998
High puberty stage (N, %)	29 (64.4)	63 (68.5)	0.637
Older adolescents (N, %)	20 (44.4)	50 (54.3)	0.276
Weight (kg)	70.5 (12.2)	77.2 (14.7)	**0.004**
Height (cm)	164.8 (10.4)	167.4 (9)	0.071
Waist circumference (cm)	81.8 (7.7)	84.3(8.4)	0.083
Body mass index (kg/m^2)	25.9 (0.5)	27.4 (0.3)	**0.012**
Obese (N, %)	6 (13.3)	31 (33.7)	**0.012**
Body fat (%)	32.4 (9.4)	35.5 (8.8)	0.065
High maternal educational level (N, %)	9 (20)	17 (18.5)	0.534
Glucose (mmol/L)	4.9 (0.3)	5.1 (0.5)	0.056
Triglycerides (mmol/L)	0.7 (0.3)	1.1 (0.6)	**<0.001**
HDL cholesterol (mmol/L)	1.4 (0.2)	1.2 (0.2)	**<0.001**
Systolic blood pressure (mmHg)	113.5 (8.0)	127.5 (15.6)	**<0.001**
Diastolic blood pressure (mmHg)	61.5 (9.1)	70.1 (8.2)	**<0.001**
Cardiorespiratory fitness (ml/kg/min)	39.9 (10.8)	36.7 (8.2)	0.148

SD: Standard deviation; HDL: High-density lipoprotein; High puberty stage equal or above IV Tanner stage; Older adolescents: age equal or above median). Student´s *t* test was used to compare mean differences between MHO and MUO groups in continuous variables, while chi-square test was used to examine mean differences in categorical variables. Although analyses were carried out with logarithmically transformed values, non-transformed data are shown in the table in order to make an easier interpretation.

Table 2 shows dietary intake in MHO and MUO adolescents. Higher fish intake (%33.3) was observed in MHO than in MUO adolescents (p<0.05, Table 2). In contrast, intake of vegetables, fruits and nuts, cereal roots, pulses, unsaturated to saturated fatty acids ratio, dairy products, meat and alcohol were similar in MHO and MUO (Table 2).

Table 2. Dietary intake in metabolically healthy overweight or obese (MHO) and metabolically unhealthy overweight or obese (MUO) adolescents.

	Overweight participants		
	MHO (n=45)	**MUO (n=92)**	**p**
	Mean (SD)	Mean (SD)	
Vegetables (g/day)	102 (79)	92 (57)	0.436
Fruits and nuts (g/day)	113 (88)	118 (77)	0.766
Cereal roots (g/day)	320 (122)	295 (110)	0.235
Pulses (g/day)	5 (14)	9 (28)	0.255
Fish (g/day)	24 (24)	16 (16)	**0.040**
FU/FS ratio (day)	0.93 (0.19)	0.93 (0.16)	0.923
Dairy products (g/day)	262 (305)	184 (164)	0.113
Meat (g/day)	140 (75)	147 (73)	0.612
Alcohol (g/day)	0.4 (0.7)	0.6 (1.2)	0.316
Mediterranean diet score (0-9)	4.5 (1.7)	3.9 (1.6)	**0.044**
High adherence (N, %)	25 (55.6)	34 (37)	**0.039**

SD: Standard deviation; FU/FS ratio: unsaturated to saturated fatty acids ratio. Although analyses were carried out with logarithmically transformed values, non-transformed data are shown in the table in order to make an easier interpretation.

The adherence to the MDP in MHO and MUO adolescents is shown in **Fig. 2**. It was observed that the MDP score was significantly higher in MHO than in MUO adolescents regardless of age, sex, energy intake and center (4.5±1.7 *vs*. 3.9±1.6, in MHO and MUO respectively, p=0.044, Model 1, Fig. 2). This difference was strengthened when body fat percentage was entered into the model (4.6±1.6 *vs*. 3.9±1.5, p=0.036, Model 2, Fig. 2), but it was diminished and became statistically non-significant after further adjustment for cardiorespiratory fitness (4.3±1.6 *vs*. 3.9±1.6, p=0.323, Model 3, Fig. 2).

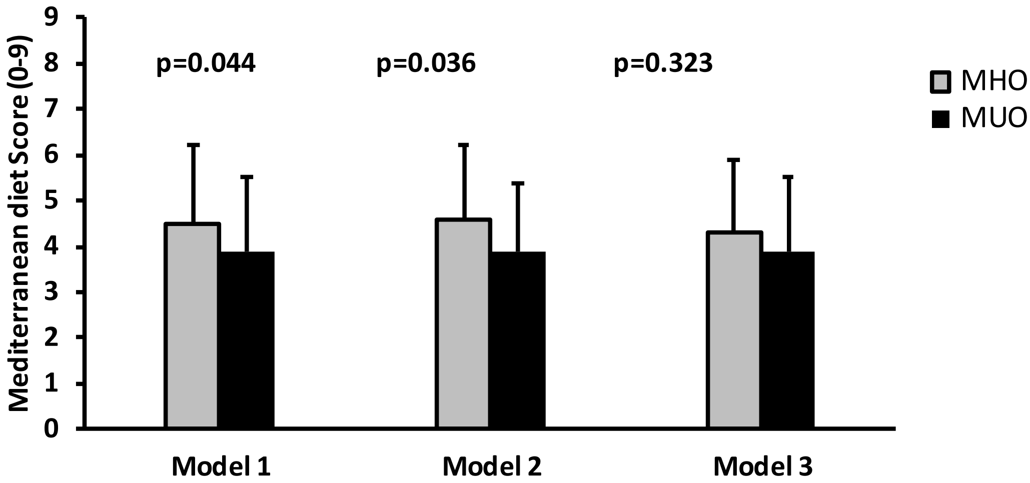


Fig. 2 Adherence to the Mediterranean diet in metabolically healthy overweight/obese (MHO, n=45) and metabolically unhealthy overweight/obese (MUO, n=92) adolescents. Bars are values of adjusted means and error bars are standard error of means. Model 1 was adjusted for sex, age, energy intake and center; Model 2: was additionally adjusted with body fat percentage (MHO/MUO n=43/88); Model 3: was further adjusted with cardiorespiratory fitness (MHO/MUO n=30/77).

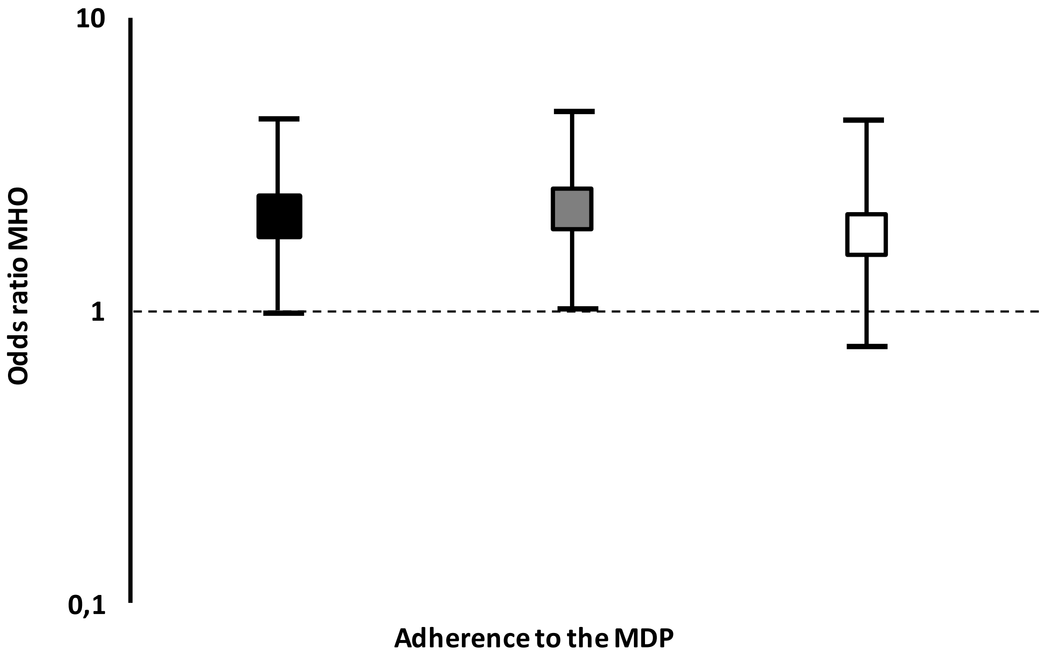


Fig. 3 Odds ratios (boxes) and 95% confidence intervals (error bars represent values) after adjusting for sex, age, energy intake and center (Model 1), additionally adjusted for body fat percentage (Model 2) and adjusted for sex, age, energy intake, center and cardiorespiratory fitness (Model 3).

Odds ratios (OR) for having obesity-associated risk factors according to the adherence to the MDP categories (low *vs*. high) are shown in **Fig. 3**. It was observed that adolescents with low adherence to the MDP (MDP score ≤4) had higher likelihood of having MUO phenotype regardless of sex, age, energy intake, center and body fat percent (OR 2.2; 95%CI, 1.01-4.81, p=0.048, Model 2, Fig. 3). However, when the analyses were further adjusted with cardiorespiratory fitness the results were attenuated and became non-significant (OR 1.8; 95%CI, 0.74-4.46, p=0.051, Model 3, Fig. 3). After sensitivity analyses with continuous OR, it was observed that the odds ratio between the adherence to the MDP and MUO metabolic phenotype was below one (OR 0.8; 95% CI, 0.58-0.97, sex, age, energy intake, center and body fat percentage adjusted p=0.032), which means that higher adherence to the MDP is associated with lower likelihood of being MUO.

DISCUSSION

The main finding of the current study is that overweight or obese adolescents with a healthier metabolic profile (i.e., MHO) present higher adherence to the MDP (~7%) compared to adolescents who have already developed cardiometabolic risk factors, and as a consequence, have a worse metabolic profile. Thus, a higher score of the MDP scale was observed among MHO than in their MUO peers, which could be due to higher fish intake observed among MHO participants. Indeed, having a low adherence to the MDP increased by twice the likelihood of having obesity-associated risk factors, and as consequence, having MUO phenotype.

As expected, the majority of the cardiometabolic risk factors were higher in MUO than in MHO adolescents. It was observed that approximately 33% of males and females did not show cardiometabolic risk factors associated to excess adiposity. These findings are in line with previous studies which reported that around a third part of teenagers with obesity could be classified as MHO [21]. In any case, the prevalence of each group studied certainly depends on the metabolic syndrome status criteria used to classify individuals into MHO and MUO [17]. Moreover, the metabolic profile seems to worsen when increasing adiposity and/or duration of obesity [22]. A recent study observed that the MHO phenotype decreased with age in both genders [22]. Thus, MHO rates ranged between 4.2% and 68% in childhood and adolescence, whereas MHO prevalence in adulthood across Europe was 7-28% and 2-19% for women and men, respectively [22,3]. In our study, on the contrary, the metabolic status did not differ across pubertal development stages or with age.

The effect of the diet quality or dietary patterns on metabolic phenotypes has already been studied. For instance, researches of the National Health and Nutrition Examination Survey examined the Healthy Eating Index in adolescents and adults with obesity and showed that the diet quality was higher among MHO compared with MUO, which stands for having a better compliance to the American Dietary Guidelines [5,23]. These findings concur with the results of the current study suggesting that the adherence to the MDP could be a protective factor to develop the MUO phenotype. In contrast, other authors did not find any significant difference in macro/micronutritional composition among middle-aged obese adults with MHO and MUO phenotypes and neither healthy obesity was associated with increased diet quality [24,25].

The benefits of adherence to the MDP on metabolic risk have been extensively studied. A higher adherence to the MDP has been related to lower BMI, glucose level and a better lipid profile in children and adolescents [26]. Similarly, Zhong et al. observed that adherence to MDP was associated with better glycemic control and lipid profile among adolescents with type 1 diabetes who were less than twenty years-old at diagnosis [27]. In this last study, adherence to the MDP was assessed using the KIDMED questionnaire, and precisely, an increase of two-points out of twelve in the KIDMED score (~%17 higher adherence) was associated with 4 mg/dL lower total cholesterol and 3.4 mg/dL lower LDL-cholesterol [27]. In another study with hypercholesterolemic children, after 12-month of nutritional intervention based on MDP, an approximately 10% decrease of total and LDL-cholesterol levels was observed [28]. Although several studies have examined the influence of the adherence to the MDP on cardiovascular health in children and adolescents [26,28], as far as we are aware, there is no previous study examining the adherence to the MDP on MHO and MUO phenotypes in youths which hampers comparisons among studies.

The present study demonstrated that adolescents with a healthy metabolic profile showed to have a higher fish intake compared to those with metabolic abnormalities. The effect of fish intake in metabolic status has previously been studied by different authors. Likewise, Kim et al. observed that high fish intake was associated with lower TG and blood pressure and higher HDL cholesterol levels [6,29]. Thus, individuals who were in the highest third of fish intake had 65% lower risk of having metabolic syndrome comparing with those in the lowest third. The possible preventive role of fish consumption in the development of the metabolic syndrome in adults was examined in a systematic review, and the authors concluded that fish consumption may improve metabolic health [31]. Dietary fatty acid composition is also likely to be involved in the protective role of metabolic syndrome [7,31]. Previous studies proposed that polyunsaturated fatty acids in fish could be the possible connectors between dietary intake and health benefits [29,32]. This observation is in accordance with the higher fish consumption (~33%) observed in our sample of MHO compared to MUO adolescents. In contrast, other studies did not observe any significant association between dietary fish intake and metabolic status [34] or showed that this preventive role of fish might be gender-related [35]. Unexpectedly, in a previous study of Danish adolescents, fish intake was associated with a poorer metabolic profile [36].

We observed that the difference in the adherence to MDP between MHO and MUO groups became non-significant after further adjustment for cardiorespiratory fitness. However, this finding should be interpreted carefully due to the missing values for cardiorespiratory fitness in the sample that could affect this observation. Cardiorespiratory fitness is an important health marker [37] which has been associated with a healthier cardiovascular status and a lower risk of metabolic factors already in children [38]. In line with our results, other studies reported that metabolic health is influenced by cardiorespiratory fitness in adolescents [39]. In this way, in a recent review Ortega et al. examined the role of fitness in MHO and suggested that higher cardiorespiratory fitness level may be a key characteristic of MHO phenotype [40]. To the opposite, other authors did not find any association between fitness and MHO phenotype in youth; nonetheless, lower levels of fatness and the lack of hepatic steatosis were strongly associated with MHO phenotype in these adolescents [41].

Strengths and limitations

This study has several limitations. First and foremost, the sample size is low due to the inclusion criteria considered in the current study (overweight/obesity status, blood samples and dietary intake data available). Nevertheless, adolescents were randomly chosen to collect blood samples in order to avoid selection bias in the HELENA study [10]. Second, the cross-sectional design of the study should be also considered as a limitation since it does not help to determine causality and directionality of the relationships. We cannot absolutely exclude that dietary data could reflect any medical or dietary advice received prior to recruitment. However, its probability is low because one of the inclusion criteria in the HELENA study was that all study participants should be at least apparently healthy. Although can indeed being dietary assessment challenging among adolescents, the 24-h dietary recall methods that was used in the HELENA study has been evaluated and has shown good validity and accuracy in adolescents [42].

In conclusion, findings of the current study suggest that MHO adolescents have a higher adherence to the MDP compared to MUO adolescents which might be mainly due to a higher fish intake, supporting the possible preventive role of the MDP and its components in the metabolic syndrome development. Moreover, cardiorespiratory fitness might also play a key role in the healthy metabolic phenotype. Hence, nutritional and lifestyle education programs focused on children and adolescents which include physical activity are needed to achieve healthy dietary habits, as well as to increase cardiorespiratory fitness, with the aim of improving metabolic health and preventing obesity-related comorbidities in later life.

CONFLICTS OF INTEREST

On behalf of all authors, the corresponding author states that there is no conflict of interest.

REFERENCES

1. Katzmarzyk PT, Barreira T V., Broyles ST et al (2015) Relationship between lifestyle behaviors and obesity in children ages 9-11: Results from a 12-country study. Obesity 23(8):1696–702.

2. Raghuveer G (2010) Lifetime cardiovascular risk of childhood obesity. Am J Clin Nutr 91(5):1514S–1519S.

3. Zamrazilova H, Weiss R, Hainer V et al (2016) Cardiometabolic health in obese adolescents is related to length of obesity exposure: A pilot study. J Clin Endocrinol Metab 101(8):3088–95.

4. Prince RL, Kuk JL, Ambler KA et al (2014) Predictors of metabolically healthy obesity in children. Diabetes Care 37(5):1462–8.

5. Wilkie HJ, Standage M, Gillison FB et al (2016) Multiple lifestyle behaviours and overweight and obesity among children aged 9–11 years: results from the UK site of the International Study of Childhood Obesity, Lifestyle and the Environment. BMJ Open 6(2):e010677.

6. Struijk EA, May AM, Wezenbeek NLW et al (2014) Adherence to dietary guidelines and cardiovascular disease risk in the EPIC-NL cohort. Int J Cardiol Elsevier Ireland Ltd ;176(2):354–9.

7. Trichopoulos A, Costacou, T, Bamia et al (2003) Adherence to a Mediterranean Diet and Survival in a Greek Population. N Engl J 348(26):2599–608.

8. The InterAct Consortium (2011) Mediterranean Diet and Type 2 Diabetes Risk in the European Prospective Investigation Into Cancer an Nutrition (EPIC) Study. Diabetes Care 34:1913–8.

9. Sofi F, Cesari F, Abbate R et al (2008) Adherence to Mediterranean diet and health status: meta-analysis. BMJ 337(a1344):1–7.

10. Moreno LA, De Henauw S, González-Gross M et al (2008) Design and implementation of the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study. Int J Obes 32(Suppl 5):S4-11.

11. Iliescu C, Censi L, Dietrich S et al (2008) Harmonization process and reliability assessment of anthropometric measurements in a multicenter study in adolescents 58–65.

12. Cole TJ, Lobstein T (2012) Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatr Obes 7(4):284–94.

13. Slaughter MH, Lohman TG, Boileau RA et al (1988) Skinfold equations for estimation of body fatness in children and youth. Hum Biol 5:709–23.

14. Breidenassel C, Go S, Ferrari M et al (2008) Sampling and processing of fresh blood samples within a European multicenter nutritional study: evaluation of biomarker stability during transport and storage. Int J Obes 32: 66–75.

15. Ortega FB, Artero EG, Ruiz JR et al (2008) Reliability of health-related physical fitness tests in European adolescents. The HELENA Study. Int J Obes (Lond) 32:49–57.

16. Leger LA, D M, Gadoury C LJ (1988) The multistage 20 metre shuttle run test for aerobic fitness. J Sport Sci 2:92–101.

17. Ortega FB, Lavie CJ, Blair SN (2016) Obesity and cardiovascular disease. Circ Res 118(11):1752–70.

18. Jolliffe CJ, Janssen I (2007) Development of Age-Specific Adolescent Metabolic Syndrome Criteria That Are Linked to the Adult Treatment Panel III and International Diabetes Federation Criteria. J Am Coll Cardiol 49(8):891–8.

19. Rey-López JP, de Rezende LF, Pastor-Valero M et al (2014) The prevalence of metabolically healthy obesity: A systematic review and critical evaluation of the definitions used. Obes Rev 15(10):781–90.

20. Vereecken CA, Covents M, Sichert-Hellert W et al (2008) Development and evaluation of a self-administered computerized 24-h dietary recall method for adolescents in Europe. Int J Obes (Lond) 32 Suppl 5:S26-34.

21. Heinzle S, Ball GDC, Kuk JL (2015) Variations in the prevalence and predictors of prevalent metabolically healthy obesity in adolescents. Pediatr Obes 1–9.

22. Phillips CM (2016) Metabolically healthy obesity across the life course: epidemiology, determinants, and implications. Ann N Y Acad Sci 1–16.

23. Camhi SM, Whitney Evans E, Hayman LL et al (2015) Healthy eating index and metabolically healthy obesity in U.S. adolescents and adults. Prev Med (Baltim). Elsevier Inc 77:23–7.

24. Hankinson AL, Daviglus ML, Horn L Van et al (2013) Diet composition and activity level of at risk and metabolically healthy obese American adults 21(3):637–43.

25. Kimokoti RW, Judd SE, Shikany JM et al (2014) Food Intake Does Not Differ between Obese Women Who Are Metabolically Healthy or abnormal. J Nutr 144:2018-26

26. Velázquez-lópez L, Santiago-díaz G, Nava-hernández J et al (2014) Mediterranean-style diet reduces metabolic syndrome components in obese children and adolescents with obesity. BMC Pediatr 14:175.

27. Zhong VW, Lamichhane AP, Crandell JL et al (2016) Association of adherence to a Mediterranean diet with glycemic control and cardiovascular risk factors in youth with type 1 diabetes: The SEARCH Nutrition Ancillary Study Victor. Eur J Clin Nutr 70(7):802–7.

28. Giannini C, Diesse L, Adamo ED et al (2014) Influence of the Mediterranean diet on carotid intima e media thickness in hypercholesterolaemic children : A 12-month intervention study. Nutr Metab Cardiovasc Dis [Internet]. Elsevier Ltd 24(1):75–82.

29. Lydakis C, Stefanaki E (2012) Correlation of blood pressure, obesity, and adherence to the Mediterranean diet with indices of arterial stiffness in children. Eur J Pediatr 171:1373–82.

30. Kim Y-S, Xun P, Iribarren C, Van Horn L et al (2016) Intake of fish and long-chain omega-3 polyunsaturated fatty acids and incidence of metabolic syndrome among American young adults: a 25-year follow-up study. Eur J Nutr 1–10.

31. Torris C, Molin M, Cvancarova Smastuen M (2014) Fish consumption and its possible preventive role on the development and prevalence of metabolic syndrome - a systematic review. Diabetol Metab Syndr 6(1):112.

32. Melanson EL, Donahoo WT (2009) The Relationship between Dietary Fat and Fatty Acid Intake and Body Weight, Diabetes, and the Metabolic Syndrome. Ann Nutr Metab 55:229–43.

33. Vanhala M, Saltevo J, Soininen P et al (2012) Serum omega-6 polyunsaturated fatty acids and the metabolic syndrome: A longitudinal population-based cohort study. Am J Epidemiol 176(3):253–60.

34. Lai YHL, Petrone AB, Pankow JS et al (2013) Association of dietary omega-3 fatty acids with prevalence of metabolic syndrome: the National Heart, Lung, and Blood Institute Family Heart Study. Clin Nutr 32(6):966–9.

35. Baik I, Abbott RD, Curb JD et al (2010) Intake of Fish and n-3 Fatty Acids and Future Risk of Metabolic Syndrome. J Am Diet Assoc 110(7):1018–26.

36. Lauritzen L, Harsløf L, Hellgren L et al (2012) Fish intake, erythrocyte n-3 fatty acid status and metabolic health in Danish adolescent girls and boys. Br J Nutr 107:697–704.

37. Liao W, Xiao D, Huang Y et al (2016) Combined Association of Diet and Cardiorespiratory Fitness with Metabolic Syndrome in Chinese Schoolchildren. Matern Child Health J. Springer US 20(9):1904–10.

38. Ruiz JR, Rizzo NS, Hurtig-wennlöf A et al (2006) Relations of total physical activity and intensity to fitness and fatness in children : the European Youth Heart Study. Am J Clin Nutr 84:299–303.

39. Neto AS, Sasaki JE, Mascarenhas LPG et al (2011) Physical activity, cardiorespiratory fitness, and metabolic syndrome in adolescents: A cross- sectional study. BMC Public Health. BioMed Central Ltd 11(1):674.

40. Ortega FB, Cadenas-sánchez C, Sui X et al (2015) Role of Fitness in the Metabolically Healthy but Obese Phenotype: A Review and Update. Prog Cardiovasc Dis. Elsevier Inc 58(1):76–86.

41. Sénéchal M, Wicklow B, Wittmeier K et al (2013) Cardiorespiratory fitness and adiposity in metabolically healthy overweight and obese youth. Pediatrics 132(1):e85-92.

42. Vereecken C, Dohogne S, Covents M et al (2010) How accurate are adolescents in portion-size estimation using the computer tool young adolescents' nutrition assessment on computer (YANA-C)? Br J Nutr 103(12):1844–50.

Supplemental Table 1. Sex-specific median intakes in the whole sample of adolescents participating in the HELENA study whose dietary data were available.

	Males (n=1077)	**Female (n=1253)**
	Median (range)	Median (range)
Vegetables (g/day)	78 (5,410)	82 (7, 538)
Fruits and nuts (g/day)	99 (8,671)	113 (5, 629)
Cereal roots (g/day)	320 (109, 719)	257 (74, 626)
Pulses (g/day)	2 (0, 319)	1 (0, 304)
Fish (g/day)	12 (0, 126)	13 (0,111)
FU/FS ratio (day)	0.87 (0.53, 1.71)	0.88 (0.55, 2.01)
Dairy products (g/day)	207 (16, 2398)	153 (7, 1296)
Meat (g/day)	157 (11, 630)	123 (13, 458)
Alcohol (g/day)	0.5 (0, 54.4)	0.3 (0, 30.4)
Mediterranean diet score (0-9)	4 (0, 8)	4 (0, 8)
High adherence (N, %)	451 (41,9%)	564 (45%)

FU/FS ratio: unsaturated to saturated fatty acids ratio.

Supplemental Table 2. Distribution of metabolically healthy overweight or obese (MHO) and metabolically unhealthy overweight or obese (MUO) adolescents and the adherence to the Mediterranean dietary pattern of participants across Center-North and South European regions.

	Metabolic phenotype			**Mediterranean diet score**	
	MHO (n)	**MUO (n)**	***P****	**Mean ± SD**	***P****
Regions					
Center-North Europe	25	52	0.915	3.9 ± 1.5	0.060
Dortmund	5	23		3.7 ± 1.5	
Ghent	3	5		4 ± 1.3	
Lille	6	6		3.5 ± 1.5	
Stockholm	8	6		4.7 ± 1.5	
Vienna	3	12		3.6 ± 1.4	
South Europe	20	40		4.5 ± 1.7	
Athens	6	20		3.5 ± 1.6	
Rome	11	16		5.1 ± 1.3	
Zaragoza	3	4		6.1 ± 0.7	

SD: Standard deviation. *P values show the difference across Center-North *vs* South Europe gradient.

3.3. Study III

Published in: Nutrients

nutrients

Article

Association of Breakfast Quality and Energy Density with Cardiometabolic Risk Factors in Overweight/Obese Children: Role of Physical Activity

Arenaza L[1], Muñoz-Hernández V[2], Medrano M[1], Oses M[1], Merchán ME[2], Cadenas-Sánchez C[2], Ortega FB[2], Ruiz JR[2], Labayen I[1].

[1]Institute for Innovation & Sustainable Development in Food Chain (ISFOOD), Department of Health Sciences, Public University of Navarra, Pamplona, Spain

[2]PROmoting FITness and Health through physical activity research group (PROFITH), Department of Physical Education and Sports, Faculty of Sport Sciences, University of Granada, Granada, Spain

Corresponding author: Lide Arenaza Etxeberria, lide.arenaza@unavarra.es

ABSTRACT

There is a general believe that having breakfast is an important healthy lifestyle factor; however, there is scarce evidence on the influence of breakfast quality and energy density on cardiometabolic risk in children, as well as on the role of physical activity in this association. The aims were (i) to examine the associations of breakfast quality and energy density from both solids and beverages with cardiometabolic risk factors, and (ii) to explore whether physical activity levels may attenuate these relationships in children with overweight/obesity from two projects carried out in the north and south of Spain. Breakfast consumption, breakfast quality index (BQI) score and BEDs/BEDb (24h-recalls and the KIDMED questionnaire), and physical activity (PA; accelerometry) were assessed, in 203 children aged 8-12 years with overweight/obesity. We measured body composition (Dual X-ray Absorptiometry), uric acid, blood pressure, lipid profile, gamma-glutamyl-transferase (GGT), glucose and insulin, and calculated HOMA and metabolic syndrome z-score. BQI score was inversely associated with serum uric acid independently of a set of relevant confounders (β=-0.172, p=0.028), but the relationship was attenuated after further controlling for total PA (p<0.07). BEDs was positively associated with total and HDL cholesterol, and systolic blood pressure regardless of confounders (all p<0.05), while BEDb was positively associated with HOMA in either active/inactive children (all p<0.03). In conclusion, higher breakfast quality and lower breakfast energy density should be promoted in overweight/obesity children to improve their cardiometabolic health.

Keywords: Breakfast quality, breakfast energy density, skipping breakfast, cardiometabolic health, childhood obesity, uric acid, HOMA, cholesterol, blood pressure, physical activity.

INTRODUCTION

Breakfast has traditionally been considered as being not only the first, but also the most important meal of the day. Skipping breakfast is associated with excess adiposity from childhood (1-3) and therefore, breakfast consumption has been suggested as a childhood obesity-preventing strategy (4). Indeed, it has been reported that children are hungrier and consume more food before lunch when skipping breakfast, while it is unclear when skipping other meals (5). The overall diet quality of children who consume breakfast is healthier compared to those who skip it (6). Skipping breakfast prevalence increases with age (7) and it is associated with increased abdominal (8) and visceral adiposity (9), metabolic syndrome (10) and lower levels of physical activity (11).

Currently, on account of the wide variety of food available, the ultra-processed food is common in children´s diet. Breakfast of Spanish children is predominantly composed of sugary and fat-rich products such as biscuits, pastries, breakfast cereals, artificial juices and milkshakes (12). In this line, a previous study reported that breakfast frequency and quality may affect glycaemia and appetite in both children and adults (13). Thus, Monteagudo et al. proposed a Breakfast Quality Index (BQI), an itemized tool based on the well-known Mediterranean dietary pattern, to assess breakfast quality in children and adolescents (14). Recently, energy density has been used as dietary parameter to evaluate the associations between diet and adiposity (15). A previous study suggested to analyze energy density from solids (BEDs) and beverages (BEDb) separately since excluding caloric beverages from BEDs calculation seems to be a useful method to avoid misinterpretation of true exposure to a high energy dense diet (16). Thereby, diet quality and energy density are important dietary factors when analyzing the adequacy of breakfast as well as of the other meals such as lunch or dinner.

Physical activity is associated with a lower risk of cardiovascular disease (17), and specially, with insulin resistance (18). Moreover, previous studies observed that high physical activity levels may reduce the detrimental effect of unhealthy dietary patterns in children and adolescents (19). Previous studies concluded that youth who seldom ate breakfast tended to be more sedentary (20) (21). According to a Swedish study, children aged 10 years consuming a breakfast which provided over 20% of their estimated daily energy requirements, performed significantly better before-lunch physical endurance tests compared to children who consumed a breakfast providing only 10% of energy requirements (22). However, there is no strong evidence on whether having a low energy intake breakfast time is detrimental to children´s physical activity levels (23).

Therefore, given that both the quality and the energy density seem to be important dimensions of the first meal of the day, to be considered for their potential relationship between obesity and related metabolic disorders, and that physical activity may attenuate the detrimental effect of unhealthy breakfast habits on cardiometabolic health, the aims of the current study were: (i) to examine the associations of breakfast quality and energy density from both solids and beverages with cardiometabolic risk factors, and (ii) to explore whether physical activity levels may attenuate these relationships in children with overweight/obesity from two projects carried out in the north and south of Spain.

MATERIAL AND METHODS

Study design and participants

The current cross-sectional pooled analysis includes baseline data from the EFIGRO (ClinicalTrials.gov ID: NCT02258126) (24) and the ActiveBrains (ClinicalTrials.gov ID: NCT02295072) (25) randomized controlled trials. Both studies involve participants with the same characteristics and were conducted between 2014 and 2017. The EFIGRO study was carried out in Vitoria-Gasteiz, (North of Spain), with the aim of examining the additional effect of supervised exercise training on hepatic fat content in children with overweight/obesity participating in a family-based healthy lifestyle educational intervention. The ActiveBrains project was conducted in the southern Spanish city of Granada with the main objective to investigate the effect of a supervised exercise training program on brain, cognition, academic achievement as well as physical and mental health in children with overweight/obesity. The study protocols were approved by the Ethic Committee of Clinical Investigation of Euskadi (PI2014045) and the Review Committee for Research Involving Human Subjects at University of Granada (Reference: 848, February 2014), respectively. Parents or legal guardians whose child was participating in the study signed an informed written consent before being enrolled in the study.

For the current cross-sectional study we used the baseline data of a total of 203 children (47.3% girls) with overweight/obesity, aged 8-12 years and with dietary data available from two non-consecutive 24h-recalls were included in the analysis.

Anthropometry, cardiometabolic risk assessment and socio-demographic data

Height (SECA 220, Hamburg, Germany) and body weight (SECA 760, Hamburg, Germany) were measured barefoot following the standard protocols. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m2). According to World Obesity Federation criteria, children were classified as having overweight or obesity (26). Waist circumference was measured by a non-elastic tape (SECA 201, Hamburg, Germany) after a normal breathing in standing position, at the middle point between the anterior iliac crest and lower border of the rib. Total and abdominal fat were assessed by dual energy X-ray absorptiometry (DXA; HOLOGIC, QDR 4500W and HOLOGIC, Discoveri Wi for EFIGRO and ActiveBrains, respectively) and FMI was calculated as fat mass divided by height squared (kg/m2). Abdominal adiposity was measured by determining three abdominal sections as described elsewhere (27).

Systolic and diastolic blood pressure (OMRON ® M6) were measured twice following recommendations for children (28). Puberty stage was determined by direct examination by a pediatrician according to Tanner and Whitehouse (29). Biochemical parameters such as serum lipid profile, gamma glutamyl transferase (GGT), glucose, insulin and uric acid were obtained from fasting blood samples as has been reported elsewhere (24) (25). Insulin resistance was determined by computing homeostatic model assessment (HOMA) as follows: (insulin (µU/mL) x glucose (mmol/dL))/22.5) (30). Maternal educational level was recorded and categorized as high (university or higher education) or low (primary, secondary or high school).

Children were categorized as having or not metabolic syndrome according to the IDF criteria (31). According to this definition, children were considered as having MetS if they presented 3 or more metabolic risk factors (central obesity; elevated systolic (≥130 mmHg) or diastolic (≥85 mmHg) blood pressure; elevated triglycerides (≥150 mg/dl) or low HDL cholesterol (<40 mg/dl); and impaired fasting blood glucose (≥100 mg/dl). We calculated the metabolic syndrome z-score based on sex- and age-specific z-scores using data from a sample of Spanish children with a similar age range and with all BMI categories (32). MetS z-score was computed by using the sum of the sex- and age-specific z-scores for the most common variables included in the metabolic syndrome definition (triglycerides, HDL-cholesterol, glucose and systolic and diastolic blood pressure) (33).

Breakfast intake, quality and energy density assessment

Two non-consecutive 24h-recalls of weekdays were recorded during a time span of a week by trained nutritionists, and thereafter, the mean of energy intake as well as food intake of both days was calculated. Children reported their detailed intake from the previous day in an interview with trained nutritionists and with the collaboration of their parents. Parents were not aware of the dietary assessment previous to the interview in order to avoid reporting bias. Pictures of food servings were used to estimate servings and food sizes (34). Nutritional composition data was obtained by the Easydiet computer software (©Biocentury, S.L.U. 2016), which is supported by the Spanish Association of Dietetics and Nutritionists. Those participants with a single questionnaire or with non-completed data on dietary intake were excluded from the analyses (N=22).

Skipping breakfast information was obtained (i) extracting an item from the KIDMED questionnaire (35), and (ii) based on meals recorded in the 24h-recalls. In addition, the quality of the breakfast was evaluated based on the modified tool "Breakfast Quality Index (BQI)" proposed by Monteagudo C et al. (14), which was slightly modified. The index used in our study is composed of 10 items related to breakfast consumption and is based on whether children meet or not the criteria of each component. The criteria to score one point in each item are the following: to consume (i) cereals and derivates, (ii) fruits and/or vegetables, (iii) dairy products, (iv) to provide <5% of total daily energy from food rich in simple sugars from breakfast, (v) to consume olive oil, (vi) monounsaturated to saturated fatty acids ratio (MUFA/SFA) ≥2:1, (vii) to provide 20-25 % of energy from breakfast in relation to total daily energy intake, (viii) to consume cereals, fruits and dairy products at the same meal, (ix) to consume ≥200-300 mg of calcium, and (x) not to consume butter or margarine. Thereby, 1 point was scored when children met the criterion in each item, while a value of 0 was given when this was not achieved. The BQI score ranged from 0 (poorest breakfast quality) to 10 points (optimal breakfast quality). Detailed information of the BQI score is available in **Supplemental table 1**.

Breakfast energy density from both solids and beverages was calculated by dividing the energy consumed (kcal) by the amount (mass in grams) of food or drinks consumed, respectively (kcal/g). Breakfast energy density from solids (BEDs) includes all solid food excluding all energy-containing and non-energy-containing beverages, whereas breakfast energy density from beverages (BEDb) included all drinkable energy-containing beverages such as soft drinks, milk, smoothies, shakes or juices.

Physical activity assessment

Accelerometers were used to objectively measure total physical activity (wActisleep-BT and wGT3X-BT, ActiGraph, Pensacola, FL, USA). Children had to wear the accelerometer on the non-dominant wrist during a seven consecutive days (24h) except during water-activities. In order to quantify the acceleration related to the movement registered and expressed in milligravity (mg), Euclidean Norm Minus One (ENMO) was analyzed using the R software (GGIR Packae, v. 1.5-12, https://cran.r-project.org/web/packages/GGIR/). Moderate-to vigorous physical activity (MVPA) was estimated by establishing age-specific cut-offs for ENMO (36) as recommended elsewhere (37). Children were considered as active when meeting the criteria of reaching a minimum of 60 minutes of MVPA per day and as inactive when MVPA<60 min/day (38).

Statistical analysis

The distribution of the variables was analyzed by exploring the skewness and kurtosis, and as triglycerides, GGT, insulin and HOMA did not have a normal distribution, all of them were logarithmically transformed. As the DXA used in both of the studies to assess total and abdominal adiposity was similar but not exactly the same model, Z-score of FMI and abdominal adiposity according to the study center was calculated in order to avoid any possible systematic error. To examine the association of breakfast quality with cardiometabolic risk factors linear regression analyses were performed considering breakfast quality as independent variable and cardiometabolic risk factors as dependent variables. For this purpose three models were performed; sex, age, center (Vitoria or Granada), maternal educational level and energy intake were used in Model 1; Model 2 was additionally adjusted for FMI and in Model 3 the analysis was further adjusted for total physical activity. This last was included in the third model to explore if physical activity plays any role in the association between breakfast dimensions and cardiometabolic risk. Linear regression analyses were also performed to examine the association of energy density from solids and beverages (independent variables) with cardiometabolic risk factors and MetS z-score (dependent variables). The same models were used for both objectives. As sensitivity analyses, all the regression models were repeated including puberty stage instead of age as confounder. Sex interaction was also examined and no significant differences were found, thus, the results are presented with boys and girls together. Statistical analyses were carried out with the statistical software SPSS version 20.0 (SPSS Inc, Chicago) with a level of significance of $\alpha=0.05$.

RESULTS

Descriptive characteristics and dimensions of breakfast intake of participants are shown in **Table 1**. Overall, it was observed that 7-21% and 4-11% of girls and boys, respectively, used to skip breakfast depending on the criteria used to define skipping breakfast. Although there were non-significant differences neither according to sex nor to study center in skipping breakfast, girls tended to skip breakfast more than boys (all $p<0.05$). Finally, boys had higher levels of total daily physical activity and MVPA than girls ($p<0.05$), while there were no significant differences according to the study center. BQI score was similar in boys and girls (Table 1), whereas children from the ActiveBrains trial had significantly higher BQI score compared to children from the EFIGRO project, and therefore, had a better breakfast quality ($p<0.001$). In contrast, BEDs and BEDb were similar in boys and girls, as well as in the two study centers (Table 1).

Table 1. Descriptive characteristics and dimensions of breakfast intake of participants.

	N	All	N	Girls	N	Boys	N	EFIGRO	N	ActiveBrains
Biological characteristics										
Age (years)	203	10.3 (1.1)[§]	96	10.3 (1)	107	10.4 (1.2)	112	10.6 (1.1)	91	10 (1.1)
BMI (kg/m^2)	203	26.1 (3.5)	96	25.9 (3.6)	107	26.3 (3.5)	112	25.5 (3.1)	91	26.9 (3.8)
Obese (N, %)	203	135 (65.5)	96	59 (61.5)	107	76 (71.0)	112	66 (58.9)	91	69 (78.8)
High maternal educational level (N, %)	203	54 (26.2)	96	25 (26)	107	29 (27.1)	112	28 (25)	91	26 (28.6)
High puberty stage (N, %)[φ]	197	45 (23.1)	88	35 (38.9)	98	10 (9.7)	102	32 (29.4)	84	13 (15.1)
Waist circumference (cm)	203	84.1 (10.5)	96	81.8 (10.4)	107	86.3 (10.2)	112	79 (7.6)	91	90.4 (10.3)
FMI (kg/m^2)	184	10.7 (2.5)	86	10.7 (2.6)	98	10.6 (2.5)	110	10.1 (2.3)	74	11.5 (2.7)
Abdominal fat (kg)	183	1.7 (0.6)	86	1.7 (0.6)	96	1.6 (0.6)	110	1.5 (0.6)	73	1.8 (0.6)
Systolic blood pressure (mmHg)	197	105.2 (15)	94	103.9 (15)	103	106.4 (14.8)	112	96.2 (10.1)	85	117 (11.6)
Diastolic blood pressure (mmHg)	197	65.4 (9.8)	94	64.6 (9.5)	103	66.1 (10)	112	61.6 (8.3)	85	70.3 (9.5)
Uric acid (mg/dl)	188	4.7 (0.9)	87	4.9 (0.9)	101	4.6 (0.8)	108	4.7 (0.8)	80	4.7 (0.9)
Cholesterol (mg/dl)	197	170.3 (30.6)	93	170.4 (32.1)	104	170.2 (29.3)	110	171.7 (28.4)	87	168.6 (33.2)
HDL-c (mg/dl)	197	51.1 (11.6)	93	49 (11.1)	104	53 (11.8)	110	51 (11.2)	87	51.3 (12.1)
LDL-c (mg/dl)	189	102.8 (24.9)	88	104.2 (24.8)	101	101.5 (25)	110	104 (23.8)	79	101 (26.4)
Triglycerides (mg/dl)	197	89.9 (50)	93	96.6 (53.3)	104	84 (46,3)	110	83.7 (39.5)	87	97.8 (60.1)
Glucose (mg/dl)	197	85.9 (6.2)	93	84.8 (6.5)	104	86.9 (5.8)	109	85.4 (5.5)	88	86.5 (7.1)
Insulin (µU/l)	194	12.9 (8.5)	93	14.4 (10.7)	101	11.5 (5.6)	110	12.2 (5)	84	13.9 (11.6)

HOMA	192	2.8 (2.1)	92	2.9 (1.7)	100	2.6 (1.3)	109	2.6 (1.1)	83	3.2 (2.1)
GGT (U/l)	184	16.9 (5.9)	85	16.9 (6.8)	99	16.9(5.1)	108	16.3 (4.8)	76	17.7 (7.3)
MetS (N, %)	181	12 (6.1)	86	7 (7.8)	95	5 (4.9)	105	4 (3.7)	76	8 (9.3)
MetS Z-score	181	2.4 (3.7)	86	2.2 (3.9)	95	2.5 (3.7)	105	0.5 (2.9)	76	4.9 (3.3)
Physical activity¥										
Total physical activity (ENMO min/day)	191	63 (15.4)	92	59.7 (13.7)	99	66.1 (16.5)	104	64.1 (16.4)	87	61.8 (14.3)
MVPA (min/day)	191	54.4 (20.9)	92	48.4 (18.3)	99	59.9 (21.7)	104	56.8 (21.7)	87	51.5 (19.6)
Skipping breakfast (N, %)										
24h-recall criteria	203	11 (5.3)	96	7 (7.2)	107	4 (3.7)	112	4 (3.5)	91	7 (7.5)
KIDMED item criteria	172	26 (12.6)	80	17 (21.3)	91	9 (11)	96	14 (14.6)	75	12 (16)
Breakfast quality										
BQI score (0-10)	191	4.2 (1.3)	90	4.1 (1.3)	100	4.2 (1.2)	109	3.8 (1)	82	4.6 (1.5)
Breakfast energy density										
From solids (BEDs)	179	3.3 (1.1)	86	3.3 (1.2)	92	3.3 (1.1)	109	3.3 (1.3)	70	3.4 (0.9)
From beverages (BEDb)	191	0.5 (0.3)	88	0.5 (0.2)	102	0.5 (0.1)	109	0.5 (0.2)	82	0.5 (0.1)

BMI, Body mass index, FMI, fat mass index; HDL-c, High density lipoprotein cholesterol, LDL-c, low density lipoprotein cholesterol, HOMA, homeostasis model assessment for insulin resistance; GGT, gamma glutamyl transferase; MetS, metabolic syndrome; MVPA, moderate-to vigorous physical activity; BEDs, breakfast energy density from solids; BEDb, breakfast energy density from beverages.

§ Values are means and standard deviations

Φ High puberty stage was considered as Tanner stage equal or above III

¥ Physical activity was obtained from raw data based on Hildebrand et al (30).

A higher percentage of boys met the criteria of having a dairy product included in the breakfast than girls ($p<0.02$, **Supplemental table 2)**. The rest of the breakfast components included in the BQI were similar according to sex. Moreover, significantly higher number of participants from the ActiveBrains project met the criteria for BQI components such as cereals ($p<0.001$), food rich in simple sugars ($p<0.05$), MUFA-rich fats ($p<0.001$) and MUFA/SFA ratio ($p<0.05$) compared to children from the EFIGRO project (Supplemental table 2).

The associations of BQI score with cardiometabolic risk factors are shown in **Table 2.** BQI score was significantly and inversely associated with serum uric acid after adjusting for sex, age, center, maternal educational level, daily energy intake and FMI ($p<0.05$, Model 2, Table 2). However, this association was attenuated after further controlling for total physical activity ($p<0.07$, Model 3, Table 2). Although there was a trend to significance between BQI score and GGT, BQI score was not significantly associated with the rest of cardiometabolic risk factors in children with overweight/obesity.

Table 2. Associations of Breakfast Quality Index (BQI) score with cardiometabolic risk factors.

	Model 1		**Model 2**		**Model 3**	
	β	**p**	**β**	**p**	**β**	**p**
FMI Z-score	0.057	0.455	-	-	-	-
Abdominal fat Z-score	0.024	0.752	-0.028	0.478	-0.047	0.235
SBP (mmHg)	0.047	0.384	0.042	0.426	0.034	0.533
DBP (mmHg)	0.034	0.625	0.029	0.674	0.041	0.553
Uric acid (mg/dl)	-0.128	0.091	-0.172	**0.028**	-0.151	0.060
Cholesterol (mg/dl)	-0.038	0.625	-0.036	0.659	-0.017	0.845
HDL-c (mg/dl)	-0.022	0.779	0.017	0.831	0.009	0.913
LDL-c (mg/dl)	-0.039	0.629	-0.046	0.582	-0.017	0.849
Triglycerides (mg/dl)	-0.008	0.913	-0.030	0.699	-0.020	0.804
HOMA	-0.016	0.845	0.022	0.779	0.020	0.809
GGT (U/l)	-0.136	0.096	-0.144	0.082	-0.161	0.061
MetS Z-score	-0.018	0.779	-0.022	0.725	-0.011	0.869

FMI, Fat mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure, HDL-c, High density lipoprotein cholesterol, LDL-c, low density lipoprotein cholesterol; HOMA, Homeostasis model assessment for insulin resistance; GGT, gamma glutamyl transferase; MetS, metabolic syndrome. Model 1 was adjusted for sex, age, center, maternal educational level and energy intake; Model 2 was additionally adjusted for fat mass index; and Model 3 was further adjusted for total physical activity.

The associations between breakfast energy density and cardiometabolic risk factors are shown in **Table 3**. BEDs was significantly associated with total cholesterol regardless of potential confounders ($p<0.05$, Model 2, Table 3) and of total physical activity ($p<0.05$, Model 3, Table 3), as well as with with HDL cholesterol regardless of potential confounders ($p<0.05$, Model 2). Similarly, BEDs was positively associated with systolic blood pressure ($p<0.05$, Model 2, Table 3). BEDb was significantly associated with HOMA after controlling for potential confounders including physical activity ($p<0.03$ for all, Model 1, 2 and 3, Table 3). Furthermore, after categorizing children as being active (MVPA ≥60 min/day, N=54) or inactive (MVPA <60 min/day, N=88), the current positive association between BEDb and HOMA remained statistically significant ($\beta=0.288$; adjusted $P=0.025$, and $\beta=0.250$; adjusted $P=0.022$ for active and inactive children, respectively).

Finally, all the analyses examining the associations of BQI and energy density of breakfast were repeated including puberty stage instead of age into the models and the results did not substantially change (data not shown).

Table 3. Associations of breakfast energy density from solids (BEDs) and beverages (BEDb) with cardiometabolic risk factors.

	BEDs				BEDb			
	Model 1		Model 2		Model 1		Model 2	
	Β	**p**	**β**	**p**	**β**	**p**	**β**	**p**
Systolic blood pressure (mmHg)	0.100	0.068	0.074	0.183	0.086	0.109	0.087	0.110
Diastolic blood pressure (mmHg)	0.009	0.900	-0.018	0.795	0.011	0.880	0.006	0.930
Uric acid (mg/dl)	0.057	0.478	0.054	0.506	0.017	0.831	0.008	0.919
Cholesterol (mg/dl)	0.163	**0.044**	0.178	**0.033**	0.021	0.794	0.022	0.793
HDL-c (mg/dl)	0.188	**0.015**	0.172	**0.031**	0.052	0.500	0.064	0.421
LDL-c (mg/dl)	0.107	0.195	0.125	0.145	0.035	0.672	0.032	0.710
Triglycerides (mg/dl)	0.027	0.725	0.037	0.647	-0.020	0.798	-0.026	0.744
HOMA-IR	0.072	0.389	0.101	0.245	0.214	**0.011**	0.216	**0.012**
GGT (U/l)	0.111	0.176	0.119	0.159	-0.026	0.753	-0.022	0.794

BEDs, Breakfast energy density from solids; BEDb, breakfast energy density from beverages; HDL-c, High density lipoprotein cholesterol, LDL-c, low density lipoprotein cholesterol; HOMA-IR, Homeostasis model assessment for insulin resistance; GGT, gamma glutamyl transferase. Model 1 was adjusted for sex, age, center, maternal educational level, energy intake and body fat percentage; and Model 2 was additionally adjusted for total physical activity.

DISCUSSION

The current study examined the association of several breakfast dimensions on cardiometabolic risk in children with overweight/obesity aged 8-12 years old. The main findings were that both breakfast quality and breakfast energy density were associated with some of the cardiometabolic risk factors studied. BQI score was inversely associated with serum uric acid, while breakfast energy density from solids was related to higher total and HDL cholesterol and higher breakfast energy density from beverages was associated with higher levels of insulin resistance independently of physical activity levels in children with overweight/obesity. To the best of our knowledge, this is the first study examining the association of breakfast quality and energy density on cardiometabolic risk in children with overweight/obesity.

In regards to skipping breakfast, a higher prevalence of skippers was reported by following the KIDMED criteria than by using the 24h-recall questionnaire since 24h-recall criteria could only reflect two of the interviewed days and there might be children who were usual skippers, but consumed breakfast in the interviewed days by chance. Previous studies reported a breakfast skipping prevalence of 10-30% in children and adolescents from the United States and Europe (39), with an even higher prevalence in individuals with obesity (40).

Taking into account that two items from the BQI were related to monounsaturated fats, children from the ActiveBrains project could have higher BQI score because in the south of Spain the consumption of virgin olive oil is higher than in other regions. Indeed, children from the ActiveBrains study (south of Spain) tended to consume higher intakes of virgin olive oil in their breakfast whereas children from the EFIGRO study (north Spain) were more likely to consume less unhealthy alternatives such as biscuits, breakfast cereals with sugar added or pastries and therefore, did not obtain a positive value in those items.

We found a negative association between BQI score and serum uric acid levels albeit no with other cardiometabolic risk markers such as lipid, glycemic profile or metabolic syndrome. Previous research showed that serum uric acid is related to cardiovascular disease (41,42), which in turn is an independent predictor for future cardiovascular mortality (43). Milk proteins seem to decrease serum uric acid concentration in adults (44) and as BQI scored positively with the presence of dairy products in the meal, they could be one of the responsible components. A cross-sectional analysis of two Caucasian adults cohorts reported lower serum uric acid levels in participants with higher consumption of carbohydrates, calcium and vitamin B2 and lower fat intake (45). The same study also examined individual food items, and high consumption of dairy products, fiber-rich bread, cereals and fruits were negatively associated with serum uric acid. Of note is that all these mentioned foods are components of BQI which score positively and therefore could partially explain our findings. However, it should be pointed that BQI was not associated with other cardiometabolic risk markers and metabolic syndrome. Overall, little is known about the influence of breakfast quality on cardiometabolic risk in children. Most studies in pediatric population examined just which were the health consequences of the consumption or skipping breakfast, whereas the few studies examining the quality of breakfast were carried out in adults (46), which hamper comparisons. Hence, as far as we are aware, there are no previous studies exploring the effect of breakfast quality in cardiometabolic health of children with overweight/obesity.

Regarding energy density, we also observed that BEDs and BEDb was positively associated with both total and HDL cholesterol as well as with systolic blood pressure. The association of energy density from solids with HDL could be explained by the fact that virgin olive oil that was added to the toasts in breakfast was considered as solid as a whole (toast with virgin olive oil), since virgin olive oil is not drinkable. Cumulative evidence suggests that virgin olive oil may have a preventive role in coronary disease due to its monounsaturated fatty acids and polyphenolic compounds content (47) (48). In addition, considering that olive oil is energetically dense, the consumption of olive oil could be the responsible of the positive association between breakfast energy density from solids and HDL cholesterol. Furthermore, the ultra-processed consists on an energy-dense food due to its high salt, fat and sugar content. The positive association we found between BEDs and systolic blood pressure could be explained by the high consumption of salt and sugars present in ultra-processed food consumed at breakfast since both sugar and salt intakes have been associated with increased blood pressure (49,50).

One important finding of the current study is that BEDb might be associated with insulin resistance in children with overweight/obesity. Interestingly, this association was found regardless of being an active (meeting PA recommendations) or inactive (not meeting PA recommendations) children. A possible explanation for this relationship could be that almost all the kids of our projects tend to add soluble cocoa or similar sweeteners to their milk cups, which in turn, increase the energy density from these beverages. Moreover, some of the participants consumed artificial juices as a substitute of fruit or natural fruit juice. This fact would be in line with a study that reported that added sugars from liquid sources is associated with impaired glucose homeostasis and insulin resistance among youth at risk of obesity (51). Similarly, Donin et al. reported a positive association between dietary energy density and insulin resistance and fat mass index in children aged 9-10 years (52), even though these associations were attenuated and became non-significant after adjustment for energy intake. According to another study in adults, total daily energy dietary energy density was associated with obesity and metabolic syndrome (53). Taken into consideration that this positive association was observed in both active and inactive children, our hypothesis about the attenuation of the detrimental effects of the unhealthy breakfast by physical activity in cardiometabolic health was not confirmed.

The current proposal for examining BQI includes an item about energy intake, but it does not include energy density of breakfast. However, taking into account our results on the association of energy density with insulin resistance, we believe that this dimension of breakfast should be also included when analyzing breakfast quality in youth.

LIMITATIONS AND STRENGTHS

The current study has several limitations. The cross-sectional design of the study should be considered as a study limitation. Dietary assessment was carried out by different researches which could bias the measurements. Nevertheless, in order to decrease the interpersonal variability, the same criteria were followed after reaching consensus. One of the strengths of the study is the homogeneity of our sample composed exclusively by children with overweight or obesity. Moreover, different dimensions of breakfast have been examined in order to have a more comprehensive study of breakfast adequacy.

CONCLUSION

In conclusion, the findings of the current study suggest that both breakfast quality and breakfast energy density might be associated with cardiometabolic risk factors such as serum uric acid, cholesterol and insulin resistance in children with overweight/obesity. Moreover, energy density from beverages was associated with insulin resistance regardless a set of potential confounders and in both children meeting and not meeting physical activity recommendations. Results of the current study suggest that breakfast energy density should be considered as an additional dimension of breakfast intake. Therefore, nutritional education programs targeting on the prevention of cardiometabolic disease in children with overweight/obesity should include strategies focused on promoting high quality and low energy density of breakfast.

Acknowledgments

We would like to acknowledge all children and families for their participation in the EFIGRO and the ActiveBrains trials as well as to professionals and pre/postgraduate students who have been involved in data collection, measurements and intervention.

Financial support

EFIGRO and ActiveBrains projects project were supported by the Spanish Ministry of Industry and Competitiveness (DEP2016-78377-R and DEP2013-47540) by "Fondos Estructurales de la Unión Europea (FEDER), Una manera de hacer Europa". EFIGRO project was also supported by the University of the Basque Country (GIU14/21). LA is supported by the grant from Education Department of the Government of the Basque Country (PRE_2016_1_0057 and PRE_2017_2_0224), MM is supported by grant from the Spanish Ministry of Education, Culture and Sport (FPU14/03329) and EST17/00210, whereas MA by the grant from the University of the Basque Country (PIF 17/186). JRR, FBO and CC-S were also supported by grants from Spanish Ministry of Economy and Competitiveness (RYC 2010-05957; RYC-2011-09011 and BES-2014-068829).

Author contributions

IL acquired and designed the study, LA, IL and VM interpreted data, LA and IL drafted the work, LA, IL, VM, MM, MO, MA, EM, CC-S, FBO and JRR have approved the submitted version and agree to be personally accountable for the author´s own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and documented in the literature.

Conflicts of interest

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

REFERENCES

1. Ahadi Z, Qorbani M, Kelishadi R, Ardalan G, Motlagh ME, Asayesh H, et al. Association between breakfast intake with anthropometric measurements, blood pressure and food consumption behaviors among Iranian children and adolescents: The CASPIAN-IV study. Public Health [Internet]. Elsevier Ltd; 2015;129(6):740–7. Available from: http://dx.doi.org/10.1016/j.puhe.2015.03.019

2. Barrett N, Riordan F, Michels N, Andersen LF, Veer P, Moreno LA, et al. Breakfast Skipping and overweight / obesity among European adolescents , a cross-sectional analysis of the HELENA dataset : a DEDIPAC study . [version 1 ; referees : awaiting peer review]. 2018;(0):1–12.

3. Szajewska H, Ruszczyński M. Systematic review demonstrating that breakfast consumption influences body weight outcomes in children and adolescents in Europe. Crit Rev Food Sci Nutr. 2010;50(2):113–9.

4. Blondin SA, Anzman-Frasca S, Djang HC, Economos CD. Breakfast consumption and adiposity among children and adolescents: an updated review of the literature. Pediatr Obes. 2016;11(5):333–48.

5. Kral TVE, Whiteford LM, Heo M, Faith MS. Effects of eating breakfast compared with skipping breakfast on ratings of appetite and intake at subsequent meals in 8- To 10-y-old children. Am J Clin Nutr. 2011;93(2):284–91.

6. Coulthard JD, Palla L, Pot GK. Breakfast consumption and nutrient intakes in 4-18-year-olds: UK National Diet and Nutrition Survey Rolling Programme (2008-2012). Br J Nutr. 2017;118(4):280–90.

7. ALBashtawy M. Breakfast Eating Habits Among Schoolchildren. J Pediatr Nurs [Internet]. Elsevier Inc.; 2017;36:118–23. Available from: http://dx.doi.org/10.1016/j.pedn.2017.05.013

8. Nurul-Fadhilah A, Teo PS, Huybrechts I, Foo LH. Infrequent Breakfast Consumption Is Associated with Higher Body Adiposity and Abdominal Obesity in Malaysian School-Aged Adolescents. PLoS One. 2013;8(3):1–6.

9. Alexander KE, Ventura EE, Spruijt-metz D, Weigensberg MJ, Goran MI, Davis JN. Association of Breakfast Skipping With Visceral Fat and Insulin Indices in Overweight Latino Youth. Obes (Silver Spring). 2010;17(8):1528–33.

10. Shafiee G, Kelishadi R, Qorbani M, Motlagh ME, Taheri M, Ardalan G, et al. Association of breakfast intake with cardiometabolic risk factors. J Pediatr (Rio J) [Internet]. Sociedade Brasileira de Pediatria; 2013;89(6):575–82. Available from: http://dx.doi.org/10.1016/j.jped.2013.03.020

11. Schembre SM, Wen CK, Davis JN, Shen E, Nguyen-Rodriguez ST, Belcher BR, et al. Eating breakfast more frequently is cross-sectionally associated with greater physical activity and lower levels of adiposity in overweight Latina and African American girls. Am J Clin Nutr. 2013;98(2):275–81.

12. Aranceta J, Serra-Majem L, Ribas L, Pérez-Rosdrigo C. Breakfast consumption in Spanish children and young people. Public Health Nutr. 2001;4(6A):1439–44.

13. Pereira MA, Erickson E, McKee P, Schrankler K, Raatz SK, Lytle LA, et al. Breakfast Frequency and Quality May Affect Glycemia and Appetite in Adults and Children. J Nutr [Internet]. 2011;141(1):163–8. Available from: http://jn.nutrition.org/cgi/doi/10.3945/jn.109.114405

14. Monteagudo C, Palacín-Arce A, Bibiloni MDM, Pons A, Tur JA, Olea-Serrano F, et al. Proposal for a Breakfast Quality Index (BQI) for children and adolescents. Public Health Nutr. 2013;16(4):639–44.

15. Hossein M, Ph R, Haghighatdoost F, Ph D, Surkan PJ, Ph D, et al. Associations between dietary energy density and obesity : A systematic review and meta-analysis of observational studies. Elsevier Inc.; 2016;32:1037–47.

16. Hebestreit A, Börnhorst C, Pala V, Barba G, Eiben G, Veidebaum T, et al. Dietary energy density in young children across Europe. Int J Obes. 2014;38:S124–34.

17. Ruiz JR, Huybrechts I, Cuenca-garcía M, Artero EG, Labayen I, Meirhaeghe A, et al. Cardiorespiratory fitness and ideal cardiovascular health in European adolescents. Heart. 2015;101:766–73.

18. Davis CL, Pollock NK, Waller JL, Allison JD, Dennis BA, Bassali R, et al. Exercise dose and diabetes risk in overweight and obese children: A randomized, controlled trial. JAMA. 2012;308(11):1103–12.

19. Cuenca-García M, Ortega FB, Ruiz JR, González-Gross M, Labayen I, Jago R, et al. Combined influence of healthy diet and active lifestyle on cardiovascular disease risk factors in adolescents. Scand J Med Sci Sport. 2014;24(3):553–62.

20. Magnusson M., Hulthén L, Kjellgren KI. Obesity , dietary pattern and physical activity among children in a suburb with a high proportion of immigrants. J Hum Nutr Diet. 2005;18:187–94.

21. Kaprio J, Rissanen A, Virkkunen M, Rose RJ. Breakfast skipping and health-compromising behaviors in adolescents and adults. Eur J Clin Nutr. 2003;(57):842–53.

22. Wyon DP, Abrahamsson L, Järtelius M, Fletcher RJ. An experimental study of the effects of energy intake at breakfast on the test performance of 10-year-old children in school. Int J Food Sci Nutr. 1997;48(1):5–12.

23. Vissers PAJ, Jones AP, Corder K, Jennings A, Van Sluijs EMF, Welch A, et al. Breakfast consumption and daily physical activity in 9-10-year-old British children. Public Health Nutr. 2013;16(7):1281–90.

24. Medrano M, Maiz E, Maldonado-Martín S, Arenaza L, Rodríguez-Vigil B, Ortega FB, et al. The effect of a multidisciplinary intervention program on hepatic adiposity in overweight-obese children: Protocol of the EFIGRO study. Contemp Clin Trials. Elsevier Inc.; 2015;45.

25. Cadenas-Sánchez C, Mora-González J, Migueles JH, Martín-Matillas M, Gómez-Vida J, Escolano-Margarit MV, et al. An exercise-based randomized controlled trial on brain, cognition, physical health and mental health in overweight/obese children (ActiveBrains project): Rationale, design and methods. Contemp Clin Trials [Internet]. Elsevier B.V.; 2016;47:315–24. Available from: http://dx.doi.org/10.1016/j.cct.2016.02.007

26. Cole T, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatr Obes. 2012;7:284–94.

27. Labayen I, Ruiz JR, Vicente-Rodríguez G, Turck D, Rodríguez G, Meirhaeghe A, et al. Early Life Programming of Abdominal Adiposity in Adolescents : The HELENA. Diabetes Care. 2009;32(11):2120–2.

28. National Heart, Lung and BI. The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents. Pediatrics [Internet]. 2004;114(2):555–76. Available from: https://www.nhlbi.nih.gov/sites/default/files/media/docs/hbp_ped.pdf

29. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child. 1976;51(3):170–9.

30. Matthews D, Hosker J, Rudenski A, Naylor B, Treacher D, Turner R. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412–9.

31. Zimmet P, Alberti KGM, Kaufman F, Tajima N, Silink M, Arslanian S, et al. The metabolic syndrome in children and adolescents? an IDF consensus report. Pediatr Diabetes [Internet]. 2007;8(5):299–306. Available from: http://doi.wiley.com/10.1111/j.1399-5448.2007.00271.x

32. Martínez-Vizcaíno V, Sánchez-López M, Salcedo-Aguilar F, Notario-Pacheco B, Solera-Martínez M, Moya-Martínez P, et al. Protocol of a Randomized Cluster Trial to Assess the Effectiveness of the MOVI-2 Program on Overweight Prevention in Schoolchildren. Rev Española Cardiol (English Ed [Internet]. 2012;65(5):427–33. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1885585712000370

33. Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: A joint interim statement of the international diabetes federation task force on epidemiology and prevention; National heart, lung, and blood institute; American heart association; World heart federation; International atherosclerosis society; And international association for the study of obesity. Circulation. 2009;120(16):1640–5.

34. Ruiz López MD. Guía para estudios dietéticos: álbum fotográfico de alimentos. Granada U de, editor. 2011. 1-128 p.

35. Cabrera SG, Fernández NH, Hernández CR, Nissensohn M. KIDMED test ; prevalence of low adherence to the Mediterranean Diet in children and young ; a systematic review. Nutr Hosp. 2015;32(6):2390–9.

36. 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):1816–24.

37. Migueles JH, Cadenas-Sanchez C, Ekelund U, Delisle Nyström C, Mora-Gonzalez J, Löf M, et al. Accelerometer Data Collection and Processing Criteria to Assess Physical Activity and Other Outcomes: A Systematic Review and Practical Considerations. Sport Med. 2017;47(9):1821–45.

38. Organization WH. 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/. 2010.

39. Rampersaud GC, Pereira MA, Girard BL, Adams R MJ. Breakfast Habits, Nutritional Status, Body Weight, and Academic Performance in Children and Adolescents. Am Diet Assoc. 2005;105:743–60.

40. Freitas Júnior IF, Christofaro DGD, Codogno JS, Monteiro PA, Silveira LS, Fernandes RA. The association between skipping breakfast and biochemical variables in sedentary obese children and adolescents. J Pediatr. 2012;161(5):871–4.

41. Maloberti A, Maggioni S, Occhi L, Triglione N, Panzeri F, Nava S, et al. Sex-related relationships between uric acid and target organ damage in hypertension. J Clin Hypertens. 2018;20(1):193–200.

42. Bassols J, Martínez-Calcerrada JM, Prats-Puig A, Carreras-Badosa G, Díaz-Roldán F, Osiniri I, et al. Uric acid, carotid intima-media thickness and body composition in prepubertal children. Pediatr Obes. 2016;11(5):375–82.

43. Zhao G, Huang L, Song M, Song Y. Baseline serum uric acid level as a predictor of cardiovascular disease related mortality and all-cause mortality: A meta-analysis of prospective studies. Atherosclerosis [Internet]. Elsevier Ltd; 2013;231(1):61–8. Available from: http://dx.doi.org/10.1016/j.atherosclerosis.2013.08.023

44. Danielle D, Maurice V, PetitClerc C, Martin C, Brulé D, Hamet P. Milk and soy protein ingestion : acute Effect on Serum Uric Acid Concentration. Am J Clin Nutr. 1991;53(July):665–9.

45. Zykova SN, Storhaug HM, Toft I, Chadban SJ, Jenssen TG, White SL. Cross-sectional analysis of nutrition and serum uric acid in two Caucasian cohorts: The AusDiab Study and the Tromsø¸ study. Nutr J [Internet]. Nutrition Journal; 2015;14(1):1–11. Available from: http://dx.doi.org/10.1186/s12937-015-0032-1

46. Iqbal K, Schwingshackl L, Gottschald M, Knüppel S, Stelmach-Mardas M, Aleksandrova K, et al. Breakfast quality and cardiometabolic risk profiles in an upper middle-aged German population. Eur J Clin Nutr. 2017;71(11):1312–20.

47. Hu FB, Ph D. The Mediterranean Diet and Mortality — Olive Oil and Beyond. N Engl J Med. 2003;348(26):2595–6.

48. Psaltopoulou T, Naska A, Orfanos P, Trichopoulos D, Mountokalakis T, Trichopoulou A. Olive oil, the Mediterranean diet, and arterial blood pressure: the Greek European Prospective Investigation into Cancer and Nutrition (EPIC) study. Am J Clin Nutr [Internet]. 2004;80(0002–9165 (Print)):1012–8. Available from: file://c/Documents and Settings/me/My Documents/Research/Literature Review/Psaltopoulou (Trichopoulou) - the Mediterranean diet BP EPIC - Am J Clin Nutr 2004.pdf

49. He FJ, Macgregor GA. Salt and sugar : their effects on blood pressure. Pflugers Arch - Eur J Physiol. 2015;467:577–86.

50. Welsh JA, Anderson CAM. Added Sugars and Cardiovascular Disease Risk in Children. World Heal Organ -WHO. 2017;

51. Wang J, Light K, Loughlin JO, Mathieu M, Paradis G, Gray-Donald K. Consumption of Added Sugars from Liquid but Not Solid Sources Predicts Impaired Glucose Homeostasis and Insulin Resistance among Youth at Risk of Obesity 1 – 3. J Nutr. 2014;144:81–6.

52. Donin AS, Nightingale CM, Owen CG, Alicja R, Jebb SA, Ambrosini GL, et al. Europe PMC Funders Group Europe PMC Funders Author Manuscripts DIETARY ENERGY INTAKE IS ASSOCIATED WITH TYPE 2 DIABETES RISK MARKERS IN CHILDREN. Diabetes Care. 2014;37(1):116–23.

53. Mendoza JA, Drewnowski A, Christakis D a. Dietary energy density is associated with obesity and the metabolic syndrome in US adults. Diabetes Care [Internet]. 2007;30(4):974–9. Available from: http://care.diabetesjournals.org/content/diacare/30/4/974.full.pdf

Table S1. Components and criteria for the calculation of Breakfast Quality Index (BQI) score (14).

		Yes		No
Cereals and derivate	**+1**	Bread, non-sugar rich breakfast cereals	**0**	Biscuits, pastries, sugar rich breakfast cereals φ
Fruits and vegetables	**+1**	Fresh fruit, natural fruit juices, tomato	**0**	Artificial juices, jam φ
Dairy products	**+1**	Whole or skimmed milk, yoghurt, cheese	**0**	Dairy desserts φ £
Food rich in simple sugars	**+1**	<5% of total daily energy from simple sugars	**0**	≥5% of total daily energy from simple sugars
MUFA-rich fats	**+1**	Olive oil added by the consumer	**0**	Olive oil from biscuits or other fats such as butter φ
MUFA/SFA ratio	**+1**	≥ 2/1	**0**	< 2
Energy intake	**+1**	20-25% of daily energy intake from breakfast	**0**	<20% or >25% of daily energy intake from breakfast
Fruits, cereals and dairy product	**+1**	To include the 3 of the components	**0**	Not to be composed of three of the components
Calcium	**+1**	≥ 200mg	**0**	<200mg
Absence of butter or margarine	**+1**	Not to include butter or margarine in the breakfast	**0**	To include butter or margarine in the breakfast

MUFA, monounsaturated fatty acids; SFA, saturated fatty acids

φ= Not to consume the foods of these item also is punctuated as 0

£=Commercial chocolate milk-shakes, smoothies and rice-puddings, mousse, ice-creams or vegetal drinks

Sugared or flavored yoghurt, commercial chocolate milk-shakes and smoothies, rice-puddings, mousse, ice-creams, vegetal drinks.

3.4. Study IV

Published in: British Journal of Nutrition

British Journal of Nutrition (2019), 121, 1158–1165 doi:10.1017/S0007114519000436

Dietary determinants of hepatic fat content and insulin resistance in overweight/obese children: a cross-sectional analysis of the Prevention of Diabetes in Kids (PREDIKID) study

Lide Arenaza[1], María Medrano[1], Maddi Oses[1], Inge Huybrechts[2], Ignacio Díez[3], Hanna Henriksson[4], Idoia Labayen[1]

[1] Institute for Innovation & Sustainable Development in Food Chain (IS-FOOD), Public University of Navarra, Pamplona 31006, Spain

[2] Nutrition and Metabolism Section, International Agency for Research on Cancer, World Health Organization, Lyon 69008, France

[3] Paediatric Endocrinology Unit, University Hospital of Araba (HUA), Vitoria-Gasteiz 01009, Spain

[4] PROmoting FITness and Health through physical activity research group (PROFITH), Department of Physical and Sports Education, Faculty of Sport Sciences, University of Granada, Granada 18071, Spain

Corresponding author: Lide Arenaza Etxeberria, lide.arenaza@unavarra.es

ABSTRACT

Paediatric non-alcoholic fatty liver disease has increased in parallel with childhood obesity. Dietary habits, particularly products rich in sugars, may influence both hepatic fat and insulin resistance (homeostatic model assessment for insulin resistance (HOMA-IR)). The aim of the study was to examine the association of the consumption of foods and foods components, dairy products desserts and substitutes (DDS), sugar-sweetened beverages (SSB) as well as total and added sugars, with hepatic fat and HOMA-IR. Dietary intake (two non-consecutive 24 h-recalls), hepatic fat (MRI) and HOMA-IR were assessed in 110 overweight/obese children (10.6 (SD 1.1) years old). Linear regression analyses were used to examine the association of dietary intake with hepatic fat and HOMA-IR adjusted for potential confounders (sex, age, energy intake, maternal educational level, total and abdominal adiposity and sugar intake). The results showed that there was a negative association between cereal intake and hepatic fat (β=–0·197, P<0·05). In contrast, both SSB consumption (β=0·217; P=0·028) and sugar in SSB (β=0·210, P=0·035), but not DDS or sugar in DDS or other dietary components, were positively associated with hepatic fat regardless of potential confounders including total sugar intake. In conclusion, cereal intake might decrease hepatic fat, whereas SSB consumption and its sugar content may increase the likelihood of having hepatic steatosis. Although these observations need to be confirmed using experimental evidence, these results suggest that healthy lifestyle intervention programs are needed to improve dietary habits as well as to increase the awareness of the detrimental effects of SSB consumption early in life.

Keywords: Hepatic steatosis, childhood obesity, HOMA-IR, dietary intake, sugar-sweetened beverages.

INTRODUCTION

In recent years, the incidence of pediatric non-alcoholic fatty liver disease (NAFLD) has increased worldwide in parallel with childhood obesity rates, and therefore constitute an emerging global health concern (1). NAFLD is one of the most common cause of liver disease among children and adolescents (2) and consists on the accumulation of lipids within the hepatocytes in the absence of excess alcohol consumption (3). This pathological disorder is strongly associated with insulin resistance and dyslipidemia and, therefore, it is considered to be the hepatic manifestation of metabolic syndrome (4,5)

Diet and other lifestyle determinants seem to modulate hepatic steatosis development and progression (6). For instance, western dietary habits (7) in both normal weight and overweight/obese adolescents, as well as energy intake in children (8) or dietary sugar intake such as fructose or sucrose in adults (9), may have an influence on hepatic fat content and other cardio-metabolic risk factors (10,11).

Sugar-sweetened beverages (SSB) are the main source of dietary added sugars among children and adolescents, representing around 10-15% of their dietary energy intake (12,13). Along with the increase of childhood obesity and hepatic steatosis, SSBs consumption has also risen in the last years (12). In this context, a systematic review and meta-analysis reported higher risk of NAFLD assessed by abdominal ultrasound or liver biopsy in patients consuming SSB (14). Other studies have also shown associations of SSB consumption with adiposity, type 2 diabetes, metabolic syndrome and cardiovascular disease risk factors (15).

Dairy products desserts and substitutes (DDS) are other common dietary sugar sources among children since they are often flavored and sugar sweetened (16,9). Nevertheless, the sugar added in DDS seems not to play such a detrimental role in cardio metabolic health in comparison with sugar from SSB (17). In contrast, the intake of other food groups and dietary components such as fruits and vegetables (18), dairy products (19) and fiber (20,21) may have a protective role against the metabolic syndrome and hepatic fat accumulation. However, the majority of the studies examining the associations of dietary intake with hepatic steatosis are conducted in adolescents (7,18) and adult populations (22). Hence, there are few studies examining the relationship between dietary habits and liver health (23,24) or insulin resistance (25) in children with overweight and obesity, and, to the best of our knowledge, none of these studies measured hepatic fat content by magnetic resonance imaging, which is considered to be the gold standard.

We hypothesized that the intake of products rich in added sugars, such as SSB consumption, would be associated with higher hepatic fat accumulation, whereas no such association would be observed between DDS and hepatic fat content. Likewise, fiber or fiber-rich foods would be beneficial in the prevention of hepatic steatosis and insulin resistance.

Therefore, the aims of the current study were to examine (i) the associations of the consumption of dietary foods and components on hepatic fat content and insulin resistance (HOMA-IR; homeostatic model assessment) and (ii) the influence of SSB and DDS, as well as their sugar content, on hepatic fat content and insulin resistance in children with overweight/obesity.

MATERIAL AND METHODS

Study participants and design

The Prevention of Diabetes in Kids (PREDIKID) study is a randomized controlled trial (ClinicalTrials.gov ID: NCT03027726) examining the effect of a family-based intervention program (26) on diabetes risk of children with overweight/obesity. The study protocol was approved by the Ethic Committee of Clinical Investigation of Euskadi (PI2014045) and the research was conducted according to the Declaration of Helsinki. Participants were recruited from the Pediatric Endocrinology Unit of the University Hospital of Araba and Primary Care Clinics from Vitoria-Gasteiz and surroundings (North of Spain) in 2017.The main outcome of the trial was insulin resistance (26), whereas the primary outcomes for this sub-study were hepatic fat content and insulin resistance. The inclusion criteria were 1) to be aged between 8 and 12 years, 2) to have overweight or obesity, and 3) to meet the international criteria for classification of type 2 diabetes risk. The current cross-sectional study includes baseline data of 110 children (10.6 ± 1.1 years old, 53.1% girls) with overweight or obesity participating in the PREDIKID study who had dietary intake and hepatic fat content data. After parents or legal guardians signed an informed written consent in order to be enrolled in the study, a total of 110 children (10.6 ± 1.1 years old, 53.1% girls) with overweight or obesity who had dietary intake and hepatic fat content data were included in the current study.

Anthropometry

Height (SECA 220) and body weight (SECA 760) were measured barefoot according to standard protocols, and waist circumference was measured with a non-elastic tape (SECA 201) following international recommendations. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m2) and, thereafter, children were classified as having overweight or obesity according to World Obesity Federation criteria (27). All the anthropometic measurements were carried out by the same trained researcher to avoid interpersonal variability bias. Pubertal stage was directly examined by a pediatrician and classified according to Tanner and Whitehouse criteria (28).

Body composition

Dual energy X-ray absorptiometry (HOLOGIC, QDR 4500W) was used to evaluate total and abdominal adiposity. Abdominal adiposity was measured by determining three abdominal sections as described elsewhere (29).

Hepatic fat

Hepatic fat content was measured by magnetic resonance imaging (MAGNETOM Avanto, Siemens Healthcare, Erlangen, Germany) with phased-array surface coil and a spine array coil equipment. This was provided by the work-in-progress software package by Siemens Medical System (version syngo.MR B17A). Briefly, two different 3D gradient-echo sequences in breath-hold and six-echo acquisition with advanced signal analysis were used for hepatic fat quantification and estimation, respectively, as reported elsewhere (30). According to the literature, liver proton density fat fraction estimated by MRI correlates well with histologic steatosis grade in children (31), and therefore MRI is considered a non-invasive and acurate method to detect hepatic steatosis in pediatric population (32).

In accordance with previous studies, the cut-off value of hepatic fat content established for categorizing children with and without hepatic steatosis was 5.5% (33,34). Therefore, children with a hepatic fat ≥5.5% were considered as having hepatic steatosis, whereas children with <5.5% hepatic fat accumulation as not having hepatic steatosis.

Insulin resistance

Insulin resistance was determined by calculating HOMA-IR using fasting blood glucose and insulin values with the following formula: (insulin (µU/mL) x glucose (mmol/L))/22.5 (35). Blood sample collection details have been published elsewhere (30).

Dietary intake assessment

Dietary intake was evaluated, by trained nutritionists, as the mean of two non-consecutive 24h-recalls during a time span of a week. Children reported, with help of their parents, all the foods and beverages consumed the day before the interview. Pictures of food servings were used to help the participants to estimate food intake. Nutritional composition data was obtained by EasyDiet software. Thereby, energy intake and macro and micronutritional composition data from both interviewed days was obtained. Regarding the food groups, each dietary recall was examined thoroughly, and thereafter, the food consumed was classified into the different food groups so as to obtain the daily food-group intake. Afterwards, the mean consumption of the dietary components consumed in both interviewed days was computed.

Fruits and vegetables were analyzed together. Detailed information about foods included in each food category and explanation about each term is available in Supplemental Table 1. Participants were asked to report product brands, particularly for SSB and DDS, in order to obtain detailed nutritional composition data, i.e., sugar and energy content of each food and beverage. SSB consumption included sugar sweetened beverages, while low- or non-caloric beverages were excluded due to the reduction of sugar and replacement for artificial non-caloric sweeteners. DDS were considered milk-derived products with added sugar when its sugar content was ≥6g/100g product. The current sugar cut-off value was established based on the natural lactose content of dairy products, assuming that dairy products with higher sugar content may have sugar added in their composition. Also, all dairy substitutes such as soya-, oat- or almond-beverages were included in DDS, but no cut-off value for sugar content was used since they are not milk-based.

Furthermore, total added sugar was calculated by removing the sugar amount in natural sugar sources such as fruits and dairy products from total dietary sugar intake.

Physical activity

Accelerometry was used as an objective measurement of total physical activity (wActisleep-BT and wGT3X-BT, ActiGraph, Pensacola, FL, USA). All participants had to wear an accelerometer on the non-dominant wrist during a week. Euclidean Norm Minus One (ENMO) was used to quantify the acceleration related to the movement registered expressed in milligravity (mg) using the R software (v. 3.1.2, www.cran.r-project.org with the GGIR package (v. 1.5-12, https://cran.r-project.org/web/packages/GGIR/).

Statistical analysis

As the variables did not show a normal distribution, non-parametrical Mann-Whitney U tests were used to examine differences in continuous variables (i.e., biological characteristics and dietary intake) between children with and without hepatic steatosis, whereas chi-square test was used for categorical variables. Linear regression analyses were used to examine the associations between dietary components (independent variables) and hepatic fat content and insulin resistance (dependent variables). Potential confounders such as sex, age, energy intake and maternal educational level (Model 1), and additionally body fat percent (Model 2) or abdominal adiposity (Model 3) and dietary simple sugar intake (Model 4) were used as covariates in the analyses. Variables with a non-normal distribution were logarithmically transformed for linear regression analyses. Sensitivity analyses were performed in dietary variables and hepatic fat content analysis adjusting additionally for HOMA-IR, physical activity and parental BMI. Collinearity diagnosis tests were also performed between dietary factors to examine if they were related to one another or not. Statistical analyses were carried out with the statistical software SPSS version 20.0 (SPSS Inc, Chicago) with the level of significance of $\alpha=0.05$.

RESULTS

Biological characteristics and dietary intake of participants with and without hepatic steatosis are shown in **Table 1**. The proportion of children with hepatic steatosis was 36.4%. Children with hepatic steatosis had significantly higher BMI zscore (P<0.03), waist circumference (P<0.01), total and abdominal adiposity (P<0.01), hepatic fat content (P<0.001) and HOMA-IR (P<0.01) compared to children without hepatic steatosis. Likewise, the percentage of children with obesity was higher in the group with hepatic steatosis (P<0.03), while age, sex, high maternal educational level, parental obesity and diabetes and total physical activity were similar between in both groups (P>0.05), non-Caucasian ethnicity was higher among children with hepatic steatosis (P<0.03). In contrast, there were no significant differences in the intakes of macronutrients, simple sugars, added sugars, fiber, cereals, fruits and vegetables, legumes, nuts, dairy products, fish and shellfish, and meat and meats products between children with and without hepatic steatosis (Ps>0.1). The consumption of SSB and the sugar content of SSB tended to be higher in children with hepatic steatosis than in their peers without hepatic steatosis (P<0.1).

Table 1. Biological characteristics and dietary intake of participants with and without hepatic steatosis of the children participating in the PREDIKID study.

	N	Children with hepatic steatosis (n=40)	N	Children without hepatic steatosis (n=70)	*P*
Age	40	10.5 (1.1)	70	10.6 (1.1)	0.485
Girls (N, %)	40	20 (50)	70	39 (55.7)	0.563
BMI zscore	40	0.29 (1.1)	70	-0.16 (0.9)	**0.028**
Obese (N, %)	40	30 (75)	70	34 (48.6)	**0.007**
Waist circumference (cm)	40	82.3 (7)	70	76.7 (6.6)	**<0.001**
Body fat (%)	40	41.2 (4.2)	69	38.4 (4.6)	**0.005**
Abdominal fat (kg)	40	3 (1.1)	69	2.3 (8.4)	**<0.001**
Hepatic fat (%)	40	9.2 (4.9)	70	3.6 (1)	**<0.001**
HOMA-IR	39	3.0 (1.3)	69	2.3 (1)	**0.005**
High maternal educational level (N, %)	39	12 (30.8)	70	16 (22.5)	0.173
Parental obesity (N, %)	40	11 (27.5)	70	15 (21.4)	0.471
Parental diabetes (N, %)	39	3 (7.5)	70	4 (5.7)	0.686
Non-Caucasian ethnicity (N, %)	38	10 (25)	69	7 (10)	**0.029**
Total physical activity (mg)	38	61.7 (14.6)	65	65.9 (17)	0.286
Energy intake (KJ/day)	40	7878 (1498)	70	7460 (1858)	0.131

Carbohydrates (g/day)	40	196 (60)	70	190 (50)	0.737
Fat (g/day)	40	86 (25)	70	79 (26)	0.148
Proteins (g/day)	40	79 (19)	70	76 (20)	0.437
Fiber (g/day)	40	14 (5)	70	14 (7)	0.921
Cereals (g/day)	40	92 (53)	70	105 (48)	0.184
Fruits and vegetables (g/day)	40	251 (183)	70	224 (148)	0.686
Legumes (g/day)	40	12.7 (15.8)	70	14.3 (20.8)	0.799
Nuts (g/day)	40	2 (6.5)	70	3 (9)	0.697
Dairy products (g/day)	40	347 (188)	70	321 (134)	0.725
Fish and shellfish (g/day)	40	47 (57)	70	34 (45)	0.260
Meat and meat products (g/day)	40	107 (70)	70	98 (76)	0.389
Simple sugars (g/day)	40	89 (37)	70	82 (26)	0.445
Added sugar (g/day)	40	60 (31)	70	51 (22)	0.146
SSB (ml/day)	40	117 (169)	70	53 (91)	0.069
Sugar from SSB (g/day)	40	9.8 (14.7)	70	4.6 (8.0)	0.094
DDS (g/day)	40	81 (77)	70	85 (93)	0.839
Sugar from DDS (g/day)	40	10.6 (10)	70	10.8 (12.0)	0.739

BMI: body mass index; DDS: dairy products desserts and substitutes; HOMA-IR: homeostatic model assessment; SSB: sugar sweetened beverages. Values are means (standard deviation).

Table 2 shows the associations of dietary components with hepatic fat content and insulin resistance. There was a negative association between cereal intake and hepatic fat content regardless of sex, age, energy intake and maternal educational level (P<0.02, Model 1). This association was diminished, but remained significant, when both total (Model 2) and abdominal fat (Model 3) were entered into the model (Ps<0.05). No statistically significant associations were found between the rest of dietary components and hepatic fat content and insulin resistance in children with overweight/obesity.

The associations of SSB and DDS intake as well as the intake of their sugar contents with hepatic fat and HOMA-IR are shown in **Table 3**. The results showed that both SSB consumption and the intake of sugar from SSB were significantly associated with hepatic fat content regardless of sex, age, energy intake and maternal educational level (P<0.02, Model 1). These relationships were still significant when either body fat percent or abdominal fat were included into the model (Ps<0.02, Models 2 and 3). Interestingly, the associations of SSB consumption and the intake of sugar from SSB with hepatic fat content remained statistically significant even after further adjustment for total simple sugar intake (P<0.05, Model 4). In a sensitivity (additional adjustment) analysis with insulin resistance (β=0.212, P=0.036 and β=0.204, P=0.045 for SSB and sugar from SSB, respectively), physical activity (β=0.217, P=0.032 and β=0.206, P=0.043 for SSB and sugar from SSB, respectively) and parental BMI (β=0.207, P =0.037 and β=0.199, P=0.047 for SSB and sugar from SSB, respectively) this associations remained statistically significant. Continuous logistic regression was performed to analyze the effect of SSB consumption in children with and without hepatic steatosis (sex, age, maternal educational level and energy intake adjustments) and even though it was not statistically significant, a trend to signification was observed (OR 1.003, 95%CI, 1.000-1.007, P=0.051). On the contrary, no significant relationships were observed between DDS consumption and sugar from DDS with hepatic fat content in children with overweight/obesity (Ps>0.05). Neither SSB nor DDS consumption were associated with insulin resistance (Ps>0.05). No collinearity was found between dietary factors.

Table 2. Associations of dietary energy and macronutrient intake, and other dietary components with hepatic fat content and insulin resistance in overweight/obese children.

	Hepatic fat content (%)						HOMA-IR					
	Model 1		Model 2		Model 3		Model 1		Model 2		Model 3	
	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*
Energy (KJ/day)	0.033	0.740	-	-	-	-	0.068	0.487	-	-	-	-
Carbohydrates (g/day)	-0.016	0.919	0.017	0.913	0.007	0.965	0.048	0.761	0.112	0.452	0.098	0.477
Fat (g/day)	0.034	0.838	0.041	0.796	0.044	0.779	-0.026	0.874	-0.063	0.693	-0.064	0.665
Proteins (g/day)	-0.017	0.907	-0.097	0.477	-0.084	0.533	-0.041	0.772	-0.092	0.485	-0.066	0.591
Fiber (g/day)	-0.118	0.261	-0.152	0.132	-0.157	0.114	0.052	0.620	0.037	0.706	0.028	0.763
Cereals (g/day)	-0.254	**0.013**	-0.211	**0.036**	-0.197	**0.048**	0.003	0.979	0.062	0.530	0.091	0.328
Fruits and vegetables (g/day)	0.028	0.771	0.044	0.638	0.062	0.501	0.063	0.511	0.080	0.381	0.116	0.173
Dairy products (g/day)	0.085	0.384	0.125	0.190	0.095	0.322	-0.073	0.454	-0.018	0.849	-0.085	0.334
Fish and shellfish (g/day)	0.152	0.114	0.112	0.230	0.103	0.263	0.110	0.250	0.079	0.389	0.069	0.422
Meat and meat products (g/day)	-0.007	0.949	-0.008	0.939	0.001	0.995	-0.076	0.492	-0.134	0.207	-0.119	0.230

HOMA-IR: homeostatic model assessment Model 1: adjusted for sex, age, energy intake and maternal educational level; Model 2: Model 1 additionally adjusted for body fat percent; Model 3: Model 2 additionally adjusted for abdominal adiposity.

Table 3. Associations of sugar-sweetened beverages (SSB) and dairy products desserts and substitutes (DDS) consumption, as well as their sugar content with hepatic fat content and insulin resistance (HOMA-IR) in overweight/obese children.

	Hepatic fat content (%)								HOMA-IR							
	Model 1		Model 2		Model 3		Model 4		Model 1		Model 2		Model 3		Model 4	
	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*
SSB (g/day)	0.263	**0.008**	0.250	**0.009**	0.243	**0.010**	0.217	**0.028**	0.108	0.276	0.102	0.282	0.085	0.336	0.094	0.318
Sugar from SSB (g/day)	0.254	**0.011**	0.243	**0.011**	0.237	**0.012**	0.210	**0.035**	0.106	0.292	0.100	0.298	0.085	0.340	0.094	0.322
DDS (g/day)	-0.051	0.612	-0.067	0.498	-0.048	0.621	-0.081	0.412	-0.054	0.585	-0.128	0.184	-0.092	0.309	-0.096	0.299
Sugar from DDS (g/day)	-0.037	0.715	-0.053	0.593	-0.036	0.715	-0.070	0.483	-0.061	0.540	-0.135	0.163	-0.099	0.274	-0.104	0.263
Simple sugar (g/day)	0.131	0.270	0.171	0.135	0.179	0.112	-	-	-0.053	0.648	-0.013	0.904	0.004	0.969	-	-
Added Sugar (g/day)	0.209	0.074	0.195	0.084	0.208	0.061	0.178	0.314	0.005	0.964	-0.024	0.827	0.002	0.986	-0.003	0.983

Model 1: adjusted for sex, age, energy intake and maternal education level; Model 2: Model 1 additionally adjusted for body fat percent; Model 3: Model 2 additionally adjusted for abdominal adiposity Model 4: Model 3 additionally adjusted for simple sugar intake.

DISCUSSION

The current study examined the influence of several food groups and dietary components on hepatic fat content and insulin resistance among pre-adolescent children with overweight/obesity. The main findings were that cereal intake was negatively associated with hepatic fat content but not with insulin resistance, whereas the rest of the food groups and macronutrients were not associated neither with hepatic fat content nor with insulin resistance. Moreover, SSB consumption and sugar from SSB were positively associated with hepatic fat accumulation, but not with insulin resistance. In contrast, we did not observe any significant relationships between DDS and hepatic fat content or insulin resistance among children aged 8-12 years with overweight/obesity.

Almost half of the children in the study (36.4% of the participants), presented hepatic steatosis. It is well known that excess adiposity increases the risk of having NAFLD (36). Our findings about prevalence are in agreement with findings from two previous studies. One of them reported that whilst the prevalence of pediatric hepatic steatosis is lower than 10% in general population, the prevalence among children with obesity can reach 80% (37). According to other study, the prevalence of NAFLD in children with normal weight was 2.6%, and increased to 20-77% among children and adolescents with obesity (38). Nevertheless, it should be pointed out that the range of prevalence might vary depending on the diagnosis method used for measuring hepatic fat content. In this study, hepatic fat content was measured by MRI, which is more accurate method compared to ultrasound, and in fact, it has been considered a non-invasive appropriate alternative for pediatric population (33). Furthermore, and in line with other studies (34), ethnic differences between Hispanic, black and white population were observed in the prevalence of hepatic steatosis. Regarding body composition, children with hepatic steatosis had higher values of waist circumference as well as total and abdominal adiposity compared to children without hepatic steatosis. On the contrary, participants with hepatic steatosis did not show higher values of BMI, which demonstrates the limitations of using BMI as a proxy of body fatness in children (39). Accordingly, basing on the strong association between abdominal adiposity and hepatic steatosis, Alisi et al reported that abdominal obesity is more powerful in predicting hepatic steatosis than BMI (40).

Overall, we found only few differences in dietary intake variables between children with and without hepatic steatosis. In contrast to our results, a previous study reported that adolescents with hepatic steatosis had higher intakes of total fat and fried foods consumption than their peers without hepatic steatosis (41). However, in the current study children with hepatic steatosis tended to consume more SSB compared to children without hepatic steatosis, which is consistent with the association between SSB consumption and NAFLD (10). Our observations suggest that cereal intake may be protective against hepatic steatosis development. On the contrary, Georgoulis et al reported that refined grains were associated with higher likelihood of having NAFLD in adults, whereas whole grain consumption favorably affected clinical characteristics of participants with NAFLD as a result of being rich in fiber and having lower glycemic index (42). Unfortunately, due to the infrequent intake of whole-grains of children from the PREDIKID study, we could not examine the influence of whole cereals on hepatic health. On the other hand, in disagreement with other studies in which whole cereal intake was inversely associated with insulin resistance among adolescents (43), no association was found between cereal intake and HOMA-IR in children with overweight/obesity from the current study.

We observed that 39.8% and 65.5% of the children consumed SSB and DDS, respectively (data not shown). Moreover, we found a positive association between SSB consumption as well as sugar in SSB and hepatic fat content regardless of sex, age, energy intake, maternal educational level, body fat percent, abdominal obesity and total simple sugar intake. Of note, while in United States SSB are primarily sweetened with high fructose corn syrup, in Europe sucrose is commonly used as the main sweetener (44), which is a disaccharide, composed of fructose and glucose. Although there are several studies reporting the association of both fructose (45) and SSB consumption (46,47) with adiposity and cardio metabolic risk factors in adults and adolescents, there are few studies focused on children to date. Furthermore, to the best of our knowledge, this is the first study examining the influence of SSB consumption on hepatic fat content in children with overweight/obesity. Interestingly, neither SSB nor sugar from SSB was associated with insulin resistance. In addition, based on the fact that impaired insulin signaling originated by dysfunctional adipose tissue induces insulin resistance, insulin resistance might be a secondary effect of excess hepatic fat. Despite the lack of the association in our study, other authors reported that daily intake of SSB was associated with increased HOMA-IR in adolescents (48). The possible mechanism of how fructose can induce NAFLD has largely been studied in animal models. Briefly, fructose is absorbed from the intestine into the portal vein and goes directly to the liver, where it stimulates de novo lipogenesis by promoting hepatic lipid accumulation (40,46), which may explain NAFLD development. Nonetheless, not only fructose, but also sucrose-containing beverages have been shown to increase hepatic fat, as well as visceral and muscle fat and triglycerides (49).

In contrast, DDS consumption was not significantly associated with hepatic fat content and insulin resistance in our sample of children with overweight/obesity. This finding is in line with previous studies suggesting that dairy fat intake, as well as other nutrients like calcium (which are mostly present in DDS, but not in SSB) could be protective against hepatic fat accumulation in adults (50), and abdominal adiposity in female adolescents (51). In addition, the fact that DDS are commonly consumed with a meal, whereas people tend to drink SSB between meals, could also explain the influence on these outcomes. Accordingly, previous studies reported that snacking promotes liver fat accumulation (52,53). As a consequence of the rise of SSB consumption in children, initiatives such as the implementation of SSB associated tax in several countries have been carried out in order to prevent obesity and related comorbidities. The purchases of SSB decreased after Mexico implemented a tax, so that this seems to be a successful intervention from global policies (54).

STRENGTHS AND LIMITATIONS

The use of magnetic resonance imaging to measure hepatic fat content should be considered as study strength for its accuracy, while ultrasonography is used in most of the other studies. Moreover, detailed nutritional composition information of SSB and DDS was obtained by analyzing the labels of products brands. However, the current study has also some limitations. These findings should be taken carefully due to its cross-sectional design and therefore, prospective studies are needed since experimental evidence would strengthen these cross section observations.

CONCLUSIONS

The main findings in the current study suggest that cereal intake seems to be associated with lower liver fat content, whereas SSB consumption and sugar from SSB might increase hepatic fat content among children with overweight/obesity. In contrast, neither DDS consumption nor sugar from DDS seems to modulate cardio metabolic risk factors, and last but not least, no associations were found between dietary intake and insulin resistance in children with excess adiposity. Our results suggest that healthy lifestyle intervention programs are needed to improve dietary habits as well as to increase the awareness of the detrimental effects of SSB consumption early in life.

ACKNOWLEDGMENTS

Firstly, we acknowledge all the children and their parents or legal guardians for participating in the study. We also thank the pediatricians for their collaboration in recruiting participants and to the pre- and post-graduate students for their collaboration in both data collection and intervention program sessions.

FINANCIAL SUPPORT

This project was supported by the Spanish Ministry of Industry and Competitiveness (DEP2016-78377-R), by “Fondos Estructurales de la Unión Europea (FEDER), Una manera de hacer Europa” and by the University of the Basque Country (GIU14/21). This work was also supported by grants from Spanish Ministry of Economy and Competitiveness (RYC 2010-05957; RYC-2011-09011), by the Education Department of the Government of the Basque Country (PRE_2016_1_0057, PRE_2017_2_0224 and PRE_2018_2_0057), by the Spanish Ministry of Education, Culture and Sports (FPU14/03329), and by the Swedish Society of Medicine.

CONFLICT OF INTEREST

None

AUTHORSHIP

IL conceived and designed the study, LA and IL drafted the manuscript, LA, MM, MO and IL collected the data and LA, MM, MO, IH, ID, HH and IL were involved in the interpretation of the results. All the authors critically revised the manuscript for important intellectual content and approved the final version of the submitted manuscript.

REFERENCES

1. Than NN, Newsome PN (2015) A concise review of non-alcoholic fatty liver diseaseAtherosclerosis 239, 192–202.

2. Yang M, Gong S, Ye SQ, Lyman B, Geng L, Chen P, et al (2014) Non-Alcoholic Fatty Liver Disease in Children: Focus on Nutritional Interventions. Nutrients 4691–705.

3. Abenavoli L, Milic N, Peta V et al (2014) Alimentary regimen in non-alcoholic fatty liver disease : Mediterranean diet. World J Gastroenterol 20, 16831–16840.

4. Ferolla SM, Silva LC, Lourdes M De et al (2015) Dietary approach in the treatment of nonalcoholic fatty liver disease. World J Hepatol 7, 2522–2534.

5. Alkhater SA. (2015) Paediatric non-alcoholic fatty liver disease : an overview. Obes Rev 16, 393–405.

6. Zivkovic AM, German JB, Sanyal AJ (2007) Comparative review of diets for the metabolic syndrome : implications for nonalcoholic fatty liver disease. Am J Clin Nutr 86, 285–300.

7. Oddy WH, Herbison CE, Jacoby P et al (2013) The Western dietary pattern is prospectively associated with nonalcoholic fatty liver disease in adolescence. Am J Gastroenterol 108 778–85.

8. Anderson EL, Howe LD, Fraser A et al (2015) Childhood Energy Intake Is Associated with Nonalcoholic Fatty Liver Disease in. Am J Clin Nutr 145, 983–989.

9. Azais-braesco V, Sluik D, Maillot M et al (2017) A review of total & added sugar intakes and dietary sources in Europe. Nutr J 16, 1–15.

10. Ma J, Fox CS, Jacques PF et al (2015) Sugar-sweetened beverage, diet soda, and fatty liver disease in the Framingham Heart Study cohorts. J Hepatol 63, 462–469.

11. Morenga LA Te, Howatson AJ, Jones RM et al (2014) Dietary sugars and cardiometabolic risk : systematic review and meta-analyses of randomized controlled trials of the effects on blood pressure and lipids. Am J Clin Nutr 100, 65–79.

12. Keller A, Bucher Della Torre S (2015) Sugar-Sweetened Beverages and Obesity among Children and Adolescents: A Review of Systematic Literature Child Obes 11, 338–346.

13. Hu FB, Malik VS (2011) Sugar-sweetened beverages and risk of obesity and type 2 diabetes : Epidemiologic evidence. Physiol Behav 100, 47–54.

14. Wijarnpreecha K, Thongprayoon C, Edmonds PJ et al (2016) Associations of sugar- and artificially sweetened soda with nonalcoholic fatty liver disease: a systematic review and meta-analysis. QJM An Int J Med, 461–466.

15. Jensen BW, Nielsen BM, Husby I et al (2013) Association between sweet drink intake and adiposity in Danish children participating in a long-term intervention study. Pediatr Obes 8,259–270.

16. Stern D, Piernas C, Barquera S et al (2014) Caloric Beverages Were Major Sources of Energy among Children and Adults in Mexico. J Nutr 144, 949–956.

17. Khan TA, Sievenpiper JL (2016) Controversies about sugars : results from systematic reviews and meta analyses on obesity, cardiometabolic disease and diabetes. Eur J Nutr 55, 25–43.

18. Cook LT, O'Reilly GA, Goran MI et al (2014) Vegetable Consumption Is Linked to Decreased Visceral and Liver Fat and Improved Insulin Resistance in Overweight Latino Youth. J Acad Nutr Diet 114, 1776–83.

19. Shin H, Yoon YS, Lee Y et al (2013) Dairy product intake is inversely associated with metabolic syndrome in Korean adults: Anseong and ansan cohort of the Korean genome and epidemiology study. J Korean Med Sci 28, 1482–8.

20. Cantero I, Abete I, Monreal JI et al (2017) Fruit fiber consumption specifically improves liver health status in obese subjects under energy restriction. Nutrients 9, 1-12

21. Davis J, Alexander K, Ventura E (2009) Inverse relation between dietary fiber intake and visceral adiposity in overweight Latino youth. Am J Clin Nutr 90, 1160–1166.

22. Jia Q, Xia Y, Zhang Q et al (2015) Dietary patterns are associated with prevalence of fatty liver disease in adults. Eur J Clin Nutr 69, 914–921.

23. Papandreou D, Karabouta Z, Pantoleon A et al (2012) Investigation of anthropometric, biochemical and dietary parameters of obese children with and without non-alcoholic fatty liver disease. Appetite 59, 939–944.

24. Felix DR, Costenaro F, Bertaso et al (2016) Non-alcoholic fatty liver disease (Nafld) in obese children- effect of refined carbohydrates in diet. BMC Pediatr 16, 1-6

25. Visuthranukul C, Sirimongkol P, Prachansuwan A et al (2015) Low-glycemic index diet may improve insulin sensitivity in obese children. Pediatr Res 78, 567–573.

26. Arenaza L, Medrano M, Amasene et al (2017) 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 18, 372.

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

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

29. Labayen I, Ruiz JR, Vicente-Rodríguez G et al (2009) Early Life Programming of Abdominal Adiposity in Adolescents : The HELENA. Diabetes Care 32, 2120–2.

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

31. Schwimmer JB, Middleton MS, Behling C et al (2015) Magnetic resonance imaging and liver histology as biomarkers of hepatic steatosis in children with nonalcoholic fatty liver disease. Hepatology 61, 1887–1895.

32. Hsu E, Murray K (2012) Is Nonalcoholic Fatty Liver Disease in Children the Same Disease as in Adults? Clin Liver Dis 16, 587–98.

33. Younossi Z, Loomba R, Rinella M et al (2017) Current and Future Therapeutic Regimens for Non-alcoholic Fatty Liver Disease (NAFLD) and Non-alcoholic Steatohepatitis (NASH). Hepatology 5, 1–36.

34. Browning JD, Szczepaniak LS, Dobbins R et al (2004) Prevalence of hepatic steatosis in an urban population in the United States: Impact of ethnicity. Hepatology 40, 1387–95.

35. Matthews D, Hosker J, Rudenski A (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28, 412–419.

36. Barshop NJ, Sirlin CB, Schwimmer JB et al (2008) Review article: Epidemiology, pathogenesis and potential treatments of paediatric non-alcoholic fatty liver disease. Aliment Pharmacol Ther 28, 13–24.

37. Alisi A, Feldstein AE, Villani A et al (2012) Pediatric nonalcoholic fatty liver disease : a multidisciplinary approach. Nat Publ Gr 9, 152–161.

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

39. Freedman DS, Sherry B et al (2009) The Validity of BMI as an Indicator of Body Fatness and Risk Among Children. Pediatrics 124 S23–34.

40. Alisi A, Manco M, Vania A et al (2009) Pediatric Nonalcoholic Fatty Liver Disease. J Pediatr 55, 469–474.

41. Macintosh AC, Hay J, Wicklow BA et al (2014) Dietary determinants of hepatic steatosis and visceral adiposity in overweight and obese youth at risk of type 2 diabetes. Am J Clin Nutr 99, 804–812.

42. Georgoulis M, Kontogianni MD, Tileli N (2014) The impact of cereal grain consumption on the development and severity of non-alcoholic fatty liver disease. Eur J Nutr 53, 1727–1735.

43. Steffen LM, Jacobs DR, Murtaugh MA et al (2003) Whole Grain Intake Is Associated with Lower Body Mass and Greater Insulin Sensitivity among Adolescents. Am J Epidemiol 158,243–50.

44. Malik VS, Hu FB (2015) Fructose and Cardiometabolic Health: What the Evidence From Sugar-Sweetened Beverages Tells Us. J Am Coll Cardiol 66, 1615-1624.

45. Alwahsh SM, Gebhardt R (2017) Dietary fructose as a risk factor for non-alcoholic fatty liver disease (NAFLD). Arch Toxicol 91, 1545–63.

46. Wang J, Light K, Loughlin JO et al (2014) Consumption of Added Sugars from Liquid but Not Solid Sources Predicts Impaired Glucose Homeostasis and Insulin Resistance among Youth at Risk of Obesity. J Nutr 144, 81–86.

47. Chan T, Lin W, Huang H et al (2014) Consumption of Sugar-Sweetened Beverages Is Associated with Components of the Metabolic Syndrome in Adolescents. Nutrients 6, 2088–2103.

48. Kondaki K, Grammatikaki E, Jime D et al (2012) Daily sugar-sweetened beverage consumption and insulin resistance in European adolescents : the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study. Public Health Nutr 16, 479–86.

49. Maersk M, Belza A, Stodkilde-jorgensen H et al (2012) Sucrose-sweetened beverages increase fat storage in the liver, muscle, and visceral fat depot : a 6-mo randomized intervention study. Am J Clin Nutr 95, 283–289.

50. Kratz M, Marcovina S, Nelson JE et al (2014) Dairy fat intake is associated with glucose tolerance, hepatic and systemic insulin sensitivity and liver fat but not b 412 -cell function in humans. Am J Clin Nutr 99, 1385–1396.

51. Castro Burbano J, Fajardo Vanegas P et al (2016) Relationship between dietary calcium intake and adiposity in female adolescents. Endocrinol Nutr 63, 58–63.

52. Piernas C, Popkin B (2010) Trends in Snacking Among U.S. Children. Heal Aff 29(3):398–404.

53. Koopman KE, Caan MWA, Nederveen AJ, Pels A, Ackermans MT, Fliers E, et al. Hypercaloric diets with increased meal frequency, but not meal size, increase intrahepatic triglycerides: A randomized controlled trial. Hepatology. 2014;60(2):545–53.

54. Colchero MA, Molina M, Guerrero-l CM (2017) After Mexico Implemented a Tax, Purchases of Sugar-Sweetened Beverages Decreased and Water Increased: Difference by Place of Residence, Household Composition, and Income Level. J Nutr 147, 1552–1557

Supplemental Table 1. Included and excluded foods in each food group and intake recommendations for children.

Food groups	Foods included	Foods excluded	Intake recommendation for children
Cereals	Whole or refined pasta, bread, rice, couscous, rusks, sliced bread.	Flours, cereals with chocolate, biscuits, pastries, energy bars, rice or corn snacks.	3 servings/day (90g/day). Preferably whole cereals (AHA) (1).
Fruits and vegetables	All kind of vegetables, home-made tomato sauce or crushed tomato. All kind of fresh fruits, natural fruit-juices.	Potatoes, commercial fried tomato sauce, canned corn, green peas. Dried or sweetened fruits (peaches in syrup, industrial fruit-salad).	4.5 servings/day (>360g/day) (AHA) (1).
Legumes	French beans, green peas, chickpeas, lentils.	Green beans, nuts.	2-3 servings/week (>80g/week) (2).
Nuts	Natural walnut, hazelnut, almond, pistachio, sunflower pipe.	Sugared or high-processed nuts.	3-7 servings/week (>60g/week) (SENC)(3).
Dairy products	Milk, yoghurt, cheese, curd	Ice-creams, vegetal drinks.	2-4 servings/day, >500ml/day to ensure calcium intake (2).
Fish and shellfish	Fresh, frozen or canned fish, mollusk, squid, octopus, crusty	Surimi.	>2 servings/week (>200g/week) (2) Preferably both white and fatty fish (AHA) (1)
Meat and meat products	White or red meat, processed meat, lunchmeat.	Vegetarian-meat alternatives, such as tofu or soy products.	<3-4 servings/week (<400g/week). Preferably lean meat (2)

Sugar-sweetened beverages	Soft drinks, sweetened soda, fizzy drinks, artificial fruit-juices, energy drinks.	Light or zero drinks (non-caloric beverages).	Try to avoid them by replacing with water or natural fruit-juices The recommended intake is 0.
Dairy desserts and substitutes	Sugared or flavored yoghurt, commercial chocolate milk-shakes and smoothies, rice-puddings, mousse, ice-creams, vegetal drinks.	Home-made smoothies or rice-puddings, yoghurts with non-caloric sweeteners, yoghurts with <6g/100g sugar content.	Try to avoid them by replacing with less-processed dairy products without added sugar. The recommended intake is 0.
Simple sugars	All dietary simple sugars; including natural sugar sources and artificially added.	Polysaccharides.	Less than 10% of total energy intake from free sugars, ideally less than 5% of total energy intake (WHO) (4)
Added sugar	Sugars and syrups put in foods during preparation or processing, or added at the table.	Natural sugar from fruits and dairy products.	<25g/day for all children aged 2-19 years regardless of age (AHA) (1).

AHA: American Heart Association; SENC: Spanish Society for Community Nutrition; WHO: World Health Organization; Perseo Program of NAOS strategy.

References

1. Lloyd-Jones DM, Hong Y, Labarthe D et al (2010) Defining and setting national goals for cardiovascular health promotion and disease reduction: The american heart association’s strategic impact goal through 2020 and beyond. Circulation 121, 586–613.

2. Estrategia NAOS (2008) Cuidar la cantidad: las raciones. Programa Perseo, Estrategia Naos 180.

3. Dapcich V, Salvador G, Ribas L et al (2004) Guía de la alimentación saludable. Senc 105.

4. World Health Organization (2014) Guideline : Sugars intake for adults and children. World Heal Organ -WHO 48.

3.5. Study V

Published in: Nutrients

nutrients

MDPI

Article

The Effect of a Family-Based Lifestyle Education Program on Dietary Habits, Hepatic Fat and Adiposity Markers in 8–12-Year-Old Children with Overweight/Obesity

Lide Arenaza [1,2,†,*], María Medrano [1,2], Maddi Oses [1,2], Maria Amasene [3], Ignacio Díez [4], Beatriz Rodríguez-Vigil [5] and Idoia Labayen [1,2]

[1] Institute for Innovation and Sustainable Development in Food Chain (IS-FOOD), Public University of Navarra, 31006 Pamplona, Navarra, Spain; maria.medrano@unavarra.es (M.M.); maddi.oses@unavarra.es (M.O.); idoia.labayen@unavarra.es (I.L.)

[2] Navarra's Health Research Institute (IdiSNA), 31008 Pamplona, Navarra, Spain

[3] Department of Pharmacy and Food Sciences, University of the Basque Country, UPV/EHU, 01006 Vitoria-Gasteiz, Araba, Spain; maria.amasene@ehu.eus

[4] Pediatric Endocrinology Unit, University Hospital of Araba (HUA), 01009 Vitoria-Gasteiz, Spain; IGNACIO.DIEZLOPEZ@osakidetza.eus

[5] Department of Magnetic Resonance Imaging, Osatek, University Hospital of Araba (HUA), 01009 Vitoria-Gasteiz, Spain; brodriguezvigil@osatek.eus

* Correspondence: lide.arenaza@unavarra.es; Tel.: +34 666-05-73-15

† Postal address: Institute for Innovation and Sustainable Development in Food Chain (IS-FOOD), Public University of Navarra, Calle Tajonar 22, Pamplona, Spain.

ABSTRACT

Healthy lifestyle education programs are recommended for obesity prevention and treatment. However, there is no previous information on the effects of these programs on the reduction of hepatic fat percentage. The aims were (i) to examine the effectiveness of a 22-week family-based lifestyle education program on dietary habits, and (ii) to explore the associations of changes in dietary intake with percent hepatic fat reduction and adiposity in children with overweight/obesity. A total of 81 children with overweight/obesity (aged 10.6 ± 1.1 years, 53.1% girls) and their parents attended a 22-week family based healthy lifestyle and psychoeducational program accompanied with (intensive group) or without (control) an exercise program. Hepatic fat (magnetic resonance imaging), adiposity (dual energy X-ray absorptiometry) and dietary habits (two non-consecutive 24 h-recalls) were assessed before and after the intervention. Energy ($p < 0.01$) fat ($p < 0.01$) and added sugar ($p < 0.03$) intake were significantly reduced in both groups at the end of the program, while, in addition, carbohydrates intake ($p < 0.04$) was reduced exclusively in the control group, and simple sugar ($p < 0.05$) and cholesterol ($p < 0.03$) intake was reduced in the exercise group. Fruit ($p < 0.03$) and low-fat/skimmed dairy consumption ($p < 0.02$), the adherence to the Mediterranean Diet Quality Index for children and teenagers (KIDMED, $p < 0.01$) and breakfast quality index ($p < 0.03$) were significantly higher in both control and intervention groups after the intervention. Moreover, participants in the exercise group increased the adherence to the Dietary Approaches to Stop Hypertension (DASH) diet ($p < 0.001$), whereas the ratio of evening-morning energy intake was significantly lower exclusively in the control group after the program ($p < 0.02$). Changes in energy intake were significantly associated with changes in fat mass index (FMI) in the exercise group, whereas changes in sugar-sweetened beverages (SSB) consumption were associated with percent hepatic fat reduction ($p < 0.05$) in the control group. A 22-week family-based healthy lifestyle program seems to be effective on improving diet quality and health in children with overweight/obesity and these should focus on SSB avoidance and physical activity.

Keywords: lifestyle program; dietary habits; hepatic fat; sugar-sweetened beverages

INTRODUCTION

The worldwide rising prevalence of childhood obesity is of public health concern [1]. Childhood obesity is associated with greater cardiometabolic risk later in life [2], and already in early ages [3]. Spain, with 26% and 12.6% of 8–17 years-old children presenting overweight and obesity, respectively, is one of the European countries with the highest prevalence of childhood obesity, reporting a worse situation in 8–13 years-old boys from low socioeconomic and educational families [4]. National strategies, where environmental and policy actions are a priority, have been launched to tackle pediatric obesity in this country [5]. Abdominal adiposity is strongly associated with non-alcoholic fatty liver disease (NAFLD) [6], which consists of the accumulation of triglycerides within hepatocytes with the absence of alcohol consumption [7]. NAFLD is also considered as the hepatic manifestation of metabolic syndrome [8].

Lifestyle changes are the main recommendation for the prevention and treatment of NAFLD. Diet quality improvement, with a special focus on sugar-sweetened beverage (SSB) avoidance, and increasing physical activity together with reducing sedentary behaviors, are recommended as the main targets to improve the spectrum of the disease [9]. Besides the importance of an early treatment of obesity and NAFLD in order to prevent adiposity-related comorbidities, lifestyle changes seem to be more effective in childhood than later life, since children are still acquiring and establishing their habits. Furthermore, the participation and active involvement of the parents in lifestyle interventions increase their effectiveness, since they are fundamental agents in their children's lifestyle habits [10–12]. As far as we are aware, there is no previous information on the effects of lifestyle education programs targeting diet quality improvement on the reduction of hepatic fat percent in prepuberal children. In adults and adolescents with obesity, lifestyle intervention based on weight loss induced by hypocaloric diets have been shown to be effective at reducing hepatic fat [13–15] or were based on exercise programs [16]. However, caloric restriction is not recommended in the pediatric population since it could affect both children's growth and development, as well as induce eating disorder behaviors development [17].

Therefore, the aims of the current study were i) to examine the effects of a family-based lifestyle educational program on dietary habits and ii) to explore the associations of changes in dietary habits with changes in percent hepatic fat and adiposity in children with overweight/obesity.

MATERIAL AND METHODS

Study Design and Participants

The EFIGRO study is a non-randomized two-arm, parallel-design controlled trial (NCT02258126, ClinicalTrials.gov), carried out between 2014 and 2017 in Vitoria-Gasteiz (Spain). The study protocol was approved by the Ethic Committee of Clinical Investigation of Euskadi (PI2014045) and was performed according to the compliance of the ethical guidelines of the Declaration of Helsinki. More detailed information and the main effects of the study have been published elsewhere [18,19]. Briefly, participants were divided into control and exercise groups, in which the main difference was that whereas children in the control group participated exclusively in the family-based healthy lifestyle program, the ones in the exercise group besides participating in the previous one additionally did in the exercise program (3 sessions per week). For the current purpose, a total of 81 children with overweight/obesity (aged 10.6 ± 1.1 years, 53.1% girls) either from the control or exercise group who (1) completed the study, (2) attended at least 50% of the lifestyle education sessions, and (3) had two 24 h-recalls at baseline and post-intervention, were included in the analyses (**Figure 1**).

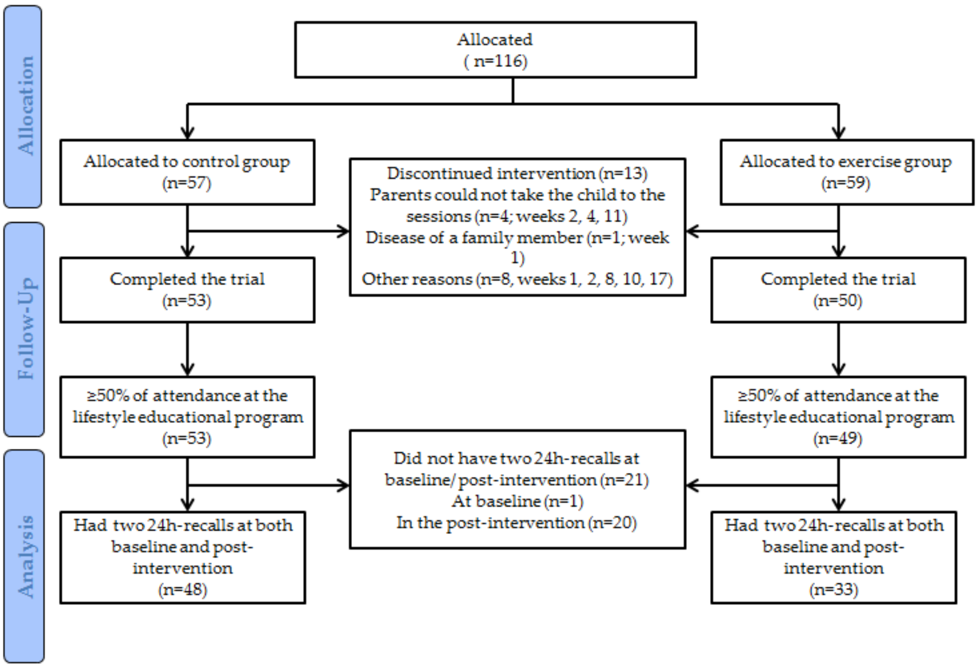


Figure 1. Flowchart of children participating in the study.

Family-based Healthy Lifestyle Program

All children and their parents or caregivers participated in a 22-week intervention focused on healthy lifestyle promotion. The program, conducted by experienced nutritionists, was composed of 11 sessions (once per two weeks), whose main aim was to improve dietary habits by increasing the intake of fruits and vegetables (F+V), enhancing breakfast habits, and reducing sugar intake and SSB consumption, as well as to make children be physically more active. Children had a booklet which included the objectives to accomplish from each session and these were commented on thereafter. In order to make easier to adopt the proposed lifestyle changes, both children and parents also attended psychoeducational sessions to achieve appropriate skills and counseling. More details about the interventions has been published elsewhere [18].

Hepatic Fat and Adiposity

Body weight (SECA 760), height (SECA 220) and waist circumference (SECA 201) were measured following standard protocols and BMI was calculated. Children were classified as overweight/obese according to the World Obesity Federation criteria [20]. Dual energy X-ray absorptiometry (DXA; HOLOGIC, QDR 4500 W) was used to measure total and abdominal adiposity [18]. Fat mass index (FMI) was calculated as fat mass divided by height squared (kg/m2). Liver fat percentage was measured by magnetic resonance imaging (MAGNETOM Avanto 1.5-T, Siemens, Munich, Germany) with a Dixon method.

Dietary Habits

Dietary Intake

Energy and nutrients intake was examined using two non-consecutive 24 h-recalls by trained dietitians and nutritional composition was analyzed with the EasyDiet software (© Biocentury, S.L.U. 2020, Spain). Thereafter, the average intake of the two reported days was calculated. We also calculated added sugar intake by subtracting natural sugar from fruits and dairy products from total simple sugar intake.

Adherence to Dietary Patterns

Adherence to dietary patterns was examined, both before and after the participation in the trial. More information about the tools and criteria used to examine the adherence to these patterns or scores is detailed in **Table S1**. Mediterranean Diet Quality Index for children and teenagers (KIDMED test) was used to examine the adherence to the Mediterranean pattern [21]; the adherence to the Dietary Approaches to Stop Hypertension (DASH) diet was assessed by following the Cohen et al. criteria [22] and HDI score by using World Health Organization (WHO) criteria [23].

Breakfast Habits

Skipping breakfast prevalence was obtained from the food recalls, and the breakfast quality index (BQI) was used to examine the children's breakfast quality before and after participating in the education program [24]. This information is available in the supplementary material (**Table S1**).

Meal Frequency and Daily Energy Distribution (Morning vs. Evening Energy Intake)

Eating frequency was obtained from the 24 h-recalls and each eating occasion was considered when meeting the criteria of (1) having ≥50 kcal/meal, and (2) spending ≥15 min from any previous eating occasion as described elsewhere [25]. The average of daily energy intake was divided into morning and evening intake. The criteria used in other studies for morning and evening classification has been before and after 12 p.m., respectively [26]. Nevertheless, taking into consideration Spanish dietary habits, in which none of the participants used to eat lunch before noon, we used another criterion. Thereby, evening/morning energy ratio (EMEratio) was calculated as evening energy intake divided by morning energy intake establishing as threshold before and after having lunch, by considering the intake of lunch as morning intake.

Statistical Analysis

Independent t tests were used to analyze baseline differences according to intervention groups for continuous variables, whereas chi-square test was used for categorical variables. To examine changes in nutritional variables between baseline and post intervention, paired sample t-test and McNemar test were used for continuous and categorical variables, respectively. Changes in biological and nutritional variables were calculated as post-value subtracted by pre-value (Δ = post-pre). Univariate linear models (ANCOVA) were performed to examine differences in changes in continuous variables (dependent variables) using study group as fixed factor and sex, age and changes in energy as covariates. Partial correlations were performed to examine the association between changes in dietary intake and changes in hepatic fat and adiposity, adjusting for sex and age and changes in height and energy intake. The interaction effect of sex and intervention group (control or exercise) was examined with repeated measures ANCOVA, by using pre- and post-intervention energy and macronutrients intake, as within subjects' variables. Statistical analyses were carried out with the statistical software SPSS version 20.0 (SPSS Inc., Chicago, IL, USA), with the significance level of α = 0.05.

RESULTS

Table 1 shows baseline characteristics of participants according to intervention group. There were no significant baseline differences between the two intervention groups in biological and sociodemographic characteristics or body composition measurements. Intervention effects on BMI and percent hepatic fat (ps < 0.01) were significantly different according to the study group, with greater effects on participants in the exercise group, as has been published elsewhere [19].

Table 1. Baseline biological and sociodemographic characteristics of study participants according to the intervention group.

	Control Group		**Exercise Group**		***p***
	N		**N**		
Age (years)	48	10.6 (1.1)	33	10.5 (1.1)	0.597
Girls (N, %)	48	26, 54.2	33	17, 51.5	0.814
High educational level of the mother (N, %)	48	39, 81.3	32	21, 63.6	0.114
Children with Spanish-origin mother (N, %)	48	43, 89.6	33	30, 90.9	0.844
Body mass index (kg/m^2)	48	25.2 (2.8)	33	25.7 (3.6)	0.536
Children with obesity (N, %)	48	26, 54.2	33	19, 58	0.762
Fat mass index (kg/m^2)	48	9.8 (2.2)	32	10.4 (2.6)	0.270
Abdominal fat (kg)	48	2.4 (1.0)	32	2.6 (1.1)	0.448
Hepatic fat (%)	48	5.1 (2.8)	33	5.8 (5.2)	0.471

Values are means (standard deviation) or frequencies and percentages (N, %).

Dietary Intake

Table 2 shows baseline differences in dietary habits between the control and exercise group, changes in dietary habits at the end of the intervention in both groups (post- vs. pre-intervention values), as well as differences in the effect of the intervention on dietary habits between control and exercise groups. It can be observed that there were no baseline differences in dietary habits between the two intervention groups ($p > 0.05$, Table 2).

It was observed that both groups significantly reduced energy and fat intake, as well as added sugar intake after participating in the program ($p < 0.05$, Table 2). Similarly, simple sugar intake was also reduced at the end of intervention in both groups, although not significantly in the control group ($p < 0.07$). Regarding food groups, fruits and low-fat/skimmed dairy consumption were significantly increased in both control and intervention groups ($p < 0.03$). Moreover, the adherence to dietary patterns was also improved regardless of the study group, as KIDMED and BQI score were significantly increased after participation in the program ($p < 0.03$). There were no significant differences between the intervention groups.

Carbohydrates intake and EME_{ratio} was significantly reduced exclusively in the control group ($p < 0.04$), whereas cholesterol intake was significantly reduced, and the DASH score increased in the exercise group, but not in the control one ($p < 0.03$). However, no statistically significant differences were found between groups in the previous variables. Although changes in cereal intake were significantly different between groups ($p < 0.03$), none of the groups showed a significant intervention effect in its intake ($p > 0.05$). A significant increase in the DASH score was observed exclusively in the exercise group ($p < 0.001$), showing a significant intervention effect between groups ($p < 0.05$).

Table 2. Dietary habits of children participating in the family-based lifestyle program (control group) and in the same plus exercise program (exercise group), before (Pre) and after (Post) the intervention.

	Control Group				Exercise Group				Baseline Control vs. Exercise	Intervention Effect Control vs. Exercise
	N	**Pre**	**Post**	***p****	**N**	**Pre**	**Post**	***p* ***	***p* ****	***p* *****
Energy, nutrients and food groups										
Energy intake (kcal/day)	48	1827 (423)	1652 (376)	**0.009**	33	1855 (430)	1622 (326)	**0.003**	0.774	0.566
Carbohydrates intake (g/day)	48	197 (53)	179 (44)	**0.039**	33	189 (53)	178 (39)	0.248	0.501	0.168
Simple sugar intake (g/day)	48	87 (32)	78 (24)	0.067	33	84 (28)	73 (25)	**0.044**	0.622	0.970
Added sugar intake (g/day)	48	55 (31)	43 (19)	**0.024**	33	58 (29)	40 (19)	**0.006**	0.955	0.517
Fat intake (g/day)	48	81 (28)	70 (24)	**0.010**	33	88 (27)	66 (19)	**<0.001**	0.229	0.062
Protein intake (g/day)	48	77 (20)	75 (20)	0.591	33	75 (18)	76 (18)	0.865	0.736	0.226
Cholesterol (mg/day)	48	306 (127)	301 (145)	0.858	33	308 (153)	249 (102)	**0.029**	0.948	0.268
Fiber (g/day)	48	14 (5)	14 (5)	0.641	33	14 (9)	16 (6)	0.281	0.720	0.400
Calcium (mg/day)	48	661 (217)	665 (201)	0.920	33	639 (203)	659 (229)	0.610	0.948	0.898
Magnesium (mg/day)	48	234 (57)	234 (58)	0.963	33	233 (121)	248 (73)	0.454	0.647	0.299
Sodium (mg/day)	48	2227 (1003)	2022 (756)	0.199	33	2191 (764)	2248 (776)	0.688	0.948	0.068
Potassium (mg/day)	48	2352 (480)	2430 (653)	0.442	33	2292 (795)	2540 (644)	0.090	0.718	0.107
Vegetables (g/day)	48	76 (58)	93 (77)	0.192	33	111 (107)	140 (85)	0.171	0.090	0.363
Fruits (g/day)	48	149 (126)	240 (168)	**0.001**	33	138 (155)	189 (166)	**0.027**	0.727	0.420
Dairy products (g/day)	48	333 (156)	336 (134)	0.910	33	306 (162)	339 (152)	0.239	0.452	0.658
Low-fat/skimmed dairy (g/day)	48	109 (144)	179 (157)	**0.001**	33	93 (138)	174 (174)	**0.014**	0.622	0.672

Cereals (g/day)	48	166 (72)	160 (61)	0.577	33	145 (57)	175 (71)	0.070	0.144	0.028
Whole cereals (g/day)	48	12 (23)	19 (32)	0.177	33	4 (11)	7 (15)	0.460	0.055	0.413
Nuts and legumes (g/day)	48	16 (21)	14 (21)	0.743	33	13 (17)	16 (19)	0.493	0.605	0.742
Fish and seafood (g/day)	48	41 (53)	41 (47)	0.958	33	34 (48)	30 (28)	0.739	0.535	0.976
Meat and meat products (g/day)	48	104 (88)	81 (64)	0.162	33	93 (62)	89 (57)	0.916	0.519	0.084
Sugar-sweetened beverages(g/day)	48	85 (146)	47 (81)	0.135	33	63 (92)	37 (101)	0.282	0.412	0.313
Adherence to dietary patterns										
KIDMED score (0–12)	45	5.7 (1.9)	8.1 (1.9)	**<0.001**	25	5.4 (2.1)	7.7 (2.0)	**<0.001**	0.552	0.652
DASH score (0–9)	48	1.3 (0.9)	1.6 (1.3)	0.182	33	1.1 (1.0)	1.9 (1.1)	**<0.001**	0.320	**0.048**
HDI score (0–7)	48	1.6 (1.1)	1.6 (1.1)	1.000	33	1.8 (1.2)	1.8 (0.9)	0.891	0.531	0.949
Breakfast habits										
Skipping breakfast (N, %)	48	0, 0	1, 2.1	0.100	33	1, 3	2, 6.1	0.100	0.407	0.652
BQI score (0–10)	48	3.9 (1.0)	5.2 (1.6)	**<0.001**	33	3.7 (0.9)	4.3 (1.2)	**0.025**	0.269	0.207
Meal frequency and daily energy distribution										
Having ≥ 4 meals/day (N, %)	48	45, 93.8	44, 91.7	0.100	33	28, 84.8	32, 97.0	0.125	0.173	0.154
Evening/morning energy intake ratio	48	0.73 (0.29)	0.61 (0.24)	**0.014**	33	0.71 (0.28)	0.69 (0.22)	0.749	0.796	0.322

KIDMED, Mediterranean Diet Quality Index for children and teenagers; DASH, Dietary Approaches to Stop Hypertension ; HDI, Healthy Diet Indicator; BQI, Breakfast Quality Index. * = *p* value refers to differences between Pre- vs. Post-intervention values for either control or exercise group analyzed by Paired-samples *t* test (continues variables) and McNemar test (categorical variables). ** = *p* value refers to differences between baseline values between control and exercise group analyzed by Independent-samples *t* test (continues variables) and chi-square test (categorical variables). *** = *p* value refers to differences in changes (Post-Pre-intervention values) between the control and the exercise groups analyzed by Univariate Linear Models adjusting with sex, age and changes in energy intake as covariates; and chi square test by using pre-post changes (categorical variables).

Association between Dietary Improvements and Changes in Hepatic Fat and Adiposity Markers

Associations of changes in dietary intake, particularly focused on those items related to the main nutritional goals of the program, and dietary patterns with hepatic fat and adiposity markers are shown in **Table 3** and separately by intervention group in **Table S2**. Changes in SSB consumption was significantly associated with changes in hepatic fat content (p < 0.05), but not with changes in FMI or abdominal fat. When the results were examined by intervention group, it was observed that the association between changes in SSB consumption and changes in percent hepatic remained statistically significant only in the control group (p < 0.05), as shown in **Table S2**. Moreover, changes in energy intake were significantly associated with changes in FMI only in the exercise group (p < 0.05, Table S2). No other significant associations were found between dietary improvements and changes in percent hepatic fat or adiposity markers. In addition, Figure S1 provides data on children's growth over the study time.

Table 3. Associations of changes in dietary intake with changes in percentage hepatic fat and adiposity markers among children with overweight/obesity.

	Δ FMI (kg/m^2)		**Δ Abdominal Fat (kg)**		**Δ Hepatic Fat (%)**	
	r	***p***	**r**	***p***	**r**	***p***
Main nutritional goals *						
Δ Energy intake (kcal/day) **	0.086	0.466	0.008	0.946	0.136	0.245
Δ Fat intake (g/day)	0.056	0.665	0.064	0.619	0.233	0.066
Δ Simple sugar (g/day)	−0.087	0.496	−0.100	0.438	0.013	0.919
Δ Fruits and vegetables (g/day)	0.136	0.288	−0.007	0.956	−0.045	0.729
Δ SSB consumption (g/day)	−0.017	0.897	−0.083	0.516	0.266	**0.035**
Δ Meal frequency (times/day)	−0.105	0.414	−0.081	0.528	−0.097	0.451
Dietary patterns						
Δ KIDMED score	0.004	0.976	−0.080	0.535	0.191	0.134
Δ DASH score	−0.166	0.194	−0.113	0.376	−0.137	0.285
Δ HDI score	−0.096	0.453	−0.043	0.740	0.157	0.220
Δ BQI score	0.004	0.976	0.022	0.864	−0.062	0.628

KIDMED, Mediterranean Diet Quality Index for children and teenagers; DASH, Dietary Approaches to Stop Hypertension; HDI, Healthy Diet Indicator; BQI, Breakfast Quality Index. Analyses were adjusted for sex, age and changes in height and energy intake. Δ means changes calculated as post-value subtracted by pre-value (Δ = post-pre). *Main nutritional goals of the family-based lifestyle education program. **Adjusted for sex, age and changes in height.

DISCUSSION

The current study showed interesting findings regarding the effect of a 22-week family-based healthy lifestyle intervention program on changes in dietary habits and adiposity markers in children with overweight/obesity. Firstly, our intervention program was effective on reducing the intakes of energy, carbohydrate, total fat and simple and added sugar intake, as well as increasing the consumption of fruits and low-fat/skimmed dairy in children with overweight/obesity. Consequently, the adherence to the Mediterranean and DASH dietary patterns improved, together with an enhancement in the breakfast quality and daily energy distribution after participating in the program. Secondly, changes in SSB consumption were associated with hepatic fat reductions, even in the absence of exercise training.

Overall, children had poor dietary habits, with only 16% of children showing optimal Mediterranean diet adherence (KIDMED score ≥8 points) at baseline. Della Corte et al. [27] reported similar results in children and adolescents with obesity; only 14.8% of participants had an optimal KIDMED score. Concerning macronutrients, the average fat percentage intake in our study sample (41%) exceeded the one established for the Mediterranean diet (30%–35%). The intake of percent added sugar (11%) was also above WHO recommendations (<10% of total energy intake, with additional health benefits if <5%) [28].

After the program, children improved their dietary habits. One of the main goals of the program was successfully achieved, as participants had lower intakes of simple and added sugar after the intervention. Moreover, total fat intake decreased among participants after the intervention, while protein intake barely changed. Due to lower carbohydrate and fat intake, despite not being the main goal of the program, the intervention induced an average reduction of ~200 kilocalories/day after the study, which might be due to healthier and less caloric dietary choices. Similarly, Reinehr et al. [29] reported reductions in energy, fat and sugar intake after a 6-month intervention based on nutritional education, behavior counselling and physical activity in 8–16 years-old overweight children.

Regarding food groups, the aim of promoting F+V consumption was partially accomplished. F+V consumption was increased in 52% and 24%, respectively, at the end of the intervention, although the latter was not significant. F+V consumption is highly recommended for all age-groups, not only because of their low caloric content, but also due to their fiber and micronutrients supply; therefore, their intake is essential for obesity prevention and treatment [30,31]. Despite the increase of vegetable consumption, participants still consumed fewer amounts than that which was recommended. In fact, after the participation in our program, the average intake of F+V among children was 332 g/day, not meeting the recommendations of the American Heart Association (>360 g/day) [32] or the WHO guidelines (>400 g/day) [33]. This is in line with a previous work conducted in the same region of our study (Basque Country) among 4–18 years-olds [34]. We included cooking workshops in two educational sessions in order to increase kids' interest and motivation regarding healthy food preparation and to make them feel involved throughout the whole process. Nonetheless, it might be interesting to additionally include F+V tasting workshops in these type of programs, since they have been to succeed in enhancing youth's interest in healthy eating [35,36].

With respect to dairy intake, low-fat/skimmed dairy consumption increased by 73.5% after the intervention, as participants tended to choose milk/yoghurts with less fat content than they used to. Nevertheless, participants still did not meet the recommendations for dairy products (500 g/day) [37]. Although these products are an appropriate source of calcium, this mineral intake was below European Food Safety Authority recommendation [38] (800–1150 mg/day for 4–17 years-olds), even after the program.

SSB consumption was not significantly changed after the program, even though the 43% reduction in its consumption should be highlighted. Another 10-week intervention in 4–10 years old children based on family meals did not find intervention effects on SSB consumption [39]. Likewise, Haerens et al. [40] found no positive effects on the consumption of SSB in 11–15 years olds after a school-based program. In contrast, another combined school/community-based water campaign intervention was successful in reducing the average SSB consumption in children [41].

The adherence to Mediterranean and DASH dietary patterns was improved after the lifestyle program. Our findings are in accordance with another study [42], reporting a 42% increase in the aforementioned score in children with abdominal obesity after 8-weeks; nonetheless, this intervention included an established energy-restricted diet. As a result of the increase in F+V and low-fat dairy consumption, the intake of micronutrients included in the DASH score, such as potassium and calcium, increased, and in turn the DASH score also did. As far as we are aware, there is no other study examining changes in the adherence to DASH and HDI dietary patterns in obese children.

Skipping breakfast was less common after the program, as its frequency reduced by 62.5%. Moreover, the greater BQI score observed after the program showed an improvement in the quality of the breakfast. Despite no changes in meal frequency, participants decreased the EMEratio, showing that the energy intake in the morning was higher compared to the afternoon after the intervention. According to Aljuraiban et al. [38], British and American adults with lower EMEratio and more eating frequents showed lower BMI and higher nutrient density, whereas those who ate less frequently and mostly in the evening showed lower intakes of F+V and lower nutrient density and higher alcohol consumption.

Concerning intervention effects on dietary habits between groups, children in the exercise group increased their cereal intake significantly, as well as their DASH score, compared to their peers in the control group. In this line, Manz et al. [43] reported a positive association of physical exercise and recommended daily physical activity with cereal intake among young boys, which suggest that nutritional adaptation as changes in food choices may occur as a response to physical activity [44]. According to the literature [45], a 15-week exercise training could help young adults pursuing healthier dietary preferences, as well as regulating their food intake in young adults. This could explain the between groups difference in DASH score, in which participants in the exercise group improved significantly more their diet quality according to DASH guidelines.

Our results pointed out a positive association between changes in energy intake and changes in FMI in the exercise group. In accordance with this, Steinsbekk et al. [46] reported that changes in energy intake from baseline to six months predicted a decrease in body fat in children with obesity. The exclusive association observed between the reduction of energy intake and the decrease in FMI in the exercise group may be explained by the additional energy expenditure due to exercise training, which would affect both energy balance and adiposity [47,48].

Changes in SSB consumption were associated with changes in percent hepatic fat. This finding is in line with our previous work [49], reporting a positive association between SSB consumption and percent hepatic fat in children with overweight/obesity, which in turn supports the evidence linking sugary drinks and liver fat [50,51]. Physical activity exerts an important effect on reducing liver fat [19], which may explain the lack of association between changes in SSB and percentage hepatic fat in the exercise group, by covering somehow the effect of SSB. However, even with the absence of exercise, the reduction of SSB consumption helps to reduce hepatic fat content. A recent systematic review confirmed the link between SSB consumption and weight gain in both children and adults [52]. Notwithstanding the number of lifestyle interventions in children with obesity, to the best of our knowledge, there are no studies targeting SSB reduction for the treatment of NAFLD in children. Findings from another study [53] suggested that a conventional low-fat or low-glycemic load diet could produce substantial decreases in liver fat and hepatocellular injury within six months in obese children.

STRENGTHS AND LIMITATIONS

This study has some strengths and limitations. The sample size could be considered as a study limitation, since it was lower than the original sample due to some missing data, especially in the post-intervention. However, our well characterized and homogeneous sample with a narrow range of age should be considered as study strength. Last but not least, the use of magnetic resonance imaging for hepatic fat determination and the study design strengthen these findings.

CONCLUSIONS

In conclusion, a 22-week family-based healthy lifestyle program mostly focused on nutritional education and accompanied by psychoeducation seemed to be effective in improving dietary habits in children with overweight/obesity by reducing energy intake; total carbohydrate, simple and added sugar intake; and total fat intake on the one hand, and by increasing the consumption of fruits and low-fat/skimmed dairy on the other. Furthermore, these changes resulted in improvements in the adherence to the Mediterranean and DASH dietary patterns, as well as in the breakfast quality and daily energy distribution after the program. The association observed between SSB consumption and hepatic fat reduction in participants who did not additionally attend the exercise program support the detrimental effects of sugary drinks on cardiometabolic health. Hence, interventions targeting healthy dietary and lifestyle habits with a special focus on the SSB avoidance and physical activity should be promoted to prevent fatty liver and cardiometabolic disorders in children with overweight/obesity.

Author Contributions: I.L. conceived and designed the study; L.A. and I.L. drafted the manuscript; L.A., M.M., M.O., M.A. and I.L. collected the data and L.A., M.M., M.O., M.A., I.D., B.R-V. and I.L. were involved in the interpretation of the results. All the authors critically revised the manuscript for important intellectual content and approved the final version of the submitted manuscript.

Funding: The current project was supported by the Spanish Ministry of Industry and Competitiveness (DEP2016-78377-R), by European Regional Development Funds (ERDF) and by the University of the Basque Country (GIU14/21). This work was also supported by grants from Spanish Ministry of Economy and Competitiveness (RYC 2010-05957; RYC-2011-09011; BES-2017-080770), by the Education Department of the Government of the Basque Country (PRE_2016_1_0057, PRE_2017_2_0224, PRE_2018_2_0057, PRE_2019_2_0004) and by the University of the Basque Country (PIF17/186). This study has been partially funded by the Junta de Andalucía, Consejería de Conocimiento, Investigación y Universidades and by ERDF, (ref. SOMM17/6107/UGR).

Acknowledgments: Firstly, we acknowledge all the children and their parents or legal guardians for participating in the study. We also thank the pediatricians for their collaboration in recruiting participants and to the pre- and post-graduate students for their collaboration in both data collection and intervention program sessions.

Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Abarca-Gómez, L.; A Abdeen, Z.; Hamid, Z.A.; Abu-Rmeileh, N.M.; Acosta-Cazares, B.; Acuin, C.; Adams, R.J.; Aekplakorn, W.; Afsana, K.; Aguilar-Salinas, C.A; 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, 2627–2642, doi:10.1016/s0140-6736(17)32129-3.
2. Llewellyn, A.; Simmonds, M.; Owen, C.G.; Woolacott, N. Childhood obesity as a predictor of morbidity in adulthood: A systematic review and meta-analysis. Obes. Rev. 2015, 17, 56–67, doi:10.1111/obr.12316.
3. Weiss, R.; Dziura, J.; Burgert, T.S.; Tamborlane, W.V.; Taksali, S.E.; Yeckel, C.W.; Allen, K.; Lopes, M.; Savoye, M.; Morrison, J.; et al. Obesity and the Metabolic Syndrome in Children and Adolescents. N. Engl. J. Med. 2004, 350, 2362–2374.
4. Sánchez-Cruz, J.-J.; José-Juan, J.-M.; Fernández-Quesada, F.; María-José, S. Prevalencia de obesidad infantil y juvenil en España en 2012. Revista Española de Cardiología 2013, 66, 371–376, doi:10.1016/j.recesp.2012.10.016.
5. Aranceta-Bartrina, J.; Pérez-Rodrigo, C.; Serra-Majem, L.; Bellido, D.; De La Torre, M.L.; Formiguera, X.; Moreno, B. Prevention of overweight and obesity: A Spanish approach. Public Health Nutr. 2007, 10, 1187–1193, doi:10.1017/s1368980007000699.
6. Ko, Y.-H.; Wong, T.-C.; Hsu, Y.-Y.; Kuo, K.-L.; Yang, S.-H. The Correlation Between Body Fat, Visceral Fat, and Nonalcoholic Fatty Liver Disease. Metab. Syndr. Relat. Disord. 2017, 15, 304–311, doi:10.1089/met.2017.0001.
7. Fang, Y.-L.; Chen, H.; Wang, C.-L.; Liang, L. Pathogenesis of non-alcoholic fatty liver disease in children and adolescence: From “two hit theory” to “multiple hit model.” World J. Gastroenterol. 2018, 24, 2974–2983, doi:10.3748/wjg.v24.i27.2974.
8. Alkhater, S.A. Paediatric non-alcoholic fatty liver disease: An overview. Obes. Rev. 2015, 16, 393–405, doi:10.1111/obr.12271.
9. Veum, V.L.; Laupsa-borge, J.; Eng, Ø.; Rostrup, E.; Larsen, T.H.; Nordrehaug, J.E. Visceral adiposity and metabolic syndrome after very high–fat and low-fat isocaloric diets: A randomized controlled trial. Am. J. Clin. Nutr. 2017, 105, 85–99.
10. Burchett, H.E.D.; Sutcliffe, K.; Melendez-Torres, G.; Rees, R.; Thomas, J. Lifestyle weight management programmes for children: A systematic review using Qualitative Comparative Analysis to identify critical pathways to effectiveness. Prev. Med. 2018, 106, 1–12, doi:10.1016/j.ypmed.2017.08.025.
11. Robertson, W.; Fleming, J.; Kamal, A.; Hamborg, T.; A Khan, K.; Griffiths, F.; Stewart-Brown, S.; Stallard, N.; Petrou, S.; Simkiss, D.; et al. Randomised controlled trial evaluating the effectiveness and cost-effectiveness of ‘Families for Health’, a family-based childhood obesity treatment intervention delivered in a community setting for ages 6 to 11 years. Health Technol. Assess. 2017, 21, 1–180, doi:10.3310/hta21010.
12. Golan, M. Parents as agents of change in childhood obesity practice. Int. J. Pediatr. Obes. 2006, 1, 66–76.
13. Hens, W.; Vissers, D.; Hansen, D.; Peeters, S.; Gielen, J.; Van Gaal, L.; Taeymans, J. 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, doi:10.1111/obr.12577.

14.Vilar-Gomez, E.; Martinez-Perez, Y.; Bertot, L.C.; Torres-Gonzalez, A.; Gra-Oramas, B.; Gonzalez-Fabian, L.; Friedman, S.L.; Diago, M.; Gómez, M.R. Weight Loss Through Lifestyle Modification Significantly Reduces Features of Nonalcoholic Steatohepatitis. Gastroenterology 2015, 149, 367–378.e5, doi:10.1053/j.gastro.2015.04.005.
15.Hens, W.; Taeymans, 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, doi:10.1123/jpah.2015-0560.
16.Medrano, M.; Cadenas-Sanchez, C.; Álvarez-Bueno, C.; Cavero-Redondo, I.; Ruiz, J.R.; Ortega, F.B.; Labayen, I. Evidence-Based Exercise Recommendations to Reduce Hepatic Fat Content in Youth- a Systematic Review and Meta-Analysis. Prog. Cardiovasc. Dis. 2018, 61, 222–231, doi:10.1016/j.pcad.2018.01.013.
17.Brown, M.L.; Wilfley, D.E. Overweight and Obesity: Implications for Treatment. Curr. Obes. Rep. 2018, 7, 235–246.
18.Medrano, M.; Maiz, E.; Maldonado-Martín, S.; Arenaza, L.; Rodríguez-Vigil, B.; Ortega, F.B.; Ruiz, J.; Larrarte, E.; López, I.D.; Sarasúa-Miranda, A.; 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, 346–355, doi:10.1016/j.cct.2015.09.017.
19.Labayen, I.; Medrano, M.; Arenaza, L.; Maíz, E.; Osés, M.; Martinez-Vizcaino, V.; Ruiz, J.R.; Ortega, F.B. Effects of Exercise in Addition to a Family-Based Lifestyle Intervention Program on Hepatic Fat in Children with Overweight. Diabetes Care 2019, 43, 306–313, doi:10.2337/dc19-0351.
20.Cole, T.; Lobstein, T. Extended international (IOTF) body mass index cut-offs for thinnes, overweight and obesity. Pediatr Obes. 2012, 7, 284–294.
21.Cabrera, S.G.; Fernández, N.H.; Hernández, C.R.; Nissensohn, M.; Roman-Viñas, B.; Serra-Majem, L. kidmed test; prevalence of low adherence to the mediterranean diet in children and young; a systematic review. Nutrición Hospitalaria 2015, 32, 2390–2399.
22.Cohen, J.F.; Lehnerd, M.E.; Houser, R.F.; Rimm, E.B. Dietary Approaches to Stop Hypertension Diet, Weight Status, and Blood Pressure among Children and Adolescents: National Health and Nutrition Examination Surveys 2003-2012. J. Acad. Nutr. Diet. 2017, 117, 1437–1444.e2, doi:10.1016/j.jand.2017.03.026.
23.A Struijk, E.; May, A.M.; Wezenbeek, N.L.; Fransen, H.P.; Soedamah-Muthu, S.S.; Geelen, A.; Boer, J.M.; Van Der Schouw, Y.T.; Bueno-De-Mesquita, H.B.; Beulens, J.W. Adherence to dietary guidelines and cardiovascular disease risk in the EPIC-NL cohort. Int. J. Cardiol. 2014, 176, 354–359, doi:10.1016/j.ijcard.2014.07.017.
24.Arenaza, L.; Muñoz-Hernández, V.; Medrano, M.; Oses, M.; Amasene, M.; Merchán-Ramírez, E.; Cadenas-Sanchez, C.; Ortega, F.B.; Ruiz, J.R.; Labayen, I. Association of Breakfast Quality and Energy Density with Cardiometabolic Risk Factors in Overweight/Obese Children: Role of Physical Activity. Nutrients 2018, 10, 1066.
25.House, B.T.; E Shearrer, G.; Miller, S.J.; E Pasch, K.; I Goran, M.; Davis, J.N. Increased eating frequency linked to decreased obesity and improved metabolic outcomes. Int. J. Obes. 2014, 39, 136–141, doi:10.1038/ijo.2014.81.
26.Aljuraiban, G.S.; Chan, Q.; Griep, L.M.O.; Brown, I.J.; Daviglus, M.L.; Stamler, J.; Van Horn, L.; Elliott, P.; Frost, G.; INTERMAP Research Group. The impact of eating frequency and time of

intake on nutrient quality and Body Mass Index: The INTERMAP Study, a Population-Based Study. J. Acad. Nutr. Diet. 2015, 115, 528–536.e1, doi:10.1016/j.jand.2014.11.017.
27.Della Corte, C.; Mosca, A.; Vania, A.; Alterio, A.; Iasevoli, S.; Nobili, V. Good adherence to the Mediterranean diet reduces the risk for NASH and diabetes in pediatric patients with obesity: The results of an Italian Study. Nutrients 2017, 39, 8–14, doi:10.1016/j.nut.2017.02.008.
28.Comments to the draft 'WHO-Guideline: Sugars intake for adults and children'. Sugar Ind. 2014, 48, 311–316, doi:10.36961/si15532.
29.Reinehr, T.; Schaefer, A.; Winkel, K.; Finne, E.; Kolip, P. Long-Term Findings from a Randomized Controlled Trial: An Effective Lifestyle Intervention in Overweight Children ("Obeldicks Light"). Posters XV 2010, 29, P2–708, doi:10.1210/endo-meetings.2010.part2.p15.p2-708.
30.Mytton, O.T.; Nnoaham, K.; Eyles, H.; Scarborough, P.; Ni Mhurchu, C. Systematic review and meta-analysis of the effect of increased vegetable and fruit consumption on body weight and energy intake. BMC Public Health 2014, 14, 886, doi:10.1186/1471-2458-14-886.
31.Puchau, B.; Ochoa, M.C.; Zulet, M.A.; Marti, A.; Martinez, J.A.; Members, G. Dietary total antioxidant capacity and obesity in children and adolescents. Int. J. Food Sci. Nutr. 2010, 61, 713–721, doi:10.3109/09637481003757860.
32.Lloyd-Jones, D.; Hong, Y.; Labarthe, D.; Mozaffarian, D.; Appel, L.J.; Van Horn, L.; Greenlund, K.; Daniels, S.; Nichol, G.; Tomaselli, G.F.; et al. Defining and Setting National Goals for Cardiovascular Health Promotion and Disease Reduction. Circulation 2010, 121, 586–613, doi:10.1161/circulationaha.109.192703.
33.World Health Organization. Diet, nutrition and the prevention of chronic diseases: Report of a Joint WHO/FAO Expert Consultation. Available online: https://apps.who.int/iris/bitstream/handle/10665/42665/WHO_TRS_916.pdf;jsessionid=7CF9C1534F0D663F22AB3E602BDBA8F1?sequence=1 (accessed on 28th January 2020).
34.Departamento de Salud del Gobierno Vasco. Encuesta de Nutrición 2005: Hábitos alimentarios y estado de salud de la población vasca de 4 a 18 años; Servicio Central de Publicaciones del Gobierno Vasco. Vitoria-Gasteiz 2006.
35.Gold, A.; Larson, M.; Tucker, J.M.; Strang, M. Classroom Nutrition Education Combined with Fruit and Vegetable Taste Testing Improves Children's Dietary Intake. J. Sch. Health 2017, 87, 106–113, doi:10.1111/josh.12478.
36.Cheng, F.W.; Monnat, S.M.; Lohse, B. Middle school-aged child enjoyment of food tastings predicted interest in nutrition education on osteoporosis prevention. J. Sch. Health 2015, 85, 467–476, doi:10.1111/josh.12268.
37.Estrategia NAOS. Cuidar la Cantidad: Las Raciones; Programa Perseo; Estrateg Naos. Agencia Española de Seguridad Alimentaria y Nutrición, Gobierno de España. Madrid, 2006.
38.Dietary Reference Values for nutrients Summary report. EFSA Support. Public 2017, 14, doi:10.2903/sp.efsa.2017.e15121.
39.Gunther, C.; Rogers, C.; Holloman, C.; Hopkins, L.C.; Anderson, S.E.; Miller, C.K.; Copeland, K.A.; Dollahite, J.S.; Pratt, K.J.; Webster, A.; et al. Child diet and health outcomes of the simple suppers program: A 10-week, 2-group quasi-experimental family meals trial. BMC Public Health 2019, 19, 1657, doi:10.1186/s12889-019-7930-7.

40.Haerens, L.; De Bourdeaudhuij, I.; Maes, L.; Vereecken, C.; Brug, J.; Deforche, B. The effects of a middle-school healthy eating intervention on adolescents' fat and fruit intake and soft drinks consumption. Public Health Nutr. 2007, 10, 443–449, doi:10.1017/s1368980007219652.
41.Van De Gaar, V.M.; Jansen, W.; Van Grieken, A.; Borsboom, G.J.J.M.; Kremers, S.P.J.; Raat, H. Effects of an intervention aimed at reducing the intake of sugar-sweetened beverages in primary school children: A controlled trial. Int. J. Behav. Nutr. Phys. Act. 2014, 11, 98, doi:10.1186/s12966-014-0098-8.
42.Ojeda-Rodríguez, A.; Zazpe, I.; Morell-Azanza, L.; Chueca, M.; Campoy, C.; Marti, A. Improved Diet Quality and Nutrient Adequacy in Children and Adolescents with Abdominal Obesity after a Lifestyle Intervention. Nutrients 2018, 10, 1500, doi:10.3390/nu10101500.
43.Manz, K.; Mensink, G.B.M.; Finger, J.D.; Haftenberger, M.; Brettschneider, A.-K.; Barbosa, C.L.; Krug, S.; Schienkiewitz, A. Associations between Physical Activity and Food Intake among Children and Adolescents: Results of KiGGS Wave 2. Nutrients 2019, 11, 1060, doi:10.3390/nu11051060.
44.King, N.; Horner, K.; Hills, A.P.; Byrne, N.M.; Wood, R.; Bryant, E.; Caudwell, P.; Finlayson, G.; Gibbons, C.; Hopkins, M.; et al. Exercise, appetite and weight management: Understanding the compensatory responses in eating behaviour and how they contribute to variability in exercise-induced weight loss. Br. J. Sports Med. 2011, 46, 315–322, doi:10.1136/bjsm.2010.082495.
45.Joo, J.; Williamson, S.A.; Vazquez, A.I.; Fernandez, J.R.; Bray, M.S. The Influence of 15-week Exercise Training on Dietary Patterns among Young Adults. Int. J. Obes. 2019, 43, 1681–1690.
46.Steinsbekk, S.; Wichstrøm, L.; Ødegård, R.; Mehus, I. Change in Body Fat during a Family-Based Treatment of Obesity in Children: The Relative Importance of Energy Intake and Physical Activity. Obes. Facts 2012, 5, 515–526, doi:10.1159/000341744.
47.Fulton, J.E.; Dai, S.; Steffen, L.M.; Grunbaum, J.A.; Shah, S.M.; Labarthe, D.R. Physical activity, energy intake, sedentary behavior, and adiposity in youth. Am. J. Prev. Med. 2009, 37, S40–S49, doi:10.1016/j.amepre.2009.04.010.
48.Das, S.K.; Roberts, S.B.; Bhapkar, M.V.; Villareal, D.T.; Fontana, L.; Martin, C.K.; Racette, S.; Fuss, P.; Kraus, W.E.; Wong, W.; et al. Body-composition changes in the Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE)-2 study: A 2-y randomized controlled trial of calorie restriction in nonobese humans. Am. J. Clin. Nutr. 2017, 105, 913–927, doi:10.3945/ajcn.116.137232.
49.Arenaza, L.; Medrano, M.; Oses, M.; Huybrechts, I.; Díez, I.; Henriksson, H.Dietary determinants of hepatic fat content and insulin resistance in overweight / obese children: A cross-sectional analysis of the Prevention of Diabetes in Kids (PREDIKID) study. Br. J. Nutr. 2019, 121, 1158–1165.
50.Assy, N.N.; Nasser, G.; Kamayse, I.; Nseir, W.; Beniashvili, Z.; Djibre, A.; Grosovski, M. Soft drink consumption linked with fatty liver in the absence of traditional risk factors. Can. J. Gastroenterol. 2008, 22, 811–816, doi:10.1155/2008/810961.
51.Abid, A.; Taha, O.; Nseir, W.; Farah, R.; Grosovski, M.; Assy, N. Soft drink consumption is associated with fatty liver disease independent of metabolic syndrome q. J. Hepatol. 2009, 51, 918–924.
52.Kajons, N.; David, M.; Gowland-Ella, J.; Lewis, P.; Batchelor, S. Thirsty? Choose Water! Behavioural interventions and water stations in secondary schools a two-by-two factorial

randomised controlled trial. BMC Public Health 2018, 18, 788, doi:10.1186/s12889-018-5685-1.
53.Ramon-Krauel, M.; Salsberg, S.L.; Ebbeling, C.B.; Voss, S.D.; Mulkern, R.V.; Apura, M.M.; Cooke, E.A.; Sarao, K.; Jonas, M.M.; Ludwig, D.S. A Low-Glycemic-Load versus Low-Fat Diet in the Treatment of Fatty Liver in Obese Children. Child Obes. 2013, 9, 252–260.

Table S1. Criteria for the dietary patterns calculation.

Dietary Approaches to Stope Hypertension (DASH)		
Nutrient	**Age 8 to 10 years**	**Age 11 to 13 years**
Total fat (%E)	≤27% of energy	≤27% of energy
Saturated fat (%E)	≤6% of energy	≤6% of energy
Protein (%E)	≥18% of energy	≥18% of energy
Cholesterol (mg)	≤150 mg	≤150 mg
Fiber (g)	≥25g	≥26 g girls; ≥31 g boys
Calcium (mg)	≥1000 mg	≥1300 mg
Magnesium (mg)	≥240 mg	≥ 240 mg
Potassium (mg)	≥3800 mg	≥4500 mg
Sodium (mg)	≤2300 mg	≤2300 mg
Healthy Diet Indicator (HDI)		
Components	**0 points**	**1 point**
Saturated fatty acids (%E)	≥10	<10
Polyunsaturated fatty acids (%E)	<6 or>10	6-10
Cholesterol (mg/day)	≥300	<300
Proteins (%E)	<10 or >15	10-15
Fiber (g/day)	<25	≥25
Fruits and vegetables (g/day)	<400	≥400
Simple sugars (%)	≥10	<10
Breakfast Quality Index (BQI)		
Components	**1 points**	**0 point**
Cereals and derivate	Bread, non-sugar rich breakfast cereals	Biscuits, pastries, sugar rich breakfast cereals
Fruits and vegetables	Fresh fruit, natural fruit juices, tomato	Artificial juices, jam
Dairy products	Whole or skimmed milk, yoghurt, cheese	Dairy desserts
Food rich in simple sugars	<5% of total daily energy from simple sugars	≥5% of total daily energy from simple sugars
MUFA-rich products	Olive oil added by the consumer	Olive oil from biscuits or other fats such as butter
MUFA/SFA ratio	≥ 2/1	< 2
Energy intake	20-25% of daily energy intake from breakfast	<20% or >25% of daily energy intake from breakfast
Fruits, cereals and dairy product	To include the 3 of the components	Not to be composed of three of the components
Calcium	≥ 200mg	<200mg
Absence of butter or margarine	Not to include butter or margarine in the breakfast	To include butter or margarine in the breakfast

%E, percentage from total energy intake; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids

Table S2. Associations of changes in dietary habits with percent hepatic fat and adiposity markers by intervention group.

	Control group						Exercise group					
	Δ FMI (kg/m^2)		Δ Abdominal fat (kg)		Δ Hepatic fat (%)		Δ FMI (kg/m^2)		Δ Abdominal fat (kg)		Δ Hepatic fat (%)	
	r	P	r	P	r	P	r	P	r	P	r	P
Main nutritional goals *												
Δ Energy intake (kcal/day)**	-0.001	0.997	0.071	0.652	0.001	0.993	0.380	**0.042**	0.059	0.759	0.265	0.165
Δ Fat intake (g/day)	-0.038	0.816	0.038	0.818	0.102	0.535	0.038	0.872	-0.028	0.908	0.336	0.148
Δ Simple sugar (g/day)	-0.087	0.597	-0.101	0.540	0.095	0.563	-0.022	0.925	-0.038	0.874	-0.092	0.700
Δ Fruits and vegetables (g/day)	0.209	0.202	0.036	0.826	0.028	0.866	0.016	0.948	-0.023	0.923	-0.232	0.325
Δ SSB consumption (g/day)	-0.058	0.726	-0.021	0.899	0.362	**0.024**	0.010	0.968	-0.351	0.129	0.100	0.675
Δ Meal frequency (times/day)	-0.155	0.346	-0.134	0.417	-0.102	0.536	0.075	0.753	0.079	0.740	-0.026	0.914
Dietary patterns												
Δ KIDMED score	0.171	0.299	0.109	0.507	0.283	0.081	-0.252	0.283	-0.375	0.103	0.110	0.645
Δ DASH score	-0.066	0.691	-0.051	0.760	0.016	0.922	-0.228	0.333	-0.191	0.420	-0.255	0.278
Δ HDI score	-0.137	0.406	-0.026	0.874	0.247	0.130	0.064	0.789	-0.018	0.940	0.155	0.514
Δ BQI score	-0.077	0.639	0.031	0.852	-0.046	0.780	-0.067	0.778	-0.020	0.935	-0.442	0.051

KIDMED, Mediterranean Diet Quality Index for children and teenagers; DASH, Dietary Approaches to Stop Hypertension; HDI, Healthy Diet Indicator; BQI, Breakfast Quality Index. Analyses were adjusted for sex, age and changes in height and energy intake. Δ means changes calculated as post-value subtracted by pre-value (Δ = post-pre). *Main nutritional goals of the family-based lifestyle education program. **Adjusted for sex, age and changes in height.

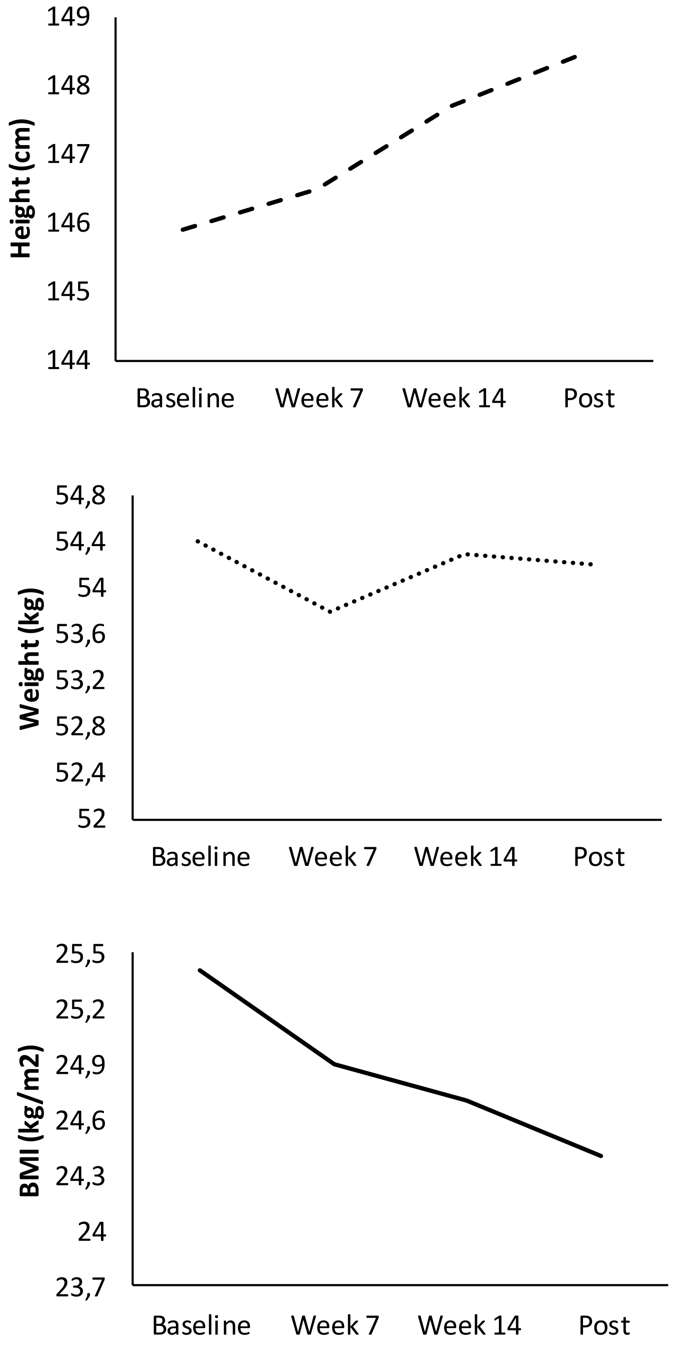


Figure S1. Children´s growth during the study. Weight, height and body mass index measurements at baseline, 7th and 14th weeks and at the end of the intervention (Post) in children participating in the study

4. GENERAL DISCUSSION

4.1. Adherence to the Mediterranean dietary pattern, adiposity and cardiometabolic health

The first two studies included in the current Thesis (Studies I and II) examined the influence of the MDP on adiposity and metabolic profile. More specifically, the Study I analysed the associations of the adherence to the MDP and CRF with total and central adiposity in pre-school children, whereas the Study II examined the relationship between the adherence to the MDP with healthy and unhealthy metabolic phenotypes in overweight/obese adolescents.

4.1.1 The influence of Mediterranean dietary pattern and cardiorespiratory fitness on total and central adiposity in pre-school children

To the best of our knowledge, the Study I is the first work examining the combined influence of the adherence to the MDP and CRF on total and central adiposity in preschool children. The main findings of this study were that 1) the adherence to the MDP among pre-school children was low to moderate, with only few children having an optimal adherence to this diet; 2) higher adherence to MDP was associated with lower values of waist circumference, whereas higher levels of CRF were associated with both lower BMI and waist circumference; and 3) children with both low adherence to the MDP and low CRF had higher waist circumference independently of age, sex, and sociodemographic factors.

In the Study I, the overall adherence to the MDP among 3-5 years-old pre-school children was 62.7%, by showing a higher adherence in girls (65%) than in boys (60%). Likewise, children were classified as having or not having optimal adherence to the MDP (≥ 80% of total MDP index), and it was observed that only less than a fourth part of children (24.3%) showed an optimal adherence to the MDP. Although the adherence to this dietary pattern was based on the KIDMED test, only those items exclusively related to Mediterranean dietary habits were considered in the total score, as items regarding breakfast habits or behaviours such as eating in fast-food restaurants are not so closely related to the MDP. This low percentage of children showing an optimal adherence to the MDP reflects that despite the fact that Spain is a Mediterranean region country, at least in the North of the country, traditional Mediterranean-based dietary habits are decreasing in early childhood. This might be of concern since findings from the current study suggest that MDP is associated with a lower waist circumference, and therefore, with a lower central adiposity, which is of high importance due to its relationship with lower CVD risk. These findings support the previous evidence on the beneficial effects of MDP on cardiovascular health (153).

We also observed that a higher CRF was associated with lower both total and central adiposity in pre-school children. These results are in line with previous findings reporting that pre-school children with excess adiposity showed lower CRF than their normal-weight peers (154). Similarly, a study conducted with 10-13 years-old children concluded that higher adiposity was associated with poorer CRF regardless of physical activity levels (155).

Although CRF is partially determined by genetics, it has been shown that regular physical activity helps enhancing it (119). Therefore, a healthy lifestyle including a diet based on Mediterranean dietary habits accompanied by regular physical activity might be helpful in maintaining a healthy body composition and preventing or avoiding excess total and abdominal adiposity in pre-school children.

4.1.2 Mediterranean dietary pattern and cardiometabolic health

The main finding of the Study II was that European adolescents with MHO phenotype showed higher adherence to the MDP than their peers with MUO phenotype. Actually, having a low adherence to the MDP increased by twice the likelihood of having obesity-related risk factors, which in turn, stands for having MUO phenotype. Despite the existing evidence on the effect of MDP adherence on cardiovascular health in paediatric population (131), as far as we are aware, this study is the first comparing the adherence to the MDP between MHO and MUO adolescents.

We found that one third of adolescents with overweight/obesity had a healthy metabolic phenotype, which means that they still did not develop obesity-related cardiovascular risk factors. The prevalence of MHO in adolescents varies depending on the criteria used to its definition. However, the prevalence of MHO among our participants seems to be close to the percentages reported in other studies (156). Regarding dietary habits of participants, we observed that MHO adolescents had around 7% higher adherence to the MDP compared to the MUO ones, supporting the beneficial effects of the MDP on metabolic health. Moreover, we observed that MHO adolescents had significantly higher (33%) intakes of fish than their MUO peers. This is in accordance with other studies reporting that fish intake is associated with lower triglyceride levels and blood pressure, as well as with lower risk of metabolic syndrome (131,157), which might be due to beneficial effects of polyunsaturated fatty acids present in fish (158–160).

Furthermore, the difference in the adherence to the MDP observed between MHO and MUO youths became non-significant after adjusting for CRF. Taking into consideration that CRF is a strong cardiovascular biomarker, this might be a key characteristic of MHO phenotype (161).

4.2. Breakfast habits and cardiometabolic health in children with overweight/obesity

The Study III examined the association of breakfast quality and energy density with cardiovascular disease risk factor among 8-12 years-old children with overweight/obesity from the North (Vitoria-Gasteiz) and South (Granada) of Spain. The main findings were the following: 1) breakfast quality, assessed by the BQI score, was negatively associated with serum uric acid concentrations; 2) higher BEDs was associated with greater levels of both total and HDL cholesterol, as well as with systolic blood pressure; and 3) BEDb was positively related to HOMA-IR in either active/inactive children with overweight/obesity.

Concerning breakfast habits, we observed that around 10% of children participating in the study used to skip breakfast. Skipping breakfast has been shown to be associated not only with obesity, but also with unhealthier lifestyle and poorer diet quality (162–164). In this line, Ramsay et al.(165) reported that 2-5 years-old children who skipped breakfast consumed nearly 40% of daily energy intake from snacks, leading to consume high amounts of added sugar.

We found an inverse association between BQI score and serum uric acid among children participating in the study. Considering that lower serum uric acid levels have been associated with lower CVD risk (65), this finding suggests that a high quality of breakfast may be beneficial for CVD prevention. Focusing on the items included in the BQI score, milk was one of them and it has been shown that the intake of milk proteins might decrease serum uric acid levels (166). Similarly, the association between BQI and serum uric acid could also be explained by dairy products, fibre-rich cereals and fruits, as all of them are items included in the BQI score and have been associated with lower serum uric acid levels (167).

Moreover, BEDs was associated with systolic blood pressure, as well as with total and HDL cholesterol. The relationship between BEDs and systolic blood pressure could be explained by the consumption of ultra-processed foods such as biscuits and pastries consumed at breakfast. Precisely, these foodstuffs are often high in sugar and salt, being both associated with increased blood pressure (168). According to a longitudinal study carried out in children aged 3-4 and 7-8 years, the consumption of ultra-processed foods in pre-school children may be predictors of increased levels of total cholesterol in school age children, which might explain the association between BEDs and total cholesterol (169). Unexpectedly, BEDs was also associated with HDL cholesterol, which is linked to a healthier cardiovascular profile. Notwithstanding, some children tended to consume bread toasts with virgin olive oil, and despite its liquid form, olive oil is not drinkable in the form it is consumed. Therefore, toasts plus virgin olive oil were considered as solids altogether. Since the consumption of virgin olive oil is associated with reduced risk of CVD and mortality, this fact can be explaining the positive association between BEDs and HDL cholesterol (170). Furthermore, BEDb was positively associated with HOMA-IR, which could be due to soluble cocoa added to milk or artificial juices consumed at breakfast, as these choices are sugar-rich, which in turn are associated with insulin resistance in children with overweight/obesity (142). This association was observed either in active or inactive children; suggesting that physical activity did not attenuate the detrimental effects of an unhealthy breakfast.

Nonetheless, to the best of our knowledge, this is the first study examining the association of breakfast quality and its energy density with CVD risk factors in children with overweight/obesity, which hampers comparisons. Indeed, although there are several studies focused on the effects of skipping breakfast on health and academic performance in children (171), the evidence on the influence of breakfast composition on CVD risk factors is scarce.

4.3. Dietary habits, hepatic steatosis and insulin resistance

The Study IV examined the influence of several food groups on hepatic fat content and insulin resistance among 8-12 years-old children with overweight/obesity. The main findings from the current study were that 1) cereal intake was negatively associated with hepatic fat content, but not with insulin resistance; 2) SSB) consumption, as well as their sugar content, were positively associated with percentage hepatic fat, but not with insulin resistance; and 3) dairy desserts and substitutes (DDS) were not associated neither with hepatic fat nor with insulin resistance in children with overweight/obesity.

We observed that 36.4% of our participants presented hepatic steatosis. A high prevalence of hepatic steatosis was expected since the study sample was composed of children with overweight/obesity. Precisely, excess adiposity increases the risk of fat deposition in the liver (72), and therefore, the prevalence of hepatic steatosis among patients with excess weight is greater compared to the general population. Thereby, children with hepatic steatosis were characterized by presenting central adiposity, as they had higher values of both waist circumference and abdominal adiposity. In contrast, we did not find differences of BMI between children with and without hepatic steatosis. This findings highlight the limitation of BMI on determining excessive adiposity, and it should be noted that abdominal adiposity might be a better predictor of hepatic steatosis than BMI (97).

Regarding dietary intake, we unexpectedly observed that cereal intake was negatively associated with hepatic fat content, which is not in accordance with findings from other studies (172). Albeit it would be of interest to examine the influence of whole cereals on hepatic fat, their intake among participants was quite low. In any case, fibre intake was not associated with percent hepatic fat. On the contrary, cereals were not associated with insulin resistance.

The most interesting findings of the current study were that SSB intake and sugar from SSB were positively associated with percent hepatic fat regardless of several confounders. This is in agreement with other studies reporting the detrimental effects of sugary drinks on liver and cardiovascular health (145,146). In our study, children with hepatic steatosis (117 ml/day of SSB consumption) consumed more than twice SSB than those without hepatic steatosis (53 ml/day of SSB consumption). Although SSB consumption has been associated with greater increase in insulin resistance in adults (173), we found no association between SSB or sugar from SSB and HOMA-IR. Given that insulin resistance might develop as a consequence of excessive hepatic fat accumulation, one explanation of this finding could be that HOMA-IR is not still altered in these children with overweight/obesity, as could be a later step in the pathogenesis of NAFLD. In contrast, there was no association of DDS with hepatic fat content or HOMA-IR in children participating in the study. One possible explanation might be that as fructose or sucrose containing SSB are liquid and do not contain fibre, the fructose from these beverages is rapidly absorbed into the portal vein and go directly to the liver (148,174). On the contrary, DDS are mostly solids and contain other nutrients such as calcium or dairy-fat, which are not present in SSB, that seems to have beneficial effects on liver and cardiovascular health (175,176).

4.4. The effect of a family-based lifestyle education program, including nutritional counselling, on dietary habits, hepatic fat and adiposity

The study V examined the effects of a 22-week family-based healthy lifestyle program on changes in dietary habits and adiposity markers in 8-12 years-old children with overweight/obesity. The main findings of the current study were that the family-based intervention program we carried out for 22 weeks was effective on 1) reducing energy, carbohydrate as well as total and simple added sugar intake, together with increasing the consumption of fruits and low-fat/skimmed dairy; and 2) improving the adherence to the Mediterranean and DASH dietary patterns, breakfast quality and energy distribution of the day in children with overweight/obesity. In addition, 3) changes in SSB consumption were associated with hepatic fat reduction, even in the absence of exercise training.

Overall, our results showed that children participating in the study had poor dietary habits. We observed that the adherence to healthy dietary patterns such as MDP, DASH or HDI at baseline was low among participants. For instance, less than one fifth part of children had an optimal adherence to the MDP. Furthermore, sugar and added sugar intake before the participation in the program was too high according to nutrition guidelines (142), which is of high concern due to its relationship with CVD risk.

However, and fortunately, dietary habits among children improved together with their participation in the program, and participants reduced their total and added sugar intake.

Besides the sugar intake, they also reduced the intakes of carbohydrates and fat; and as a consequence, participants consumed less energy per day at the end of the lifestyle program. Although the reduction of energy intake of the diet was not a specific aim of the nutritional intervention, by the end of the program most of children had learnt to make nutritionally more adequate dietary choices, and therefore, these healthier options seemed to be lower in energy.

The nutrition program was not only focused on promoting the consumption of fruits, vegetables and fibre-rich foods such as whole-grain bread/cereals and improving breakfast and snacks habits, but it was also on reducing artificially-added sugar-rich products. Before the intervention, most of kids tended to consume SSB, flavoured yoghurts, biscuits or candies every single day, and hence, their intake of sugar was too high by leading to weight gain. On the other hand, as some participants used to refuse vegetables before trying them, we organised a vegetable-based cooking workshop to make children veggies more attractive. We also wanted to offer families healthier snack alternatives in order to avoid the consumption of ultra-processed foods, so the program included another healthy snacks-workshop based on fruits, plain yoghurts and whole cereals. Thus, children learnt that by adding pieces of fruit to plain yoghurt they could obtain a sweet-flavour yoghurt but much healthier than the artificially-flavoured ones. They also tried adding oatmeal or nuts to yoghurts, made shakes and ice-creams with fruits and milk or designed healthy toasts with whole-grain bread. Interestingly, the consumption of fruit and low-fat/skimmed dairy increased at the end of the program; even though, children did not meet their recommendations yet (177–179). As a result of these dietary improvements, the adherence to the MDP and DASH diet was also increased. In addition, although not significantly, SSB consumption among participants reduced considerably after the program. It is of note that improvements of dietary habits of children depend a great deal on their parents awareness of health and diet, as parents are still the responsible for grocery shopping, meals planning, and overall, feeding their children. On the other hand, the distribution of the daily energy was improved since participants decreased the EME_{ratio} which according to the literature, seems to have benefits on adiposity (180). The lack of studies examining the adherence to the DASH or HDI diet in children after participating in a lifestyle program hampers comparisons.

We also observed that changes in SSB consumption were associated with changes in percent hepatic fat. This finding is in line with our previous study (181) in which SSB were positively associated with percent hepatic fat. As previously mentioned, the evidence regarding the detrimental effects of SSB consumption on liver health is strong (142,182). These negative effects of sugary drinks on liver are mostly attributed to the fast-absorbable fructose, as this monosaccharide increases *de novo* lipogenesis, TG production and fat accumulation in the liver, and increase blood TG and cholesterol levels (183–186). Importantly, the decrease in SSB consumption was associated with hepatic fat reduction, even in the absence of exercise training.

5. CONCLUSIONS AND CLINICAL HEALTH IMPLICATIONS/

ONDORIOAK ETA INPLIKAZIO KLINIKOAK

5.1. Conclusions

The conclusions of the current International Doctoral Thesis are the following:

I) High adherence to the MDP might prevent pre-school children from abdominal adiposity and later CVD, whereas high CRF seems to have benefits not only preventing abdominal adiposity, but also total adiposity in pre-school children. These findings highlight the importance of promoting a healthy lifestyle including MDP as well as physical activity since early childhood.

II) High adherence to the MDP and its components, such as the intake of fish, seem to have a protective role against adiposity-related CVD risk factors development in adolescents with overweight/obesity, leading to present a metabolically healthy obesity. Moreover, CRF may also play a key role in the healthy metabolic phenotype.

III) Breakfast with high quality and with low energy density from both solids and beverages might be beneficial to prevent CVD risk factors in children with overweight/obesity. Therefore, besides breakfast components and its quality, breakfast energy density should also be considered as an additional dimension in nutrition-based education programs for an early prevention of CVD risk factors.

IV) A diet moderate in cereals intake and low in SSB and their sugars content seems to be related to lower hepatic fat content in children with overweight/obesity. A 22-week family-based healthy lifestyle program mostly focused on nutritional education is effective on improving dietary habits and reducing SSB consumption in 8-12 years-old children with overweight/obesity. In addition, the reduction of SSB consumption was shown to be effective on reducing hepatic fat content in these children, by supporting the detrimental effects of sugary drinks on hepatic steatosis. Interventions in children with overweight/obesity should include specific strategies focused on the reduction and avoidance of SSB for the prevention and treatment of hepatic steatosis and its consequent CVD comorbidities.

5.2. Clinical health implications

The findings from the current International Doctoral Thesis regarding the influence of dietary habits on hepatic and cardiovascular health in children might have clinical implications in the design and development of future healthy lifestyle education programs focused on the prevention of obesity and its related comorbidities. Nutritional education programs should include contents targeting the promotion of MDP, the consumption of a healthy breakfast, a diet low in total and added sugars, and particularly, SSB avoidance for the prevention and treatment of obesity, hepatic steatosis and CVD risk factors in children.

SSB require special attention when focusing on sugars and health. The present work emphasizes the relation between the consumption of SSB and hepatic fat content by supporting the cumulative evidence regarding the detrimental effects of these sugary drinks on hepatic steatosis and cardiovascular health. However, the consumption of SSB, such as soft drinks and artificial fruit-juices, is common among children, supposing an important contribution of added sugar. Lifestyle programs aiming the reduction of hepatic fat content should set the goal of avoiding the consumption of SSB. Teaching children how to interpret nutritional labels and weighting the amount of sugar that these products contain could also help to think more critically by increasing the awareness of the total amount of sugar they consume.

Physical activity improves CRF and body composition, which are both related to a healthier metabolic phenotype. Based on this, it is of high importance to promote a physically active lifestyle and the practice of regular physical activity among children with overweight/obesity to prevent obesity-related comorbidities.

In summary, health policies targeting healthy lifestyle promotion and sugar-rich food and beverages advertising restriction might additionally help to prevent and tackle obesity, hepatic steatosis and CVD, together with increasing the well-being of people and reducing the public health costs.

5.1. Ondorioak

Nazioarteko Doktorego Tesi honen ondorioak ondorengoak dira:

I) PDM-kiko atxikidurak eskolaurreko haurretan gantz-pilaketa abdominala eta etorkizuneko GKB izatea prebeni lezakeen bitartean, bihotz-arnas gaitasunak gantz-pilaketa abdominala zein totala prebenitzeko onurak izan litzake. Aurkikuntza hauek PDM eta ariketa fisikoa barnebiltzen dituen txikitatik bizi-ohitura osasungarriak sustatzearen garrantzia azpimarratzen dute.

II) PDM-kiko atxikidurak eta arraina bezalako dietako elikagaiek adipositateari loturiko GKB-ren arrisku faktoreen garapenaren aurrean funtzio babesgarria izan dezateke gainpisua/obesitatea pairatzen duten nerabeetan, metabolikoki osasuntsua den obesitatea izatera daramatelarik. Era berean, bihotz-arnas gaitasunak rol garrantzitsua bete lezake metabolikoki osasuntsua den fenotipoan.

III) Kalitate altuko eta solido zein edari jatorriko dentsitate energetiko baxuko gosaria GKB-ren arrisku faktoreak prebenitzeko onuragarria izan liteke gainpisua/obesitatea pairatzen duten haurretan. Ondorioz, gosariaren osagaiak eta honakoaren kalitateaz haratago, gosariaren dentsitate energetikoa ere oinarri nutrizionaleko hezkuntza programetan dimentsio gehigarritzat hartu beharko litzateke GKB-ren arrisku faktoreen prebentzio goiztiarra bermatzeko.

IV) Zerealen neurrizko kontsumoa duen eta azukredun edarietan eta hauen azukre edukian urria den dieta bat gibeleko gantz-pilaketa txikiago batekin erlazionatzen da gainpisua/obesitatea duten haurretan. Hezkuntza nutrizionalean oinarritutako 22 astetako bizi-ohituren hezkuntza programa familiar bat elikadura-ohiturak hobetzeko eta azukredun edarien kontsumoa murrizteko eraginkorra izan daiteke gainpisua/obesitatea pairatzen duten 8-12 urte bitarteko haurretan. Horrez gain, azukredun edarien kontsumoaren murrizketa haur hauen gibeleko gantz edukia murrizteko eraginkorra dela behatu da, azukreetan aberatsak diren edariek esteatosi hepatikoan duten eragin kaltegarria azpimarratuz. Gainpisua/obesitatea pairatzen duten haurrei bideratutako esteatosi hepatikoaren eta GKB-ren prebentzioa helburu duten esku-hartze programek azukredun edarien kontsumoaren murrizketa eta ekiditean oinarritutako berariazko estrategiak barnebildu beharko lituzkete.

5.2. Inplikazio klinikoak

Haurren elikadura-ohiturek esteatosi hepatikoan eta osasun kardiobaskularrean duten eraginaren inguruko Nazioarteko Doktorego Tesi honen aurkikuntzek obesitatea eta ondoriozko erikortasunaren prebentzioa helburu duten etorkizuneko bizi-ohitura osasuntsuen hezkuntza programen diseinua eta garapenerako inplikazio klinikoak izan ditzake. Haurrei zuzendutako hezkuntza nutrizionaleko programek PDM, gosari osasungarri baten kontsumoa, azukre eta gehitutako azukreetan urria den, eta bereziki, azukredun edarien saihestea sustatzen duten edukiak barnebildu beharko lituzkete obesitatea, esteatosi hepatikoa eta GKB-ren arrisku faktoreen haurren prebentzio eta tratamendurako.

Azukreak eta osasunaren arteko arloan, azukredun edariek arreta berezia eskatzen dute. Lan honek azukredun edarien kontsumoak eta gibeleko gantz edukiaren arteko erlazioa azpimarratzen du, edari gozo hauek esteatosi hepatikoan eta osasun kardiobaskularrean duten eragin kaltegarriaren inguruko ebidentzia euskarri duelarik. Hala ere, edari freskagarriak eta fruta-zuku artifizialak bezalako azukredun edarien kontsumoa ohikoa da haurretan, gehitutako azukreen ekarpen garrantzitsu bat suposatuz. Gibeleko gantz edukia murriztea helburu duten bizi-ohituretan oinarritutako programek azukredun edariak saihestearen helburua jarri beharko lukete. Horrez gain, haurrei informazio nutrizionala biltzen duten etiketak interpretatzen eta produktuek duten azukre kantitatea pisatzen irakasteak, kontsumitzen duten azukre kantitatearen inguruko kontzientzia handitzen eta modu kritikoagoan pentsatzen lagunduko lieke.

Ariketa fisikoak bihotz-arnas gaitasuna eta gorputz-konposizioa hobetzen ditu, biak fenotipo metaboliko osasuntsuago batekin erlazionatzen direlarik. Honakoan oinarrituz, obesitateari egozten zaizkion erikortasuna prebenitzeko, fisikoki aktiboa den bizimodua eta modu ohikoan ariketa fisikoa burutzea sustatzeak garrantzi handia gainpisua/obesitatea duten haurretan.

Laburbilduz, bizi-ohitura osasungarriak eta azukreetan aberatsak diren elikagaien iragarkien murrizketak helburu dituzten osasun politikek obesitatea, esteatosi hepatikoa eta GKB prebenitu eta hauei aurre egiten lagun lezakete. Ekimen hauek pertsonen ongizatea areagotu ez ezik, osasun publikoaren gastua ere murriztuko lukete.

REFERENCES

1. Abarca-Gómez L, Abdeen ZA, Hamid ZA, Abu-Rmeileh NM, Acosta-Cazares B, Acuin C, 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):2627–42.

2. World Health Organization. Childhood overweight and obesity [Internet]. 2020. Available from: https://www.who.int/dietphysicalactivity/childhood/en/

3. Ahrens W, Pigeot I, Pohlabeln H, De Henauw S, Lissner L, Molnár D, et al. Prevalence of overweight and obesity in European children below the age of 10. Int J Obes. 2014;38:S99–107.

4. Sánchez-Cruz J-J, Jiménez-Moleón JJ, Fernández-Quesada F, Sánchez MJ. Prevalencia de obesidad infantil y juvenil en España en 2012. Rev Española Cardiol. 2013;66(5):371–6.

5. Bray GA, Kim KK, Wilding JPH. Obesity: a chronic relapsing progressive disease process. A position statement of the World Obesity Federation. Obes Rev. 2017;18(7):715–23.

6. Simmonds M, Llewellyn A, Owen CG, Woolacott N. Predicting adult obesity from childhood obesity: A systematic review and meta-analysis. Obes Rev. 2016;17(2):95–107.

7. Global Obesity Observatory. Presentation maps. World Obesity Federation.

8. World Health Organization. Obesity. Health topics.

9. Rankin J, Matthews L, Cobley S, Han A, Sanfers R, Wiltshire HD, et al. Psychological consequences of childhood obesity : psychiatric comorbidity and prevention. Adolesc Health Med Ther. 2016;7:125–46.

10. World Health Organization. Why does childhood overweight and obesity matter? Consequences of an unhealthy lifestyle during childhood.

11. Reis JP, Loria CM, Lewis CE, Powell- TM, Wei GS, Carr JJ, et al. Association Between Duration of Overall and Abdominal Obesity Beginning in Young Adulthood and Coronary Artery Calcification in Middle Age. JAMA. 2013;310(3):280–8.

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

13. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, et al. Nonalcoholic Fatty Liver Disease: A feature of the Metabolic Syndrome. Diabetes. 2001;50:1844–50.

14. Ayer J, Charakida M, Deanfield JE, Celermajer DS. Lifetime risk: Childhood obesity and cardiovascular risk. Eur Heart J. 2015;36(22):1371–6.

15. Goossens GH. The Metabolic Phenotype in Obesity: Fat Mass, Body Fat Distribution, and Adipose Tissue Function. Obes Facts. 2017;10(3):207–15.

16. Gishti O, Gaillard R, Durmus B, Abrahamse M, Van Der Beek EM, Hofman A, et al. BMI,

total and abdominal fat distribution, and cardiovascular risk factors in school-age children. Pediatr Res. 2015;77(5):710–8.

17. Iwashima S, Nakagawa Y, Ishikawa T, Satake SSE, Nagata E, Ohzeki T. Abdominal obesity is associated with cardiovascular risk in Japanese children and adolescents. J Pediatr Endocrinol Metab. 2011;24(1–2):51–4.

18. Lee SJ, Bacha F, Arslanian SA. Waist circumference, blood pressure, and lipid components of the metabolic syndrome. J Pediatr. 2006;149(6):809–16.

19. Zimmet P, Alberti G, Kaufman F, Tajima N, Silink M, Arslanian S, et al. The metabolic syndrome in children and adolescents. Lancet. 2007;369(9579):2059–61.

20. Matsushita Y, Nakagawa T, Yamamoto S, Takahashi Y, Yokoyama T, Noda M, et al. Associations of visceral and subcutaneous fat areas with the prevalence of metabolic risk factor clustering in 6,292 Japanese individuals: The Hitachi health study. Diabetes Care. 2010;33(9):2117–9.

21. Goran MI, Bergman RN, Gower BA. Influence of total vs. Visceral fat on insulin action and secretion in African American and white children. Obes Res. 2001;9(8):423–31.

22. Maffeis C, Morandi A. Body composition and insulin resistance in children. Eur J Clin Nutr [Internet]. 2018;72(9):1239–45. Available from: http://dx.doi.org/10.1038/s41430-018-0239-2

23. Chiarelli F, Marcovecchio ML. Insulin resistance and obesity in childhood. Eur J Endocrinol. 2008;159(SUPPL. 1):67–74.

24. Caprio S, Perry R, Kursawe R. Adolescent Obesity and Insulin Resistance: Roles of Ectopic Fat Accumulation and Adipose Inflammation. Gastroenterology [Internet]. 2017;152(7):1638–46. Available from: http://dx.doi.org/10.1053/j.gastro.2016.12.051

25. Neeland IJ, Poirier P, Després JP. Cardiovascular and Metabolic Heterogeneity of Obesity: Clinical Challenges and Implications for Management. Circulation. 2018;137(13):1391–406.

26. Maffeis C, Silvagni D, Bonadonna R, Grezzani A, Banzato C, Tatò L. Fat Cell Size, Insulin Sensitivity, and Inflammation in Obese Children. J Pediatr. 2007;151(6):647–52.

27. Matthews D, Hosker J, Rudenski A, Naylor B, Treacher D, Turner R. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412–9.

28. Rosner B, Cook NR, Daniels S, Falkner B. Childhood blood pressure trends and risk factors for high blood pressure: The NHANES experience 1988-2008. Hypertension. 2013;62(2):247–54.

29. Members F, Mancia G, Fagard R, Germany RES, Anton P, Uk PS. 2013 ESH / ESC Guidelines for the management of arterial hypertension TheT ask Force for the management of arterial hypertension of the European Society of Hypertension (ESH)

and of the European Society of Cardiology (ESC). J Hypertens. 2013;1281–357.

30. Webb NJ, Wu E. 2016 European Society of Hypertension guidelines for the management of high blood pressure in children and adolescents. J Hypertens. 2016;34:1887–920.

31. Yang Y, Dong B, Wang S, Dong Y, Zou Z, Fu L, et al. Prevalence of high blood pressure subtypes and its associations with BMI in Chinese children: A national cross-sectional survey. BMC Public Health. 2017;17(1):2–9.

32. Sharma AK, Metzger DL, Rodd CJ. Prevalence and severity of high blood pressure among children based on the 2017 American academy of pediatrics guidelines. JAMA Pediatr. 2018;172(6):557–65.

33. Sánchez Alvarado ASY. Relationships between Obesity and Cardiovascular Diseases in Four Southern States and Colorado. J Heal Care Poor Underserved. 2011;22(4):61–72.

34. Raghuveer G. Lifetime cardiovascular risk of childhood obesity. Am J Clin Nutr. 2010;91(5):1514–9.

35. Nielsen TRH, Gamborg M, Fonvig CE, Kloppenborg J, Hvidt KN, Ibsen H, et al. Changes in lipidemia during chronic care treatment of childhood obesity. Child Obes. 2012;8(6):533–41.

36. Deeb A, Attia S, Mahmoud S, Elhaj G, Elfatih A. Dyslipidemia and Fatty Liver Disease in Overweight and Obese Children. J Obes. 2018;2018.

37. l'Allemand D, Wiegand S, Reinehr T, Müller J, Wabitsch M, Widhalm K, et al. Cardiovascular risk in 26,008 European overweight children as established by a multicenter database. Obesity. 2008;16(7):1672–9.

38. Nielsen TRH, Lausten-Thomsen U, Fonvig CE, Bøjsøe C, Pedersen L, Bratholm PS, et al. Dyslipidemia and reference values for fasting plasma lipid concentrations in Danish/North-European White children and adolescents. BMC Pediatr. 2017;17(1):1–11.

39. Juhola J, Magnussen CG, Viikari JS, Kähönen M, Hutri-Kähönen N, Jula A, et al. Tracking of Serum Lipid Levels, Blood Pressure, and Body Mass Index from Childhood to Adulthood: The Cardiovascular Risk in Young Finns Study. J Pediatr. 2011;159(4):584–90.

40. Fang YL, Chen H, Wang CL, Liang L. Pathogenesis of non-alcoholic fatty liver disease in children and adolescence: From "two hit theory" to "multiple hit model." World J Gastroenterol. 2018;24(27):2974–83.

41. Fabbrini E, Magkos F. Hepatic Steatosis as a Marker of Metabolic Dysfunction. Nutrients. 2015;7:4995–5019.

42. Pacifico L, Martino M Di, Catalano C, Panebianco V, Bezzi M, Anania C, et al. T1-weighted dual-echo MRI for fat quantification in pediatric nonalcoholic fatty liver

disease. Wolrd J Gastroenterol. 2011;17(25):3012–9.

43. Vos MB, Abrams SH, Barlow SE, Caprio S, Daniels SR, Kohli ÃR, et al. CLINICAL GUIDELINES NASPGHAN Clinical Practice Guideline for the Diagnosis and Treatment of Nonalcoholic Fatty Liver Disease. J Pediatr Gastroenterol Nutr. 2017;64(2):319–34.

44. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, et al. Prevalence of hepatic steatosis in an urban population in the United States: Impact of ethnicity. Hepatology. 2004;40(6):1387–95.

45. Ittmeier KRDMW, Yner LAR. Metabolic Consequences of Hepatic Steatosis in Overweight and Obese. Diabetes Care. 2012;35(905–910).

46. Younossi Z, Loomba R, Rinella M, Bugianesi E, Marchesini B. Metabolic Features of Nonalcoholic Fatty Liver (NAFL) in Obese Adolescents: Findings from a Multi-ethnic Cohort. Hepatology. 2018;68(4):1376–90.

47. Shin HJ, Kim HG, Kim M, Koh H, Kim HY, Roh YH. Normal Range of Hepatic Fat Fraction on Dual- and Triple-Echo Fat Quantification MR in Children. PLoS One [Internet]. 2015;10(1):1–10. Available from: http://dx.doi.org/10.1371/journal.pone.0117480

48. Qayyum A, Nystrom M, Noworolski SM, Chu P, Mohanty A, Merriman R. MRI steatosis grading: Development and initial validation of a color mapping system. Am J Roentgenol. 2012;198(3):582–8.

49. Mayer-Davis EJ, Lawrence JM, Dabelea D, Divers J, Isom S, Dolan L, et al. Incidence Trends of Type 1 and Type 2 Diabetes among Youths, 2002–2012. N Engl J Med. 2017;376(15):1419–1429.

50. Schofield CJ, Sutherland C. Disordered insulin secretion in the development of insulin resistance and Type 2 diabetes. Diabet Med. 2012;29(8):972–9.

51. Lillioja S, Mott DM, Spraul M, Ferraro R, Foley J, Ravussin E, et al. Insulin resistance and insulin secretory disfunction as precursors of non-insulin-dependent diabetes mellitus. Prospective Studies of Pima Indians. N Engl J Med. 1993;329(27):1988–92.

52. Amutha A, Mohan V. Diabetes complications in childhood and adolescent onset type 2 diabetes - A review. J Diabetes Complications [Internet]. 2016;30(5):951–7. Available from: http://dx.doi.org/10.1016/j.jdiacomp.2016.02.009

53. Rhodes ET, Prosser LA, Hoerger TJ, Lieu T, Ludwig DS, Laffel LM. Estimated morbidity and mortality in adolescents and young adults diagnosed with Type 2 diabetes mellitus. Diabet Med. 2012;29(4):453–63.

54. Pulgaron ER, Delamater AM. Obesity and type 2 diabetes in children: Epidemiology and treatment. Curr Diab Rep. 2014;14(8):1–21.

55. Einarson TR, Acs A, Ludwig C, Panton UH. Prevalence of cardiovascular disease in type 2 diabetes: A systematic literature review of scientific evidence from across the world in 2007-2017. Cardiovasc Diabetol [Internet]. 2018;17(1):1–19. Available from:

https://doi.org/10.1186/s12933-018-0728-6

56. Lavine J, Doo E, Bass NM. Association between diabetes, family history of diabetes and risk of nonalcoholic steatohepatitis and fibrosis. Hepatology. 2012;56(3):943–51.

57. Kitade H, Chen G, Ni Y, Ota T. Nonalcoholic fatty liver disease and insulin resistance: New insights and potential new treatments. Nutrients. 2017;9(4):1–13.

58. L. Sheng, M. Christopher AM. Prevalence of Type 2 Diabetes and Prediabetes in Children with Nonalcoholic Fatty Liver Disease. JAMA. 2016;176(1):100–106.

59. World Health Organization. Cardiovascular diseases. 2020.

60. Bridger T. Childhood obesity and cardiovascular disease. Paediatr Child Health (Oxford). 2009;14(3):177–82.

61. Slyper AH, Rosenberg H, Kabra A, Weiss MJ, Blech B, Gensler S, et al. Early atherogenesis and visceral fat in obese adolescents. Int J Obes [Internet]. 2014;38(7):954–8. Available from: http://dx.doi.org/10.1038/ijo.2014.11

62. Bassols J, Martínez-Calcerrada JM, Prats-Puig A, Carreras-Badosa G, Díaz-Roldán F, Osiniri I, et al. Uric acid, carotid intima-media thickness and body composition in prepubertal children. Pediatr Obes. 2016;11(5):375–82.

63. Giuseppe M, Fabiana A, Silvia L, Manuela F, Carmelo B, Rao M, et al. Lifestyles and Cardiovascular Prevention in Childhood and Adolescence. Pediatr Cardiol [Internet]. 2019;40(6):1113–25. Available from: https://doi.org/10.1007/s00246-019-02152-w

64. Kanellis J, Kang DH. Uric acid as a mediator of endothelial dysfunction, inflammation, and vascular disease. Semin Nephrol. 2005;25(1):39–42.

65. Wu AH, Gladden JD, Ahmed M, Ahmed A, Filippatos G. Relation of serum uric acid to cardiovascular disease. Int J Cardiol [Internet]. 2016;213:4–7. Available from: http://dx.doi.org/10.1016/j.ijcard.2015.08.110

66. Hyun Lee J. Prevalence of hyperuricemia and its association with metabolic syndrome and cardiometabolic risk factors in korean children and adolescents: Analysis based on the 2016–2017 korea national health and nutrition examination survey. Korean J Pediatr. 2019;62(8):317–23.

67. Than NN, Newsome PN. A concise review of non-alcoholic fatty liver disease. Atherosclerosis. 2015;239(1):192–202.

68. Vos MB, Abrams SH, Barlow SE, Caprio S, Daniels SR, Kohli R, 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 Nu. J Pediatr Gastroenterol Nutr. 2017;64(2):319–34.

69. Méndez-Sánchez N, Arrese M, Zamora-Valdés D, Uribe M. Current concepts in the

pathogenesis of nonalcoholic fatty liver disease. Liver Int. 2007;423–33.

70. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global Epidemiology of Nonalcoholic Fatty Liver Disease—Meta-Analytic Assessment of Prevalence, Incidence, and Outcomes. Hepatology. 2016;64(1):73–84.

71. Yang M, Gong S, Ye SQ, Lyman B, Geng L, Chen P, et al. Non-Alcoholic Fatty Liver Disease in Children: Focus on Nutritional Interventions. 2014;4691–705.

72. 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 [Internet]. 2015;10(10):e0140908. Available from: http://dx.plos.org/10.1371/journal.pone.0140908

73. 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(947).

74. Yu EL, Golshan S, Harlow KE, Jorge E, Durelle J, Goyal NP, et al. Prevalence of Nonalcoholic Fatty Liver Disease in Children with Obesity. J Pediatr. 2019;207:64–70.

75. Mitra S, De A, Chowdhury A. Epidemiology of non-alcoholic and alcoholic fatty liver diseases. Transl Gastroenterol Hepatol. 2020;5:1–17.

76. Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. Pediatrics. 2006;118(4):1388–93.

77. Schwimmer JB, McGreal N, Deutsch R, Finegold MJ, Lavine JE. Influence of gender, race, and ethnicity on suspected fatty liver in obese adolescents. Pediatrics. 2005;115(5).

78. Wiegand S, Keller KM, Röbl M, L'Allemand D, Reinehr T, Widhalm K, et al. Obese boys at increased risk for nonalcoholic liver disease: Evaluation of 16 390 overweight or obese children and adolescents. Int J Obes. 2010;34(10):1468–74.

79. Ballestri S, Nascimbeni F, Baldelli E, Marrazzo A, Romagnoli D, Lonardo A. NAFLD as a Sexual Dimorphic Disease : Role of Gender and Reproductive Status in the Development and Progression of Nonalcoholic Fatty Liver Disease and Inherent Cardiovascular Risk. Adv Ther. 2017;34(6):1291–326.

80. Goran MI, Walker R, Le K, Mahurkar S, Vikman S, Davis JN, et al. Effects of PNPLA3 on Liver Fat and Metabolic Profile in Hispanic Children and Adolescents. Diabetes. 2010;59:3127–30.

81. Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet. 2008;40(12):1461–5.

82. Davis JN, Walker RW, Vikman S, Spruijt-metz D, Weigensberg MJ, Allayee H, et al. Increased hepatic fat in overweight Hispanic youth influenced by interaction between genetic variation in PNPLA3 and high dietary carbohydrate and sugar consumption 1 – 4. Am J Clin Nutr. 2010;92:1522–7.

83. Martínez LA, Larrieta E, Calva JJ, Kershenobich D, Torre A. The Expression of PNPLA3 Polymorphism could be the Key for Severe Liver Disease in NAFLD in Hispanic Population. Ann Hepatol [Internet]. 2019;16(6):909–15. Available from: http://dx.doi.org/10.5604/01.3001.0010.5282

84. Blachier M, Leleu H, Peck-Radosavljevic M, Valla DC, Roudot-Thoraval F. The burden of liver disease in Europe: A review of available epidemiological data. J Hepatol [Internet]. 2013;58(3):593–608. Available from: http://dx.doi.org/10.1016/j.jhep.2012.12.005

85. Caballería L, Pera G, Auladell MA, Torán P, Muñoz L, Miranda D, 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.

86. Younossi ZM. Non-alcoholic fatty liver disease – A global public health perspective. J Hepatol [Internet]. 2019;70(3):531–44. Available from: https://doi.org/10.1016/j.jhep.2018.10.033

87. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, 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. Hepatology. 2012;55(6):2005–23.

88. Younossi ZM, Loomba R, Anstee QM, Rinella ME, Marchesini G, Neuschwander-tetri BA, et al. Dianostic Modalities for Nonalcoholic Fatty Liver Disease, Nonalcoholic Steatohepatitis, and Associated Fibrosis. Hepatology. 2018;68(1):349–60.

89. Patton HM, Sirlin C, Behling C, Middleton M, Schwimmer JB, Lavine JE. Pediatric nonalcoholic fatty liver disease: A critical appraisal of current data and implications for future research. J Pediatr Gastroenterol Nutr. 2006;43(4):413–27.

90. Schwimmer JB, Middleton MS, Behling C, Newton KP, Awai HI, Paiz MN, et al. Magnetic resonance imaging and liver histology as biomarkers of hepatic steatosis in children with nonalcoholic fatty liver disease. Hepatology. 2015;61(6):1887–95.

91. Springer F, Machann J, Claussen CD, Schick F, Schwenzer NF. Liver fat content determined by magnetic resonance imaging and spectroscopy. World J Gastroenterol. 2010;16(13):1560–6.

92. Murphy P, Hooker J, Ang B, Wolfson T, Gamst A, Bydder M, et al. Associations Between Histologic Features of Nonalcoholic Fatty Liver Disease (NAFLD) and Quantitative Diffusion-Weighted MRI Measurements in Adults. J Magn Reson Imaging. 2014;00.

93. Schwimmer JB, Middleton MS, Behling C, Newton KP, Awai HI, Paiz MN, et al. Magnetic Resonance Imaging and Liver Histology as Biomarkers of Hepatic Steatosis in Children with Nonalcoholic Fatty Liver Disease Running title: Hepatic Steatosis in MRI vs Histology Authors: Hepatology. 2015;61(6):1887–95.

94. Labayen I, Ruiz JR, Ortega FB, Davis CL, Rodríguez G, González-Gross M, et al. Liver enzymes and clustering cardiometabolic risk factors in European adolescents: The

HELENA study. Pediatr Obes. 2015;10(5):361–70.

95. Kang Y, Park S, Kim S, Koh H. Normal serum alanine aminotransferase and non-alcoholic fatty liver disease among Korean adolescents: A cross-sectional study using data from KNHANES 2010-2015. BMC Pediatr. 2018;18(1):1–5.

96. Molleston JP, Schwimmer JB, Yates KP, Murray KF, Cummings OW, Lavine JE, et al. Histological abnormalities in children with nonalcoholic fatty liver disease and normal or mildly elevated alanine aminotransferase levels. J Pediatr [Internet]. 2014;164(4):707-713.e3. Available from: http://dx.doi.org/10.1016/j.jpeds.2013.10.071

97. Alisi A, Manco M, Vania A, Nobili V. Pediatric Nonalcoholic Fatty Liver Disease in 2009. J Pediatr [Internet]. 2009;155(4):469–74. Available from: http://dx.doi.org/10.1016/j.jpeds.2009.06.014

98. Katsiki N, Mikhailidis DP, Mantzoros CS. Non-alcoholic fatty liver disease and dyslipidemia: an update. Metabolism [Internet]. 2016;65(8):1109–23. Available from: http://dx.doi.org/10.1016/j.metabol.2016.05.003

99. Schwimmer JB, Zepeda A, Newton KP, Stavra A. Longitudinal Assessment of High Blood Pressure in Children with Nonalcoholic Fatty Liver Disease. PLoS One. 2014;1–17.

100. Al M, Silverman MG, Nasir K, Budoff MJ, Blankstein R, Szklo M, et al. The association of nonalcoholic fatty liver disease, obesity, and metabolic syndrome, with systemic inflammation and subclinical atherosclerosis: The Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis [Internet]. 2015;1–5. Available from: http://dx.doi.org/10.1016/j.atherosclerosis.2015.02.011

101. Alkhater SA. Paediatric non-alcoholic fatty liver disease: an overview. Obes Rev. 2015;16:393–405.

102. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: The multiple parallel hits hypothesis. Hepatology. 2010;52(5):1836–46.

103. Tian S, Liu Y, Feng A, Lou K, Dong H. Metabolically healthy obesity and risk of cardiovascular disease, cancer, and all-cause and cause-specific mortality: A protocol for a systematic review and meta-analysis of prospective studies. BMJ Open. 2019;9(10):1–6.

104. Ortega FB, Lavie CJ, Blair SN. Obesity and cardiovascular disease. Circ Res. 2016;118(11):1752–70.

105. Jolliffe CJ, Janssen I. Development of Age-Specific Adolescent Metabolic Syndrome Criteria That Are Linked to the Adult Treatment Panel III and International Diabetes Federation Criteria. J Am Coll Cardiol. 2007;49(8):891–8.

106. World Health Organization. Noncommunicable diseases. 2018.

107. World Health Organization. What are the causes? Diet, physical activity & health. 2020.

108. Nassau F Van, Singh AS, Cerin E, Salmon J, Mechelen W Van, Brug J. The Dutch Obesity Intervention in Teenagers (DOiT) cluster controlled implementation trial : intervention effects and mediators and moderators of adiposity and energy balance-related behaviours. Int J Behav Nutr. 2014;11(158).

109. Anderson EL, Fraser ÃA, Howe ÃLD, Callaway MP, Sattar N, Day C, et al. Physical Activity Is Prospectively Associated With Adolescent Nonalcoholic Fatty Liver Disease. Hepatology. 2016;62(1):110–7.

110. Tarp J, Child A, White T, Westgate K, Bugge A, Grøntved A, et al. Physical activity intensity , bout-duration , and cardiometabolic risk markers in children and adolescents. Int J Obes [Internet]. 2018;42:1639–50. Available from: http://dx.doi.org/10.1038/s41366-018-0152-8

111. Organization WH. Physical activity. 2018.

112. Welk GJ, Corbin CB, Dale D. Measurement issues in the assessment of physical activity in children. Res Q Exerc Sport. 2000;71:59–73.

113. Migueles JH, Cadenas-Sanchez C, Ekelund U, Delisle Nyström C, Mora-Gonzalez J, Löf M, et al. Accelerometer Data Collection and Processing Criteria to Assess Physical Activity and Other Outcomes: A Systematic Review and Practical Considerations. Sport Med. 2017;47(9):1821–45.

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

115. Al-mallah MH, Sakr S, Al-qunaibet A. Cardiorespiratory Fitness and Cardiovascular Disease Prevention: an Update. Curr Atheroscler Rep. 2018;20(1).

116. Agostinis-Sobrinho C, Ruiz JR, Moreira C, Abreu S, Lopes L, Oliveira-Santos J, et al. Cardiorespiratory Fitness and Blood Pressure: A Longitudinal Analysis. J Pediatr [Internet]. 2018;192:130–5. Available from: https://doi.org/10.1016/j.jpeds.2017.09.055

117. Medrano M, Arenaza L, Migueles JH, Rodríguez-Vigil B, Ruiz JR, Labayen I. Associations of physical activity and fitness with hepatic steatosis, liver enzymes, and insulin resistance in children with overweight/obesity. Pediatr Diabetes. 2020;21(4):565–74.

118. Yu H jie, Cai L biao, Yang X hao, Yuan S, Li Q xiao, He Q qiang. Cardiorespiratory Fitness Attenuates the Obesity Risk in Chinese Children Who Have Parents with Overweight/Obesity. J Pediatr [Internet]. 2018;200:150-154.e1. Available from: https://doi.org/10.1016/j.jpeds.2018.05.015

119. Pozuelo-Carrascosa DP, García-Hermoso A, Álvarez-Bueno C, Sánchez-López M, Martinez-Vizcaino V. Effectiveness of school-based physical activity programmes on cardiorespiratory fitness in children: A meta-Analysis of randomised controlled trials. Br J Sports Med. 2018;52(19):1234–40.

120. Rice MH, Howell CC. Measurement of physical activity, exercise, and physical fitness in children: Issues and concerns. J Pediatr Nurs. 2000;15(3):148–56.

121. Léger LA, Mercier D, Gadoury C, Lambert J. The multistage 20 metre shuttle run test for aerobic fitness. J Sports Sci. 1988;6(2):93–101.

122. Ruiz JR, Ramirez-Lechuga J, Ortega FB, Castro-Piñero J, Benitez JM, Arauzo-Azofra A, et al. Artificial neural network-based equation for estimating VO2max from the 20 m shuttle run test in adolescents. Artif Intell Med. 2008;44(3):233–45.

123. Johnson L, Mander AP, Jones LR, Emmett PM, Jebb SA. Energy-dense, low-fiber, high-fat dietary pattern is associated with increased fatness in childhood. Am J Clin Nutr. 2008;87:846–54.

124. Tognon G, Hebestreit A, Lanfer A, Moreno LA, Pala V, Siani A, et al. Mediterranean diet, overweight and body composition in children from eight European countries: Cross-sectional and prospective results from the IDEFICS study. Nutr Metab Cardiovasc Dis. 2014;24(2):205–13.

125. 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(628–635):628–35.

126. Lopez-garcia E, Schulze MB, Fung TT, Meigs JB, Rifai N, Manson JE, et al. Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction 1 – 3. Am J Clin Nutr. 2004;80(1029–35).

127. Garcia-arellano A, Ramallal R, Ruiz-canela M, Salas-salvadó J, Hé JR, Ros E, et al. Dietary Inflammatory Index and Incidence of Cardiovascular Disease in the PREDIMED Study. Nutrients. 2015;7:4124–38.

128. Struijk EA, May AM, Wezenbeek NLW, Fransen HP, Soedamah-Muthu SS, Geelen A, et al. Adherence to dietary guidelines and cardiovascular disease risk in the EPIC-NL cohort. Int J Cardiol [Internet]. 2014;176:354–9. Available from: http://dx.doi.org/10.1016/j.ijcard.2014.07.017

129. Trichopoulos A, Costacou, T, Bamia C TD. Adherence to a Mediterranean Diet and Survival in a Greek Population. N Engl J Med. 2003;348(26):2599–608.

130. Della Corte C, Mosca A, Vania A, Alterio A, Salvatore I, Nobili V. Good adherence to the Mediterranean diet reduces the risk for NASH and diabetes in pediatric patients with obesity : The results of an Italian Study. Nutrition [Internet]. 2017;39–40:8–14. Available from: http://dx.doi.org/10.1016/j.nut.2017.02.008

131. Giannini C, Diesse L, Adamo ED, Chiavaroli V. Influence of the Mediterranean diet on carotid intima-media thickness in hypercholesterolaemic children: A 12-month intervention study. Nutr Metab Cardiovasc Dis [Internet]. 2014;24(1):75–82. Available from: http://dx.doi.org/10.1016/j.numecd.2013.04.005

132. Psaltopoulou T, Naska A, Orfanos P, Trichopoulos D, Mountokalakis T, Trichopoulou A.

Olive oil, the Mediterranean diet, and arterial blood pressure: the Greek European Prospective Investigation into Cancer and Nutrition (EPIC) study. Am J Clin Nutr. 2004;80:1012–8.

133. Cohen JFW, Scm S, Ms MEL, Houser RF, Scd EBR. Dietary Approaches to Stop Hypertension Diet, Weight Status, and Blood Pressure among Children and Adolescents: National Health and Nutrition Examination Surveys 2003-2012. J Acad Nutr Diet [Internet]. 2017;117(9):1437-1444.e2. Available from: http://dx.doi.org/10.1016/j.jand.2017.03.026

134. Hekmatdoost A, Shamsipour A, Meibodi M, Eslamparast T, Poustchi H. Adherence to the Dietary Approaches to Stop Hypertension (DASH) and risk of Nonalcoholic Fatty Liver Disease. Int J Food Sci Nutr. 2016;67(8):1024–1029.

135. Siervo M, Lara J, Chowdhury S, Ashor A, Oggioni C, Mathers JC. Systematic Review with Meta-Analysis Effects of the Dietary Approach to Stop Hypertension (DASH) diet on cardiovascular risk factors : a systematic review and meta-analysis. Br J Nutr. 2015;113:1–15.

136. Saneei P, Hashemipour M, Kelishadi R, Rajaei S, Esmaillzadeh A. Effects of recommendations to follow the Dietary Approaches to Stop Hypertension (DASH) diet v . usual dietary advice on childhood metabolic syndrome : a randomised cross-over clinical trial. Br J Nutr. 2013;110:2250–9.

137. Hajna S, Liu J, Leblanc PJ, Faught BE, Merchant AT, Cairney J, et al. Association between body composition and conformity to the recommendations of Canada ' s Food Guide and the Dietary Approaches to Stop Hypertension (DASH) diet in peri-adolescence. Public Health Nutr. 2012;15(10):1890–6.

138. Anderson EL, Howe LD, Fraser A, Macdonald-wallis C, Callaway MP, Sattar N, et al. Childhood Energy Intake Is Associated with Nonalcoholic Fatty Liver Disease in. J Nutr. 2015;145(5):983–9.

139. Rouhani MH, Haghighatdoost F, Surkan PJ, Azadbakht L. Associations between dietary energy density and obesity: A systematic review and meta-analysis of observational studies. Nutrition [Internet]. 2016;32(10):1037–47. Available from: http://dx.doi.org/10.1016/j.nut.2016.03.017

140. Azaïs-Braesco V, Sluik D, Maillot M, Kok F, Moreno LA. A review of total & added sugar intakes and dietary sources in Europe. Nutr J. 2017;16(6):1–15.

141. World Health Organization. Guideline: Sugars intake for adults and children. 2015;48.

142. Vos M, Kaar JL, Welsh JA, Van Horn L V, Feig DI, Anderson CA, et al. Added Sugars and Cardiovascular Disease Risk in Children. A Scientific Statement From the American Heart Association. Circulation. 2017;135:e1017–e1034.

143. Hu FB, Malik VS. Sugar-sweetened beverages and risk of obesity and type 2 diabetes : Epidemiologic evidence. Physiol Behav. 2011;100(1):47–54.

144. Keller A, Bucher Della Torre S. Sugar-Sweetened Beverages and Obesity among Children and Adolescents: A Review of Systematic Literature Reviews. Child Obes. 2015;11(4):338–46.

145. Ma J, Fox CS, Jacques PF, Speliotes EK, Hoffmann U, Smith CE, et al. Sugar-sweetened beverage, diet soda, and fatty liver disease in the Framingham Heart Study cohorts. J Hepatol. 2015;63(2):462–9.

146. Wijarnpreecha K, Thongprayoon C, Edmonds PJ, Cheungpasitporn W. Associations of sugar- and artificially sweetened soda with nonalcoholic fatty liver disease: a systematic review and meta-analysis. QJM An Int J Med. 2016;461–6.

147. Jin R, Welsh JA, Le N, Holzberg J, Sharma P, Martin DR, et al. Dietary Fructose Reduction Improves Markers of Cardiovascular Disease Risk in Hispanic-American Adolescents with NAFLD. 2015;(Ldl):3187–201.

148. Jegatheesan P, De Bandt J-P. Fructose and NAFLD: The Multifaceted Aspects of Fructose Metabolism. Nutrients. 2017;9:1–13.

149. Ahadi Z, Qorbani M, Kelishadi R, Ardalan G, Motlagh ME, Asayesh H, et al. Association between breakfast intake with anthropometric measurements, blood pressure and food consumption behaviors among Iranian children and adolescents: The CASPIAN-IV study. Public Health [Internet]. 2015;129(6):740–7. Available from: http://dx.doi.org/10.1016/j.puhe.2015.03.019

150. Alexander KE, Ventura EE, Spruijt-metz D, Weigensberg MJ, Goran MI, Davis JN. Association of Breakfast Skipping With Visceral Fat and Insulin Indices in Overweight Latino Youth. Obes (Silver Spring). 2010;17(8):1528–33.

151. Blondin SA, Anzman-Frasca S, Djang HC, Economos CD. Breakfast consumption and adiposity among children and adolescents: an updated review of the literature. Pediatr Obes. 2016;11(5):333–48.

152. Freitas Júnior IF, Christofaro DGD, Codogno JS, Monteiro PA, Silveira LS, Fernandes RA. The association between skipping breakfast and biochemical variables in sedentary obese children and adolescents. J Pediatr. 2012;161(5):871–4.

153. Liyanage T, Ninomiya T, Wang A, Neal B, Jun M, Wong MG, et al. Effects of the mediterranean diet on cardiovascular outcomes-a systematic review and meta-analysis. PLoS One. 2016;11(8).

154. Tuan SH, Li CH, Sun SF, Li MH, Liou IH, Weng TP, et al. Comparison of cardiorespiratory fitness between preschool children with normal and excess body adipose-An observational study. PLoS One. 2019;14(10):1–12.

155. Tsiros MD, Coates AM, Howe PRC, Walkley J, Hills AP, Wood RE, et al. Adiposity is related to decrements in cardiorespiratory fitness in obese and normal-weight children. Pediatr Obes. 2016;11(2):144–50.

156. Heinzle S, Ball GDC, Kuk JL. Variations in the prevalence and predictors of prevalent metabolically healthy obesity in adolescents. Pediatr Obes. 2016;11(5):425–33.

157. Velázquez-lópez L, Santiago-díaz G, Nava-hernández J, Muñoz-torres A V, Medina-bravo P, Torres-tamayo M. Mediterranean-style diet reduces metabolic syndrome components in obese children and adolescents with obesity. BMC Pediatr. 2014;14:175.

158. Torris C, Molin M, Cvancarova Smastuen M. Fish consumption and its possible preventive role on the development and prevalence of metabolic syndrome - a systematic review. Diabetol Metab Syndr. 2014;6(1):112.

159. Kim Y-S, Xun P, Iribarren C, Van Horn L, Steffen L, Daviglus ML, et al. Intake of fish and long-chain omega-3 polyunsaturated fatty acids and incidence of metabolic syndrome among American young adults: a 25-year follow-up study. Eur J Nutr [Internet]. 2016;1–10. Available from: http://www.scopus.com/inward/record.url?eid=2-s2.0-84955609806&partnerID=tZOtx3y1

160. Baik I, Abbott RD, Curb JD, Shin C. Intake of Fish and n-3 Fatty Acids and Future Risk of Metabolic Syndrome. J Am Diet Assoc [Internet]. 2010;110(7):1018–26. Available from: http://dx.doi.org/10.1016/j.jada.2010.04.013

161. Ortega FB, Cadenas-sánchez C, Sui X, Blair SN, Lavie CJ. Role of Fitness in the Metabolically Healthy but Obese Phenotype : A Review and Update. Prog Cardiovasc Dis [Internet]. 2015;58(1):76–86. Available from: http://dx.doi.org/10.1016/j.pcad.2015.05.001

162. Tambalis KD, Panagiotakos DB, Psarra G, Sidossis LS. Breakfast skipping in Greek schoolchildren connected to an unhealthy lifestyle profile. Results from the National Action for Children’s Health program. Nutr Diet. 2019;76(3):328–35.

163. Smith KJ, Breslin MC, McNaughton SA, Gall SL, Blizzard L, Venn AJ. Skipping breakfast among Australian children and adolescents; findings from the 2011–12 National Nutrition and Physical Activity Survey. Aust N Z J Public Health. 2017;41(6):572–8.

164. Ardeshirlarijani E, Namazi N, Jabbari M, Zeinali M, Gerami H, Jalili RB, et al. The link between breakfast skipping and overweigh/obesity in children and adolescents: a meta-analysis of observational studies. J Diabetes Metab Disord. 2019;18(2):657–64.

165. Ramsay SA, Bloch TD, Marriage B, Shriver LH, Spees CK, Taylor CA. Skipping breakfast is associated with lower diet quality in young US children. Eur J Clin Nutr [Internet]. 2018;72(4):548–56. Available from: http://dx.doi.org/10.1038/s41430-018-0084-3

166. Danielle D, Maurice V, PetitClerc C, Martin C, Brulé D, Hamet P. Milk and soy protein ingestion : acute Effect on Serum Uric Acid Concentration. Am J Clin Nutr. 1991;53:665–9.

167. Zykova SN, Storhaug HM, Toft I, Chadban SJ, Jenssen TG, White SL. Cross-sectional analysis of nutrition and serum uric acid in two Caucasian cohorts: The AusDiab Study and the Tromsø¸ study. Nutr J [Internet]. 2015;14(1):1–11. Available from:

http://dx.doi.org/10.1186/s12937-015-0032-1

168. He FJ, Macgregor GA. Salt and sugar : their effects on blood pressure. Pflugers Arch - Eur J Physiol. 2015;467:577–86.

169. Rauber F, Campagnolo PDB, Hoffman DJ, Vitolo MR. Consumption of ultra-processed food products and its effects on children's lipid profiles: A longitudinal study. Nutr Metab Cardiovasc Dis [Internet]. 2015;25(1):116–22. Available from: http://dx.doi.org/10.1016/j.numecd.2014.08.001

170. Guasch-Ferré M, Hu FB, Martínez-González MA, Fitó M, Bulló M, Estruch R, et al. Olive oil intake and risk of cardiovascular disease and mortality in the PREDIMED Study. BMC Med. 2014;12(1):1–11.

171. Rampersaud GC, Pereira MA, Girard BL, Adams R MJ. Breakfast Habits, Nutritional Status, Body Weight, and Academic Performance in Children and Adolescents. Am Diet Assoc. 2005;105:743–60.

172. Georgoulis M, Kontogianni MD, Tileli N. The impact of cereal grain consumption on the development and severity of non-alcoholic fatty liver disease. Eur J Nutr. 2014;53:1727–35.

173. Ma J, Jacques PF, Meigs JB, Fox CS, Rogers GT, Smith CE, et al. Sugar-Sweetened Beverage but Not Diet Soda Consumption Is Positively Associated with Progression of Insulin Resistance and. J Nutr. 2016;146(12):2544–50.

174. Vos MB, Goran MI. Sugar, Sugar … Not So Sweet for the Liver. Gastroenterology. 2017;153(3):642–5.

175. Castro Burbano J, Fajardo Vanegas P, Robles Rodríguez J, Pazmiño Estévez K. Relationship between dietary calcium intake and adiposity in female adolescents. Endocrinol Nutr. 2016;63(2):58–63.

176. Kratz M, Marcovina S, Nelson JE, Yeh MM, Kowdley K V, Callahan HS, et al. Dairy fat intake is associated with glucose tolerance , hepatic and systemic insulin sensitivity , and liver fat but not b -cell function in humans. Am J Clin Nutr. 2014;99:1385–96.

177. Lloyd-Jones DM, Hong Y, Labarthe D, Mozaffarian D, Appel LJ, Van Horn L, et al. Defining and setting national goals for cardiovascular health promotion and disease reduction: The american heart association's strategic impact goal through 2020 and beyond. Circulation. 2010;121(4):586–613.

178. Estrategia NAOS. Cuidar la cantidad: las raciones. Programa Perseo, Estrateg Naos. 2008;180.

179. World Health Organization. Diet, nutrition and the prevention of chronic diseases: Report of a Joint WHO/FAO Expert Consultation. WHO Tech Rep Ser. 2003;

180. Aljuraiban GS, Chan Q, Griep LMO, Brown IJ, Daviglus ML, Stamler J, et al. The Impact of Eating Frequency and Time of Intake on Nutrient Quality and Body Mass Index: The

INTERMAP Study, a Population-Based Study. J Acad Nutr Diet [Internet]. 2015;115(4):528-536.e1. Available from: http://dx.doi.org/10.1016/j.jand.2014.11.017

181. Arenaza L, Medrano M, Oses M, Huybrechts I, Díez I, Henriksson H. Dietary determinants of hepatic fat content and insulin resistance in overweight / obese children : a cross-sectional analysis of the Prevention of Diabetes in Kids (PREDIKID) study. Br J Nutr. 2019;121:1158–65.

182. Ma J, Fox CS, Jacques PF, Speliotes EK, Hoffmann U, Smith CE, et al. Sugar-sweetened beverage , diet soda , and fatty liver disease in the Framingham Heart Study cohorts. J Hepatol [Internet]. 2015;63(2):462–9. Available from: http://dx.doi.org/10.1016/j.jhep.2015.03.032

183. Collison KS, Saleh SM, Bakheet RH, Al-rabiah RK, Inglis AL, Makhoul NJ, et al. Diabetes of the Liver : The Link Between Nonalcoholic Fatty Liver Disease and HFCS-55. Obes (Silver Spring) [Internet]. 2009;17(11):2003–13. Available from: http://dx.doi.org/10.1038/oby.2009.58

184. Ferder L, Ferder MD, Inserra F. The Role of High-Fructose Corn Syrup in Metabolic Syndrome and Hypertension. Curr Hypertens Rep. 2010;12:105–12.

185. Assy N, Nasser G, Kamayse I, Nseir W. Soft drink consumption linked with fatty liver in the absence of traditional risk factors. Can J Gastroenterol. 2008;22(10):811–6.

186. Malik VS, Popkin BM, Bray GA, Després J, Hu FB, Malik VS, et al. Sugar-Sweetened Beverages, Obesity, Type 2 Diabetes Mellitus, and Cardiovascular Disease Risk. Circulation. 2010;121:1356–64.

OSASUN ZIENTZIETAKO DOKTOREGO PROGRAMA

Haurren elikadura-ohituren eragina gibel-esteatosian eta osasun kardiobaskularrean

Lide Arenaza Etxeberria 2015-2020

Universidad Pública de Navarra
Nafarroako Unibertsitate Publikoa