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Silvia Bel Serrat

Dieta, estilos de vida y factores de riesgo cardiovascular en niños y adolescentes europeos

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DIETA, ESTILOS DE VIDA Y FACTORES DE RIESGO CARDIOVASCULAR EN NIÑOS Y ADOLESCENTES EUROPEOS

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Diet, lifestyle and cardiovascular disease risk factors in European children and adolescents

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Facultad de Ciencias de la Salud

UNIVERSIDAD DE ZARAGOZA

SILVIA BEL SERRAT

ZARAGOZA, JULIO DE 2013

A Cinta y Antonio, mis padres,

y a Gemma

A Guille

“Hacer lo que te gusta es libertad; que te guste lo que haces, felicidad”

(Anónimo)

“Doing what you like is freedom, liking what you do is happiness”

(Anonymous)

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Fdo.: Theodora Mouratidou

En Zaragoza, a 22 de julio de 2013

Lista de publicaciones [List of publications]

La presente Tesis Doctoral es un compendio de trabajos científicos previamente publicados, aceptados para su publicación o sometidos a revisión. Las referencias de los artículos que componen este documento se detallan a continuación:

I. Börnhorst C, **Bel-Serrat S**, Pigeot I, Huybrechts I, Ottevaere C, Sioen I, De Henauw S, Mouratidou T, Mesana MI, Westerterp K, Bammann K, Lissner L, Eiben G, Pala V, Rayson M, Krogh V, Moreno LA. Validity of 24-h recalls in (pre-)school aged children: Comparison of proxy-reported energy intakes with measured energy expenditure. *Clin Nutr* 2013. doi:pii: S0261-5614(13)00096-4. 10.1016/j.clnu.2013.03.018.

II. **Bel-Serrat S**, Mouratidou T, Pala V, Huybrechts I, Börnhorst C, Fernández-Alvira JM, Hadjigeorgiou C, Eiben G, Hebestreit A, Lissner L, Molnár D, Siani A, Veidebaum T, Krogh V, Moreno LA. Relative validity of the Children's Eating Habits Questionnaire-food frequency section among young European children: the IDEFICS Study. *Public Health Nutr* 2013:1-11.

III. **Bel-Serrat S**, Mouratidou T, Huybrechts I, Cuenca-García M, Manios Y, Gómez-Martínez S, Molnár D, Kafatos A, Gottrand F, Widhalm K, Sjöström M, Wästlund A, Stehle P, Azzini E, Vyncke K, González-Gross M, Moreno LA. The role of dietary fat on the association between dietary amino acids and serum lipid profile in European adolescents participating in the HELENA Study. *Eur J Clin Nutr (submitted)*.

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V. **Bel-Serrat S**, Mouratidou T, Börnhorst C, Peplies J, De Henauw S, Marild S, Molnár D, Siani A, Tornaritis M, Veidebaum T, Krogh V, Moreno LA. Food consumption and cardiovascular risk factors in European children: the IDEFICS study. *Pediatr Obes* 2013;8(3):225-36.

VI. **Bel-Serrat S**, Mouratidou T, Jiménez-Pavón D, Huybrechts I, Cuenca-García M, Mistura L, Gottrand F, González-Gross M, Dallongeville J, Kafatos A, Manios Y, Stehle P, Kersting M, De Henauw S,Castillo MJ, Hallstrom L, Molnár D, Widhalm K, Marcos A, Moreno LA. Is dairy consumption associated with low cardiovascular diseases risk in European adolescents? Results from the HELENA Study. *Pediatr Obes (accepted)*.

VII. **Bel-Serrat S**, Mouratidou T, Santaliestra-Pasías AM, Iacoviello L, Kourides YA, Marild S, Molnár D, Reisch L, Siani A, Stomfai S, Vanaelst B, Veidebaum T, Pigeot I, Ahrens W, Krogh V, Moreno LA. Clustering of multiple lifestyle behaviours and its association to cardiovascular risk factors in children: the IDEFICS study. *Eur J Clin Nutr* 2013;67(8):848-854.

VIII. Rey-López JP, **Bel-Serrat S**, Santaliestra-Pasías A, de Moraes AC, Vicente-Rodríguez G, Ruiz JR, Artero EG, Martínez-Gómez D, Gottrand F, De Henauw S, Huybrechts I, Polito A, Molnar D, Manios Y, Moreno LA. Sedentary behaviour and clustered metabolic risk in adolescents: The HELENA study. *Nutr Metab Cardiovasc Dis* 2012.

Contenidos

Proyectos de investigación 23

Listado de abreviaturas 25

Resumen general 27

1. Introducción 35

1.1 Valoración de la dieta en niños y adolescentes 35

1.1.1 Cuestionario de frecuencia de consumo de alimentos 39

1.1.2 Recuerdo dietético de 24 horas 40

1.1.3 Registro de alimentos 41

1.1.4 Historia dietética 43

1.1.5 Validación de los métodos de valoración de la dieta 44

1.2. Riesgo cardiovascular en niños y adolescentes 45

1.3 Relación entre riesgo cardiovascular y estilos de vida 50

1.3.1 Dieta 50

1.3.2 Actividad física 53

1.3.3 Comportamientos sedentarios 54

2. Objetivos 57

3. Material y Métodos 61

3.1 Comités de Ética 61

3.2 Muestra y diseño del estudio 62

3.3 Métodos de medida 66

3.3.1 Factores sociodemográficos (Estudio IDEFICS) 66

3.3.2 Gasto energético total (Estudio IDEFICS) 66

3.3.3 Dieta e ingesta de energía (Estudio IDEFICS) 67

3.3.4 Examen físico (Estudio IDEFICS) 69

3.3.5 Actividad física (Estudio IDEFICS) 69

3.3.6 Comportamientos sedentarios (Estudio IDEFICS) 69

3.3.7 Muestras biológicas (Estudio IDEFICS) 70

3.3.8 Factores socio-demográficos (Estudio HELENA) 71

3.3.9 Dieta e ingesta de energía y nutrientes (Estudio HELENA) 71

3.3.10 Examen físico (Estudio HELENA) 72

3.3.11 Maduración sexual (Estudio HELENA) 72

3.3.12 Actividad física (Estudio HELENA) 72

3.3.13 Comportamientos sedentarios (Estudio HELENA) 73

3.3.14 Muestras biológicas (Estudio HELENA) 73

3.3.15 Condición física 74

3.3.16 Indicador de riesgo cardiovascular 74

3.4 Análisis estadísticos: consideraciones generales 75

4. Resultados 77

4.1 Artículo 1: Validity of 24-h recalls in (pre-)school aged children: Comparison of proxy-reported energy intakes with measured energy expenditure. 79

4.2 Artículo 2: Relative validity of the Children's Eating Habits Questionnaire-food frequency section among young European children: the IDEFICS Study. 87

4.3 Artículo 3: The role of dietary fat on the association between dietary amino acids and serum lipid profile in European adolescents participating in the HELENA Study. 101

4.4 Artículo 4: Associations between macronutrient intakes and serum lipid profile depend on body fat in European adolescents: the HELENA study. 125

4.5 Artículo 5: Food consumption and cardiovascular risk factors in European children: the IDEFICS study. 153

4.6 Artículo 6: Is dairy consumption associated with low cardiovascular diseases risk in European adolescents? Results from the HELENA Study. 167

4.7 Artículo 7: Clustering of multiple lifestyle behaviours and its association to cardiovascular risk factors in children: the IDEFICS study. 181

4.8 Artículo 8: Sedentary behaviour and clustered metabolic risk in adolescents: The HELENA study. 191

5. Discusión 201

5.1 Validez de los métodos de valoración de la dieta 201

5.2 Dieta y factores de riesgo cardiovascular 204

5.3 Estilos de vida y factores de riesgo cardiovascular 209

5.4 Implicaciones para la salud pública 211

6. Aportaciones principales de la Tesis Doctoral 217

7. Conclusiones 221

8. Referencias 225

Apéndice 239

Agradecimientos 241

Anexo 245

Contents

Research projects 23

List of abbreviations 25

General abstract 31

1. Introduction *[Section in Spanish]* 35

1.1 Dietary assessment in children and adolescents 35

1.1.1 Food frequency questionnaire 39

1.1.2 24-hour dietary recall 40

1.1.3 Food record 41

1.1.4. Diet history 43

1.1.5 Validation studies of dietary assessment methods 44

1.2 Cardiovascular disease risk in children and adolescents 45

1.3 Association between cardiovascular disease risk and lifestyle behaviours 50

1.3.1 Diet 50

1.3.2 Physical activity 53

1.3.3 Sedentary behaviours 54

2. Objectives 59

3. Materials and Methods [Section in Spanish] 61

3.1 Ethics Committee 61

3.2 Sample and study design 62

3.3 Measurement methods 66

3.3.1 Socio-demographic factors (IDEFICS study) 66

3.3.2 Total energy expenditure (IDEFICS study) 66

3.3.3 Dietary and energy intake (IDEFICS study) 67

3.3.4 Physical examinations (IDEFICS study) 69

3.3.5 Physical activity (IDEFICS study) 69

3.3.6 Sedentary behaviours (IDEFICS study) 69

3.3.7 Biological samples (IDEFICS study) 70

3.3.8 Socio-demographic factors (HELENA study) 71

3.3.9 Dietary and energy intake (HELENA study) 71

3.3.10 Physical examinations (HELENA study) 72

3.3.11 Sexual maturation (HELENA study) 72

3.3.12 Physical activity (HELENA study) 72

3.3.13 Sedentary behaviours (HELENA study) 73

3.3.14 Biological samples (HELENA study) 73

3.3.15 Physical fitness 74

3.3.16 Clustered cardiovascular disease risk score 74

3.4 Statistical analysis: general considerations 75

4. Results 77

4.1 Paper 1: Validity of 24-h recalls in (pre-)school aged children: Comparison of proxy-reported energy intakes with measured energy expenditure. 79

4.2 Paper 2: Relative validity of the Children's Eating Habits Questionnaire-food frequency section among young European children: the IDEFICS Study. 87

4.3 Paper 3: The role of dietary fat on the association between dietary amino acids and serum lipid profile in European adolescents participating in the HELENA Study. 101

4.4 Paper 4: Associations between macronutrient intakes and serum lipid profile depend on body fat in European adolescents: the HELENA study. 125

4.5 Paper 5: Food consumption and cardiovascular risk factors in European children: the IDEFICS study. 153

4.6 Paper 6: Is dairy consumption associated with low cardiovascular diseases risk in European adolescents? Results from the HELENA Study. 167

4.7 Paper 7: Clustering of multiple lifestyle behaviours and its association to cardiovascular risk factors in children: the IDEFICS study. 181

4.8 Paper 8: Sedentary behaviour and clustered metabolic risk in adolescents: The HELENA study. 191

5. Discussion [Section in Spanish] 201

5.1 Dietary assessment methods validity 201

5.2 Diet and cardiovascular disease risk factors 204

5.3 Lifestyle behaviours and cardiovascular disease risk factors 209

5.4 Public health implications 211

6. Main thesis contributions 219

7. Conclusions 223

8. References 225

Appendix 239

Acknowledgments 241

Annex 245

Proyectos de investigación [Research projects]

El trabajo que se desarrolla a continuación, así como los artículos que forman parte de esta investigación, están basados en los siguientes proyectos de investigación:

1. **Estudio IDEFICS** (*Identification and prevention of dietary- and lifestyle-induced health effects in children and infants*). Proyecto financiado por la Unión Europea: European Union Sixth RTD Framework Programme (Contract FOOD-CT-2006-016181-2).
   Página web: www.idefics.eu
   Coordinador: Wolfgang Ahrens

2. **Estudio HELENA** (*Healthy Lifestyle in Europe by Nutrition in Adolescence*). Proyecto financiado por la Unión Europea: European Union Sixth RTD Framework Programme (Contract FOOD-CT-2005-007034).
   Página web: www.helenastudy.com
   Coordinador: Luis A. Moreno

Asimismo, **Silvia Bel Serrat** ha recibido una **beca destinada a la formación y contratación de personal investigador** (B079/08), concedida por el Departamento de Ciencia, Tecnología y Universidad del **Gobierno de Aragón** desde enero de 2008 hasta diciembre de 2011.

Listado de abreviaturas [List of abbreviations*]

AAP	Academia Americana de Pediatría
AF	Actividad Física
ANCOVA	Análisis de la Covarianza
ANOVA	Análisis de la Varianza
CC	Circunferencia de la Cintura
CEHQ-FFQ	Children's Eating Habits Questionnaire – Food Frequency section
CEICA	Comité Ético de Investigación Clínica de Aragón
CFCA	Cuestionario de Frecuencia de Consumo de Alimentos
ECV	Enfermedad Cardiovascular
FAS	Family Affluence Scale
GET	Gasto Energético Total
HELENA	Healthy Lifestyle in Europe by Nutrition in Adolescence
HELENA-DIAT	HELENA-Dietary Assessment Tool
HDL-c	Lipoproteína de alta densidad [High Density Lipoprotein cholesterol]
HOMA	Homeostatic Model Assessment
IE	Ingesta de Energía
IDEFICS	Identification and prevention of dietary- and lifestyle induced health effects in children and infants
IMC	Índice de Masa Corporal
NCEPT-ATP III	National Cholesterol Education Program's Adult Treatment Panel III
ISCED	Clasificación Internacional Normalizada de Educación [International Standard Classification of Education]

LDL-c	Lipoproteína de baja densidad [Low Density Lipoprotein-cholesterol]
MSM	Multiple Source Method
OMS	Organización Mundial de la Salud
PASW	Predictive Analytics Sowtware
POTG	Prueba Oral de Tolerancia a la Glucosa
SACINA	Self Administered Children and Infants Nutrition Assessment
SM	Síndrome Metabólico
TCA	Tablas de Composición de Alimentos
TG	Triglicéridos

*Abbreviations in English language are shown in the scientific papers included in the present Doctoral Thesis.

Resumen general

La infancia y la adolescencia no sólo se caracterizan por ser periodos de crecimiento rápido y maduración, sino también porque se adquiere el comportamiento alimentario para toda la vida. También se ha observado que las primeras manifestaciones de arteriosclerosis se dan de forma temprana durante la infancia y que, además, están relacionadas con la dieta y otros estilos de vida como la actividad física y los comportamientos sedentarios, entre otros factores. Por todo ello, es de gran importancia valorar de forma precisa la dieta de los niños y adolescentes para poder establecer relaciones entre dieta y enfermedad, así como identificar posibles asociaciones entre los factores de riesgo cardiovascular y el estilo de vida en este grupo poblacional, que permitan desarrollar estrategias dirigidas a mejorar el estilo de vida de los niños y adolescentes y así prevenir la aparición de enfermedades crónicas en el futuro.

A nivel general, los objetivos de la presente Tesis Doctoral son: 1) determinar el grado de validez de dos métodos de valoración de la dieta para ser usados en niños y 2) analizar la asociación de la dieta y otros estilos de vida con los factores de riesgo cardiovascular en niños y adolescentes para ampliar el conocimiento científico en este grupo de la población e identificar posibles brechas dentro de este área de investigación.

Para la validación de los métodos de valoración de la dieta se obtuvieron mediciones de niños participantes en el estudio IDEFICS (Identification and prevention of dietary- and lifestyle induced health effects in children and infants). Un total de 36 niños (4-10 años) de España y Bélgica fueron medidos para determinar la validez de un recuerdo dietético de 24-horas en comparación con la técnica del agua doblemente marcada. En la validación del cuestionario de frecuencia de consumo de alimentos semi-cuantitativo se incluyeron 2.508 niños (2-8 años) procedentes de ocho países europeos (Italia, Estonia, Chipre, Bélgica, Suecia, Alemania, Hungría, España). Para la consecución del segundo objetivo se obtuvieron

mediciones tanto de niños europeos (n=5.548) participantes en el estudio IDEFICS como de adolescentes europeos (n=511) de entre 12,5-17,5 años que participaron en el estudio HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) el cual se llevó a cabo en diez ciudades europeas (Atenas en Grecia, Dortmund en Alemania, Gante en Bélgica, Heraklion en Grecia, Lille en Francia, Pécs en Hungría, Roma en Italia, Estocolmo en Suecia, Viena en Austria y Zaragoza en España).

Los resultados del presente trabajo muestran que la capacidad del cuestionario de frecuencia de consumo de alimentos para clasificar a los individuos en función de su ingesta fue diferente en función de grupo de alimentos considerado; sin embargo, el recuerdo dietético de 24-horas resultó ser un buen método para determinar la ingesta de energía a nivel del grupo, pero no a nivel individual. En cuanto al riesgo cardiovascular, la ingesta elevada de algunos alimentos como los frutos secos y semillas y los cereales de desayuno, entre otros, en niños y como los lácteos en adolescentes se asoció inversamente con el riesgo cardiovascular. Aparte de la dieta, los estilos de vida, concretamente el sedentarismo, también se asociaron con el riesgo cardiovascular ya que jugar a los videojuegos durante más de cuatro horas los fines de semana aumentó al doble el riesgo de enfermedad cardiovascular en chicos adolescentes. Además, el tener un tamaño de muestra suficientemente amplio permitió valorar la relación entre conjuntos o clusters de algunos estilos de vida y el riesgo cardiovascular. Por ejemplo, el tener un comportamiento caracterizado por niveles bajos de sedentarismo y baja ingesta de bebidas azucaradas se asoció con un menor riesgo cardiovascular. La influencia de los macronutrientes en el perfil lipídico sérico también fue investigada en la presente Tesis Doctoral. De hecho, los resultados mostraron que una ingesta elevada de hidratos de carbono y baja de lípidos estaba asociada con un peor perfil lipídico; además, cabe destacar que dichas asociaciones fueron dependientes de la masa grasa del

individuo. Así mismo, también se observó que la asociación entre la ingesta de aminoácidos y la concentración de lípidos plasmáticos era dependiente de la ingesta de grasa del individuo.

El diseño transversal utilizado en todos los artículos supone una de las principales limitaciones de la presente Tesis Doctoral puesto que no nos permite determinar relaciones causales. La información sobre la dieta auto-declarada siempre está sujeta a una variedad de errores de medida no intencionados. En el caso del estudio IDEFICS, toda la información acerca de la ingesta de alimentos fue facilitada por los padres, lo cual disminuiría la precisión de los datos obtenidos, principalmente en cuanto a las ingestas que no tienen lugar bajo la supervisión de los padres. En lo que respecta a los recuerdos de alimentos de 24 horas empleados tanto en el estudio IDEFICS como en el HELENA, únicamente se incluyeron dos días de medición no consecutivos. Hubiera sido deseable un incremento de los días de medición de la dieta para lograr una mayor disminución de la variación intra-persona. Otra limitación importante sería que los comportamientos sedentarios y la actividad física en el estudio IDFICS fueron también obtenidos a través de un cuestionario rellenado por los padres, con lo que no se puede descartar el fenómeno de la deseabilidad social y la posible infra o sobredeclaración de los valores respondidos en cuanto a estas variables.

En resumen, estos resultados ponen de manifiesto la necesidad de desarrollar métodos de valoración de la dieta capaces de estimarla de forma más precisa. Además, existe evidencia de que tanto la dieta como los comportamientos sedentarios están asociados con el riesgo cardiovascular durante la infancia y la adolescencia, por ello es necesario diseñar estrategias destinadas a prevenir el desarrollo de riesgo cardiovascular a edades tan tempranas promoviendo el consumo de alimentos saludables y la práctica de actividad física, a expensas de la disminución de los niveles de sedentarismo, tanto en niños como en adolescentes.

General abstract

Childhood and adolescence are not only characterized as being periods of rapid growth and maturation, but also periods during which dietary habits are formed. There is also existing evidence indicating that the onset of atherosclerosis occur at early stages and that is related to lifestyle-related behaviours such as diet, physical activity and/or sedentary behaviours, among others. For these reasons, it is of great importance to accurately assess diet in both children and adolescents to detect diet-disease relationships, as well as to identify associations between cardiovascular disease risk factors and lifestyle in these population groups.

The aims of the present Doctoral Thesis are: 1) to examine the validity of two dietary assessment methods to be applied in children, and 2) to assess the association of diet expressed as dietary intakes and food consumption, and other lifestyle behaviours with cardiovascular disease risk factors in children and adolescents in order to enhance the scientific knowledge and identify research gaps in this area.

To investigate the validity of two dietary assessment methods, measurements were obtained in children participating in the IDEFICS (Identification and prevention of dietary- and lifestyle induced health effects in children and infants) study. A total of 36 children (4-10 years) from Belgium and Spain were measured to determine the validity of the 24-hour dietary recall method against the doubly labeled water technique. The second validation study included the examination of 44-item semi-quantitative food frequency questionnaire in 2,508 children (2-8 years) from eight European countries (Italy, Estonia, Cyprus, Belgium, Sweden, Germany, Hungary, Spain). To address the second objective related to examining the association of diet and other lifestyle behaviours with cardiovascular disease risk factors, measurements were obtained from European children (n=5,548) participating in the IDEFICS

study and from European adolescents (n=511) aged 12.5-17.5 years taking part in the HELENA study (Healthy Lifestyle in Europe by Nutrition in Adolescence) carried out in ten European cities (Athens in Greece, Dortmund in Germany, Ghent in Belgium, Heraklion in Greece, Lille in France, Pécs in Hungary, Rome in Italy, Stockholm in Sweden, Vienna in Austria and Zaragoza in Spain).

The findings of this study showed that the food frequency questionnaire's ability to rank individuals according to their intakes differed by food groups assessed. The second validation study conducted as part of this Thesis showed that the 24-hour dietary recall method provided valid estimates of energy intake at group level but not at the individual level. Regarding cardiovascular disease risk, higher intakes of certain foods such as nuts and seeds and breakfast cereals, among others, in children and dairy among adolescents were inversely associated with cardiovascular disease risk. Aside dietary factors, lifestyle, specifically indicators of sedentary behaviours, was found to be associated with cardiovascular disease risk, i.e. playing videogames for more than four hours at weekends increased the risk of cardiovascular disease in male adolescents by two folds. Furthermore, the large pool of data obtained in the studies enabled the authors to examine the association between clusters of several individual lifestyle behaviours and risk of cardiovascular diseases. For instance, low sedentary behaviours and low sugar sweetened beverages intake were associated with lower risk of cardiovascular disease. In this thesis, the influence of macronutrients on blood lipid profile was also addressed. Findings showed that high intake of carbohydrates and low intake of fat were associated with a worse serum lipid profile. It is noteworthy that such associations are body fat status-dependent. Likewise, the observed association between amino acids intake and serum lipids concentrations is also dependent of the individual's fat intake.

The cross-sectional design of all the manuscripts is one of the main limitations of the present Doctoral Thesis as it does not allow us to draw causal associations. Self-reported dietary data is always subject to a variety of unintentional measurement errors. Indeed, data was proxy-reported within the IDEFICS study, which decreased the accuracy of the reported data, mainly considering those food intakes that take place out of parental supervision. Regarding the 24-hour dietary recalls applied in both the IDEFICS study and the HELENA study, only two measurement days were collected. It would have been desirable to increase the number of recording days in order to reduce the within-person variability. Another important limitation is that, within the IDEFICS study, sedentary behaviours and physical activity were assessed through proxy-reported questionnaires; therefore, the effect of social desirability on the reported data as well as certain degree of under- or over-reporting cannot be precluded when considering these data.

In conclusion, these findings highlight the need of developing more accurate dietary measurement methods to assess diet more precisely. Besides, there is evidence that both diet and sedentary behaviours are associated with cardiovascular disease risk during childhood and adolescence; for that reason, it is necessary to design strategies aimed to prevent from suffering cardiovascular disease at early stages of life by means of promoting the intake of healthy foods and engagement in physical activity by reducing sedentary behaviours in both children and adolescents.

1. Introducción [Introduction]

La infancia y la adolescencia se caracterizan por ser periodos de rápido crecimiento y desarrollo, lo cual conlleva un aumento de los requerimientos nutricionales. Aunque la nutrición constituye un pilar fundamental a lo largo de toda la vida, un suministro adecuado de nutrientes durante estas etapas es esencial para alcanzar un crecimiento y desarrollo óptimos (1). Los procesos de crecimiento y maduración física y de desarrollo de la personalidad que tienen lugar durante la infancia y la adolescencia no influyen únicamente en la cantidad y forma de los nutrientes ingeridos, sino también en la actitud que el niño toma ante los alimentos (1). De hecho, durante la infancia y la adolescencia se establecen los hábitos alimentarios, se definen las preferencias y, en general, se forma la base del comportamiento alimentario para toda la vida (1). Esto pone de manifiesto aún más si cabe la importancia de una correcta alimentación durante edades tempranas como la niñez y la adolescencia.

1.1 Valoración de la dieta en niños y adolescentes

Valorar la ingesta de alimentos en niños y adolescentes de forma precisa es un factor primordial para conocer el grado de adecuación nutricional de su dieta (2) así como para llevar a cabo investigación de origen clínico y epidemiológico para detectar asociaciones reales entre la dieta y la salud (3). Sin embargo, se ha observado que la obtención de datos dietéticos fiables y precisos en este grupo de población supone una gran dificultad (3). De hecho, valorar la dieta en niños y adolescentes se considera mucho más complicado que hacerlo en adultos puesto que tienden a tener una alimentación altamente variable día a día y, además, sus hábitos alimentarios pueden cambiar muy rápidamente (4). Además, los niños más pequeños tienen una reducida capacidad para recordar, para estimar el tamaño de las porciones y para cooperar en el proceso de valoración de la dieta (4). Se considera que a partir de los 8 años los niños ya son capaces de decir lo que comen puesto que ya han

alcanzado el nivel de desarrollo necesario para ser conscientes de su ingesta de alimentos (4). Mientras tanto, es en los padres, madres y/o tutores sobre quienes recae la difícil tarea de informar sobre la dieta de los niños (4). Se ha mostrado que los padres suelen dar información precisa de la ingesta de alimentos de sus hijos cuando ésta tiene lugar en casa (5-8), sin embargo, a menudo no saben qué es lo que el niño consume fuera de casa, por lo que la información que puedan dar en este sentido no es del todo fiable (8). En algunas ocasiones se acude a otros cuidadores, como por ejemplo maestros o cuidadores de guardería, para obtener datos de la ingesta que tiene lugar en el colegio o guardería, pero, en este caso, los niveles de motivación e interés pueden variar ampliamente (3), con lo que también variará la calidad de la información proporcionada. Los adolescentes, por su lado, son completamente capaces de dar información sobre su dieta; sin embargo, a menudo muestran poco interés en dar datos precisos sobre la misma (4). La **Tabla 1** muestra aspectos de la valoración de la dieta tanto en niños como en adolescentes que tienen que tener en cuenta los encuestadores a la hora de valorarla.

Tabla 1. Aspectos sobre el encuestado-encuestador en la valoración de la dieta en niños y adolescentes (9).

Infancia	Adolescencia
Habilidades cognitivas	
- Baja habilidad para leer y escribir - Limitado concepto del tiempo - Memoria limitada	- Capacidad cognitiva completa
- Conocimiento limitado de los alimentos y de la preparación de los mismos	- Conocimiento extenso de los alimentos, pero ¿cuál es su conocimiento acerca de su preparación?
- La información sobre la dieta debe ser facilitada por los padres y madres	- Tienen la responsabilidad de dar ellos mismos la información sobre su dieta
Hábitos alimentarios	
- Hábitos alimentarios que cambian rápidamente, pero patrones de alimentación (más) estructurados	- Hábitos alimentarios que cambian rápidamente y patrones de alimentación desestructurados
- Mayor frecuencia de ingestas en casa	- Mayor frecuencia de ingestas fuera de casa
- Bajo la supervisión de adultos	- Menor supervisión por parte de adultos
- Importante influencia de los padres	- Importante influencia de los amigos/as
Psicológicos	
- Los alimentos satisfacen el hambre	- Los alimentos son una forma de auto-expresión

A pesar de los retos metodológicos que existen, se asume que los métodos de valoración de la dieta disponibles actualmente y que han sido diseñados para su uso en adultos son apropiados para recopilar datos en poblaciones pediátricas (9). Actualmente se dispone de cuatro métodos de valoración de la dieta: el registro de alimentos, el recuerdo dietético de 24 horas, el cuestionario de frecuencia de consumo de alimentos (CFCA) y la historia dietética. A la hora de elegir un instrumento dietético, independientemente del grupo de población al que va dirigido, deben de tenerse en cuenta los objetivos del estudio, el número y características de la población a estudio así como los recursos de los que se dispone (9). Debido a que todos los métodos dietéticos están sujetos a limitaciones (4), es necesario tener una idea muy clara desde un inicio sobre cuál es la información que se quiere medir para no cometer ningún error en la elección del método dietético y así obtener la información deseada. Por esta razón, a menudo se tienden a utilizar de forma combinada para maximizar las ventajas de cada instrumento (4) y compensar sus limitaciones con el uso de otro instrumento distinto. Una combinación bastante frecuente en estudios transversales es la utilización de los recuerdos de 24 horas junto con los CFCA. En la **Tabla 2** se pueden observar las ventajas y desventajas de los instrumentos de valoración de la dieta.

Tabla 2. Ventajas y desventajas de los instrumentos de valoración de la dieta (4).

Instrumento	Ventajas	Desventajas
Registro de alimentos	1. Cuantificación de la ingesta	1. Alto coste para el investigador
	2. Puede incrementar la auto-supervisión para el control del peso o del cambio de otro comportamiento	2. Alta responsabilidad para el encuestado
	3. No requiere recordar los alimentos consumidos	3. Entrenamiento extenso del encuestado y necesidad de motivación
		4. Necesidad de muchos días de medida para recopilar la dieta habitual del individuo
		5. Afecta al comportamiento alimentario
		6. Frecuente infra-declaración de la ingesta
		7. El grado de información sobre la dieta disminuye con el tiempo
		8. El grado de agotamiento incrementa con el número de registros diarios solicitados
		9. Puede dar lugar a una muestra no representativa con el subsiguiente sesgo de no respuesta
Recuerdo dietético de 24 horas	1. Cuantificación de la ingesta	1. Alto coste para el investigador
	2. Apropiado para ser usado en distintas poblaciones, dando lugar a menor grado de sesgo de no respuesta	2. Necesidad de muchos días de medida para recopilar la dieta habitual del individuo
	3. Responsabilidad relativamente baja para el encuestado	3. Frecuente infra-declaración de la ingesta
	4. No afecta al comportamiento alimentario	
Cuestionario de frecuencia de consumo de alimentos	1. Se pregunta a menudo por la ingesta habitual del individuo	1. No se cuantifica de forma precisa
	2. Bajo coste para el investigador	2. Tarea cognitiva complicada para el encuestado
	3. Baja responsabilidad para el encuestado	3. Frecuente sobre-declaración de la ingesta
	4. No afecta al comportamiento alimentario	
Historia dietética	1. Se pregunta a menudo por la ingesta habitual del individuo	1. No se cuantifica de forma precisa
	2. Obtención de información total sobre la dieta	2. Tarea cognitiva complicada para el encuestado
	3. Información disponible a menudo acerca de los alimentos ingeridos por toma	3. Frecuente infra/sobre-declaración de la ingesta
	4. Puede suponer un bajo coste para el investigador	4. Puede suponer una alta responsabilidad para el investigador
	5. No afecta al comportamiento alimentario	

1.1.1 Cuestionario de frecuencia de consumo de alimentos

Los CFCAs se usan generalmente para clasificar a los individuos según su ingesta de alimentos, grupos de alimentos o nutrientes, más que para estimar niveles absolutos de ingesta, y se usan con mucha frecuencia en estudios caso-control o estudios de cohortes para valorar la asociación entre la dieta y el riesgo de enfermedad (10-12). En el CFCA, los sujetos encuestados deben indicar la frecuencia con la que consumen cada uno de los alimentos incluidos en una lista durante un periodo de tiempo específico (4). Aunque se recopila información sobre la frecuencia, en ocasiones también se incluye el tamaño de las porciones ingeridas, lo cual permite una posterior estimación relativa o absoluta de la ingesta de nutrientes mediante la asociación de los alimentos que componen el cuestionario a bases de datos de composición de alimentos (4).

El CFCA cuenta con varias ventajas. Entre ellas está su bajo coste de administración y el hecho de que puede estimar la ingesta habitual de alimentos del sujeto durante un periodo de tiempo extenso (4). A diferencia de otros métodos, el CFCA puede ser usado para evitar cambios recientes en la dieta por medio de la obtención de información acerca de la dieta del individuo sobre un largo periodo de tiempo anterior (4). La mayoría de los CFCAs están diseñados para ser autoadministrados y se requieren entre 30 y 60 minutos para ser rellenados en función del cuestionario y del encuestado (4). Debido a que los costes de obtención de datos y procesado así como la carga que le supone al encuestador son bastante bajos comparados con los de otros instrumentos, los CFCAs se han convertido en un método habitual para estimar la ingesta dietética en grandes estudios epidemiológicos (4).

En cuanto a las limitaciones, la más importante es que presenta un elevado error de medición (13, 14). Existen muchos detalles sobre la ingesta dietética que no llegan a medirse y la cuantificación de la ingesta no es tan precisa como con los recuerdos o los registros (4). Estas imprecisiones son debidas a listas de alimentos incompletas y a errores en las

estimaciones de la frecuencia y de los tamaños de las porciones habituales y, como resultado, pueden dar lugar a una estimación poco exacta de la ingesta media del grupo (4).

1.1.2 Recuerdo dietético de 24 horas

El principal uso del recuerdo dietético de 24 horas es describir la ingesta media dietética de un grupo porque las medias son robustas y no están afectadas por la variación intra-persona (4). La obtención de múltiples recuerdos permite valorar mejor la ingesta habitual del individuo, pero requiere procedimientos estadísticos especiales diseñados para tal finalidad (4). En el recuerdo dietético de 24 horas el encuestado debe de recordar e informar acerca de todos los alimentos y bebidas que ha consumido en las 24 horas previas o durante el día previo (4). El recuerdo se lleva a cabo a través de una entrevista, en persona o por vía telefónica (15, 16), también asistida por ordenador (17) o por medio de papel y lápiz y está a menudo estructurada, habitualmente con preguntas de sondeo, para ayudar al entrevistado a recordar todos los alimentos consumidos a lo largo del día (4). Es crucial la presencia de entrevistadores bien entrenados a la hora de llevar a cabo la entrevista puesto que mucha de la información acerca de la ingesta de alimentos se obtiene a través de sondeo (4). El entrevistador debe tener conocimientos sobre alimentos y nutrición, y debe estar informado acerca de los alimentos disponibles en el mercado y de la forma de preparación de los mismos, incluyendo alimentos regionales y étnicos (4).

Entre las ventajas del recuerdo dietético 24 horas está el hecho de que el encuestador administra el instrumento y anota las respuestas, lo que hace que la alfabetización del encuestado no sea imprescindible (4). Debido a que el periodo acerca del cual hay que recordar la ingesta es cercano, el encuestado es capaz de recordar la mayoría de los alimentos y bebidas que consumió (4). También es un instrumento muy útil para ser usado a lo largo de un amplio rango de poblaciones y donde los entrevistadores pueden ser entrenados para capturar el detalle necesario (4). Por otro lado, debido a que los recuerdos tienen lugar

después que los alimentos han sido ingeridos, es menos probable que el instrumento de medición interfiera en el comportamiento alimentario (4). El realizar la entrevista a través de programas informáticos proporciona una reducción en el coste para el procesamiento de los datos, menor pérdida de información y mayor estandarización de las entrevistas (18, 19).

La mayor limitación del recuerdo de alimentos de 24 horas es que los individuos pueden no reportar su dieta de forma precisa debido a diversas razones relacionadas con el conocimiento, la memoria y la situación en la que tiene lugar la entrevista (4). La dieta de la mayoría de los individuos varía en gran medida día a día, por lo que no es apropiado utilizar datos obtenidos de un solo recuerdo para caracterizar la dieta habitual de un individuo (4). De la misma manera, la ingesta de un solo día tampoco se debe usar para estimar la proporción de la población que consume dietas adecuadas o inadecuadas (4). Ello se debe a que la distribución verdadera de la dieta habitual es mucho más estrecha que la distribución de la dieta diaria, i.e. hay variación no solo entre las personas en cuanto a su dieta habitual (variación entre-persona) sino también la hay día a día para cada persona (variación intra-persona) (4).

1.1.3 Registro de alimentos

En un registro de alimentos hay que registrar los alimentos y bebidas así como las cantidades consumidas durante uno o más días (4). Dichas cantidades pueden ser medidas, usando una báscula o medidas caseras como vasos, cucharadas, etc., o estimadas usando modelos, fotos o sin usar ninguna ayuda en particular (4). Si se registra información de varios días, suelen ser consecutivos y no se incluyen más de 3 ó 4 días, puesto que cuando se superan los 4 días de registro disminuyen las ingestas registradas (20) debido a la fatiga del encuestado. Teóricamente, la información es anotada cuando tiene lugar la ingesta (4). Para completar un registro de alimentos es necesario entrenar al encuestado en cuanto al nivel de detalle requerido para describir de forma adecuada los alimentos y las cantidades

consumidas, incluyendo el nombre del alimento (si es posible el nombre de la marca), el método de preparación, recetas para platos combinados y tamaño de las porciones (4). Al final del periodo de registro, un entrevistador entrenado debe de revisar los registros con el encuestado para clarificarlos y comprobar que no se ha olvidado ningún alimento (4).

El registro de alimentos tiene la capacidad de proporcionar información cuantitativa precisa acerca de los alimentos consumidos durante el periodo de registro de los mismos (21). El hecho de registrar los alimentos a la vez que se van consumiendo disminuye el problema de la omisión y los alimentos están mejor descritos (4). Así mismo, la medición de las cantidades consumidas en cada ocasión debería de proporcionar tamaños de porciones más precisos que si el encuestado tuviera que recordar el tamaño de la porción consumido previamente (4). Además, cuenta con la ventaja de que puede ser rellenado por otra persona distinta del sujeto de interés, por lo que es el método que se usa frecuentemente en personas institucionalizadas o en niños (4).

Una de las desventajas más importantes de este método es que está sujeto a error en cuanto a la selección de la muestra y la medición de la dieta (4). El registro de alimentos requiere que la persona que lo complete esté motivada y sepa leer y escribir (si se hace sobre papel), lo que potencialmente limita el uso de este método en algunos grupos poblacionales (por ejemplo: personas con bajo nivel socioeconómico, personas con un nivel bajo de alfabetización, inmigrantes recientes, niños y algunos grupos de mayores) (4). Los requerimientos para la cooperación del individuo a la hora de completar un registro de alimentos puede limitar la generalización de los resultados obtenidos a una población más amplia de la cual ha sido extraída la muestra estudiada (4). Por otro lado, hay un incremento en el número de registros incompletos conforme aumentan los días de registro, y la validez de la información recogida disminuye en los últimos días de un periodo de registro de siete días en comparación con la recogida durante los primeros días (20). Además, registrar los alimentos conforme se van consumiendo puede afectar al tipo de alimento elegido y a las

cantidades consumidas (22). El grado de conocimiento requerido para completar un registro de alimentos así como el grado de exigencia de la tarea puede alterar el comportamiento dietético que se está intentado medir (23).

1.1.4 Historia dietética

En el sentido más general, una historia dietética es cualquier valoración dietética que le pide al encuestado que registre su dieta pasada (4). Según Burke, el término "historia dietética" se refiere a la recogida de información no solo acerca de la frecuencia de consumo de varios alimentos sino también acerca de la preparación típica del alimento (24, 25). Por lo tanto, el término "historia dietética" se reserva para denominar a aquellos métodos de valoración de la dieta diseñados para determinar la ingesta habitual de alimentos de una persona en los que se valoran bastantes detalles acerca de las características de los alimentos consumidos habitualmente además de la frecuencia y cantidad del alimento consumido (4). De hecho, la historia dietética diseñada por Burke incluye tres elementos: una entrevista detallada acerca del patrón de consumo habitual, un listado de alimentos en el que hay que indicar frecuencia y cantidad consumida habitualmente y un registro de alimentos de tres días (24, 25).

La mayor fortaleza de la historia dietética es la valoración de los patrones de comidas y de detalles acerca de la ingesta de alimentos en lugar de ingestas durante un corto periodo de tiempo (como en los registros de alimentos o en los recuerdos dietéticos de 24 horas) o únicamente frecuencias de consumo de alimentos (4). Los detalles sobre la forma de preparación de los alimentos pueden ser útiles para caracterizar mejor la ingesta de nutrientes, así como la exposición a otras sustancias que pueden estar presentes en los alimentos (4).

Sin embargo, una desventaja de este método es que el encuestado tiene que realizar demasiadas valoraciones acerca de los alimentos que consume habitualmente y de las

cantidades consumidas (4). Estas tareas tan subjetivas pueden resultar muy difíciles para algunas personas. De hecho, Burke advirtió que las ingestas de nutrientes estimadas con este método debían de ser consideradas ingestas relativas en lugar de absolutas (4). Cabe destacar que la historia dietética comparte todas estas limitaciones con el CFCA (4). Además, la validez de la historia dietética es difícil de valorar debido a la falta de conocimiento independiente de la ingesta habitual a largo plazo del individuo (4).

1.1.5 Validación de los métodos de valoración de la dieta

La validez de un instrumento de valoración de la dieta se refiere a la capacidad que éste tiene para valorar la ingesta verdadera del individuo (3). Un método se define válido si la ingesta dietética declarada no difiere significativamente de la ingesta actual (3). Para comprobar la validez de un instrumento de valoración de la dieta se tiende a compararlo con otro método similar o por observación directa del consumo del alimentos (2). Sin embargo, esta técnica es bastante limitada puesto que el método al que se compara también está sujeto a limitaciones similares a las del instrumento de prueba (2). Para evitar este problema, es deseable el empleo de biomarcadores puesto que suponen una medida objetiva y son independientes del error del método evaluado (2).

De entre todos los biomarcadores, el agua doblemente marcada se considera el método de referencia para la validación de medidas de ingesta de energía (IE) por medio de la estimación del gasto energético total (GET) (2). El uso del agua doblemente marcada como biomarcador de la IE está basado en la hipótesis de que los individuos están en situación de balance energético: en condiciones estables de peso, la IE y el GET son equivalentes (3). Durante las etapas de crecimiento y desarrollo los niños y adolescentes están normalmente en balance energético positivo, de hecho está aumentado en un 1-2% (26).

La validez de los recuerdos dietéticos de 24 horas se suele estudiar por medio de la comparación de las ingestas declaradas por el encuestado con aquellas medidas de ingestas

registradas de forma discreta por observadores entrenados o por el uso de marcadores biológicos, entre ellos el agua doblemente marcada y la excreción de nitrógeno por la orina (4).

El estudio de validación definitivo para un CFCA consistiría en observar la dieta habitual del individuo durante un largo periodo de tiempo de forma no intrusiva; sin embargo, todavía no se han llevado a cabo estudios de estas características (4). Por ello, el método más aceptado para examinar la concordancia de las respuesta de frecuencia de consumo de alimentos y la dieta habitual es la utilización de registros de alimentos y recuerdos dietéticos múltiples durante un periodo determinado como indicadores de la dieta habitual (4). Este método se ha utilizado en diversos estudios para evaluar la validez de los CFCAs (27).

1.2 Riesgo cardiovascular en niños y adolescentes

La Organización Mundial de la Salud (OMS) ha determinado que las enfermedades cardiovasculares (ECV) son la causa principal de muerte a nivel mundial, es decir, un mayor número de personas mueren anualmente a causa de ECV que de ninguna otra causa (28). De hecho, se estima que en el año 2008 un total de 17,3 millones de personas murieron debido a ECV, lo que representa un 30% de las muertes globales y se prevé que estas cifras alcanzarán los 23,3 millones de personas para el año 2030, por lo que las ECV seguirán siendo la causa de muerte más destacada (28). Además, los países de bajo y medio nivel socioeconómico están afectados de forma desproporcionada puesto que alrededor del 80% de las muertes por ECV tienen lugar en estos países y ocurren de forma equitativa tanto en hombres como en mujeres (28).

La obesidad, la hipertensión, la diabetes y la dislipemia son conocidos factores de riesgo cardiovascular (28). Su aparición conjunta en un mismo individuo se denomina "Síndrome Metabólico" (SM). Este término se usa generalmente para indicar una situación

clínica en la que co-ocurren trastornos metabólicos y cardiovasculares que son factores de riesgo para el desarrollo de diabetes mellitus tipo 2 y de ECV (29). Diversos estudios han puesto de manifiesto que cuatro factores: obesidad (especialmente la obesidad central), intolerancia a la glucosa, dislipidemia aterogénica (niveles altos de triglicéridos (TG) y niveles bajos de lipoproteínas de alta densidad (HDL-c)) e hipertensión co-ocurren en algunos individuos en un mayor grado de lo esperado sólo por casualidad (29). Además, parece ser que la prevalencia así como las interacciones entres estos componentes varían según el sexo, la edad y el grupo étnico (29). Por otro lado, existen diversos problemas relacionados con la definición del SM: 1) todos los componentes del SM son variables continuas, lo que implica la necesidad de definir unos puntos de corte; sin embargo no hay todavía un consenso sobre cuales deben de ser los umbrales para establecer el diagnóstico de cada componente; 2) todas estas variables están interrelacionadas, pero la pato-fisiología de su relación todavía no se conoce de forma completa; y 3) la inclusión de la resistencia a la insulina o diabetes como componente de diagnóstico todavía es una cuestión que genera controversia, aunque desde el punto de vista fisiopatológico parece ser el factor central (30).

Los dos criterios más utilizados para el diagnóstico de SM en adultos son los establecidos por la OMS (31) y el *National Cholesterol Education Program's Adult Treatment Panel III* (NCEPT-ATP III) (32), sin embargo, existen algunas diferencias entre ellos. La definición de la OMS requiere la evaluación de la resistencia a la insulina o del trastorno del metabolismo de la glucosa. Por otro lado, la definición de la NCEPT-ATP III no exige la medida de la resistencia a la insulina, lo que facilita su utilización en estudios epidemiológicos (33).

Las ECV se han convertido también en un problema pediátrico puesto que se ha observado que las primeras manifestaciones de arteriosclerosis podrían darse de forma temprana durante la infancia (34). Además, también se ha observado tanto en niños como en adolescentes la aparición conjunta de factores de riesgo metabólico y cardiovasculares mencionados previamente como la obesidad central, la tensión arterial elevada, la resistencia

a la insulina, TG elevados y niveles bajos de HDL-c (35), pero, lo más preocupante, es que estos trastornos metabólicos tienden a continuar desde la infancia a la edad adulta (36).

Los cambios que tienen lugar durante el crecimiento y el desarrollo hacen que la identificación de criterios apropiados así como de puntos en corte para diagnosticar el SM en niños y adolescentes sea mucho más complicada aún si cabe que en el caso de los adultos (37). De hecho, actualmente no existe una definición del SM en niños y adolescentes, por lo que muchos de los estudios proponen el uso de la definición de la NCEPT-ATP III modificada, pero todavía no existe un consenso a la hora de establecer los umbrales para cada componente (38). En la revisión sistemática de Moraes et al. (33) la prevalencia de SM en adolescentes fue mayor en aquellos estudios que usaron los criterios de la OMS que en aquellos que usaron la definición dada por la NCEPT-ATP III. Sin embargo, no solo el uso de una definición u otra puede llevar a diferencias en la estimación de la prevalencia, sino que con el uso de la misma definición también pueden observarse diferencias en dicha estimación (38). No obstante, lo que realmente importa es que independientemente de la definición utilizada, Olza et al. (39) diagnosticaron el SM tanto en niños como en adolescentes obesos, aunque es importante destacar que la frecuencia fue mayor en estos últimos. La **Tabla 3** muestra los criterios que se han usado hasta ahora para diagnosticar el SM en niños y adolescentes.

Tabla 3. Criterios para el diagnóstico del SM en poblaciones pediátricas (39).

Estudio	Población (sexo, edad y grupo étnico)	Exceso de adiposidad	Presión arterial	Lípidos	Glucosa (insulina)
Cook et al., 2003[40]	Chicos y chicas 12-19 años Americanos blancos, de color y mexicanos	CC ≥ percentil 90	Presión sistólica o diastólica ≥ percentil 90	TG ≥ 110 mg/dl HDL-c ≤ 40 mg/dl	≥ 110 mg/dl
de Ferranti et al., 2004[41]	Chicos y chicas 12-19 años Americanos blancos no hispánicos, de color y mexicanos	CC > percentil 75	Presión sistólica > percentil 90	TG ≥ 100 mg/dl HDL-c < 50 mg/dl	≥ 110 mg/dl
Weiss et al., 2003[30]	Chicos y chicas 4-20 años Blancos, de color e hispánicos	IMC > percentil 97 o z-score > 2	Presión sistólica o diastólica ≥ percentil 95	TG > percentil 95 y HDL-c < percentil 5 según edad, sexo y raza	POTG > 140 y < 200 mg/dl a las 2 horas
Cruz et al., 2003[42]	Chicos y chicas 8-13 años Hispánicos (americanos mexicanos, americanos de la zona central, o mezclados)	CC ≥ percentil 90	Presión sistólica o diastólica ≥ percentil 90	TG ≥ percentil 90 y HDL-c ≤ percentil 10 según edad, sexo y raza	POTG: glucosa a los 120 minutos ≥ 140 mg/dl y < 200 mg/dl
Viner et al., 2003[43]	Chicos y chicas 2-18 años Blancos, de color, surasiáticos, y otros o etnias mixtas	IMC > percentil 95	Presión sistólica ≥ percentil 95	Cualquiera de los siguientes: TG elevados (≥ 150 mg/dl), bajo HDL-c (< 35 mg/dl) o colesterol total elevado (≥ percentil 95)	(Cualquiera de los siguientes: hiperinsulinemia en ayunas [pre- ≥ 15 mU/l, medio- (estadíos 2-4) ≥ 30 mU/l y pospuberal ≥ 20 mU/l]}, glucosa en ayunas alterada (≥ 110 mg/dl)) o tolerancia a la glucosa alterada: glucosa a los 120 minutos ≥ 140 mg/dl
Ford et al., 2003[44]	Chicos y chicas 12-17 años	CC ≥ percentil 90	Presión sistólica o diastólica ≥ percentil 90	TG ≥ 110 mg/dl HDL-c ≤ 40 mg/dl	≥ 110 mg/dl
IDF, 2007[a][45]	≥ 10 a < 16 años	CC ≥ percentil 90	Presión sistólica ≥ 130 o presión diastólica ≥ 85 mm Hg	TG ≥ 150 mg/dl HDL-c < 40 mg/dl	≥ 110 mg/dl

CC, circunferencia de la cintura; HDL-c, lipoproteína de alta densidad; IMC, índice de masa corporal; TG, triglicéridos; POTG, prueba oral de tolerancia a la glucosa.

[a]Esta definición considera a la circunferencia de la cintura una condición *sine qua non* para el síndrome metabólico.

Debido a que todavía no se ha llegado a un acuerdo común para establecer una definición de SM en poblaciones pediátricas puesto que los puntos de corte para cada uno de los factores de riesgo cardiovascular varían en función de la edad, el género y el estadío

puberal (39), diversos estudios epidemiológicos focalizados en factores de riesgo cardio-metabólicos en niños y adolescentes han utilizado diferentes estrategias para calcular un indicador continuo del SM (46). Tanto las variables incluidas en el indicador como el enfoque estadístico varían de forma considerable (46). A la hora de interpretar el indicador de SM, se considera que los valores bajos son indicativos de un mejor perfil mientras que valores elevados indicarían un perfil peor. Según Andersen et al. (47) la agrupación de los factores de riesgo cardiovascular en un solo indicador sería una mejor medida de la salud cardiovascular en poblaciones pediátricas que los factores de riesgo por sí solos. Además, el uso de este tipo de indicadores compensaría las fluctuaciones que se dan día a día en cada uno de los factores de riesgo de forma individual (48).

Por otro lado, está incrementando la evidencia que apoya el uso de un indicador continuo en lugar de un enfoque dicotómico puesto que la capacidad para mostrar asociaciones entre factores de exposición y variables dicotómicas del SM a través de regresión logística limitaría la fortaleza de la asociación (46). El hecho de que el riesgo cardiovascular es una función progresiva de varios factores de riesgo del SM, y que aumenta con el número de factores de riesgo del SM, respalda la importancia de un indicador de riesgo cardio-metabólico, puesto que considera a todos los componentes de forma independiente a la combinación de dos o tres de ellos, y asume que todos los factores de riesgo son igual de importantes y de responsables para definir el riesgo de SM o cardiovascular (46). Sin embargo, el uso de un indicador de riesgo continuo no está exento de limitaciones puesto que es específico de la muestra en la que se usa y no se puede comparar con otros estudios a no ser que las características sociodemográficas, la distribución de los datos, las medidas de tendencia central y la variabilidad sean similares en las dos muestras (46).

1.3 Relación entre el riesgo cardiovascular y los estilos de vida

Según la OMS, una proporción importante de las ECV podrían ser prevenidas con la modificación de sus factores de riesgo, principalmente a través de una dieta saludable y el control del peso, la actividad física, el uso limitado del alcohol y el tabaco, y de mantener la presión arterial y los lípidos plasmáticos dentro de los niveles normales (28). Los factores de riesgo relacionados con el estilo de vida son responsables de alrededor del 80% de las ECV, incluyendo las cerebrovasculares (28). Los efectos de una dieta no saludable y de la inactividad física se ponen de manifiesto con un incremento de la tensión arterial, aumento de la glucosa en sangre, lípidos plasmáticos elevados y sobrepeso y obesidad (28). Son estos factores "intermedios" los que indican un riesgo incrementado de desarrollar ECV como infartos, fallo cardiaco y otras complicaciones (28). Se ha observado que el dejar de fumar, la reducción del contenido de sal de la dieta, el consumo de frutas y vegetales, la actividad física regular y el evitar el uso dañino del alcohol reducen el riesgo cardiovascular en adultos (28). El riesgo cardiovascular también puede reducirse con la prevención o el tratamiento de los factores de riesgo "intermedios" (28). Sin embargo, si los estilos de vida, como la dieta y la actividad física, influyen o no en la expresión del SM o de los factores de riesgo cardiovascular en niños y adolescentes no se sabe todavía con certeza (49).

1.3.1 Dieta

Diversos estudios han puesto de manifiesto que la ingesta elevada de grasa y de azúcares añadidos junto con baja ingesta de fibra son factores de riesgo para el síndrome metabólico en adultos; sin embargo hay poca información disponible con respecto a la asociación entre dieta y riesgo cardiovascular o síndrome metabólico en poblaciones infantiles (50). Dado que las ECV parecen iniciarse durante la infancia (34) y que los patrones alimentarios que se forman durante la infancia tienden a persistir durante la adolescencia

(51) y hasta la edad adulta; las intervenciones dietéticas para prevenir la obesidad y los factores de riesgo de enfermedad crónica deberían estar dirigidas a la edad infantil (52).

Algunos estudios han investigado la asociación entre la dieta y los factores de riesgo cardiovascular en niños y adolescentes. Williams y Strobino (53) observaron que tras el seguimiento durante 4 años de niños neoyorquinos, la ingesta de energía estaba directamente relacionada con los niveles plasmáticos de colesterol total. Sin embargo, la ingesta de grasa monoinsaturada y de fibra dietética fueron variables protectoras del colesterol total. Así mismo, la ingesta de sacarosa mostró una asociación inversa con los niveles de HDL-c. La ingesta de hidratos de carbono se relacionó de forma adversa con la circunferencia de la cintura, los niveles de TG y glucosa en una muestra de niños norteamericanos de entre 7 y 12 años (49). En niños de origen latino con sobrepeso (50), se observó que la ingesta de fibra era significativamente mayor en aquellos participantes con ninguna característica del SM (5,2 gramos de fibra/día) comparados con aquellos con más de 3 características propias del SM (4,1 gramos de fibra/día). En el caso de los adolescentes, ingestas elevadas de fibra fueron relacionadas con el SM; sin embargo no se halló ninguna relación entre una baja ingesta de grasa o colesterol y el SM (54).

A pesar del gran interés y trascendencia que tienen los resultados mencionados anteriormente, cabe destacar que los nutrientes son consumidos habitualmente en conjunto a través de los alimentos que conforman nuestra dieta, y que, a la hora de dar recomendaciones a la población general, es mucho más sencillo hacerlo en términos de consumo de alimentos que de nutrientes. Por ello, cobran mayor interés aquellos estudios focalizados en la ingesta de alimentos y su relación con el SM o los factores de riesgo cardiovascular. La ingesta de bebidas azucaradas se relacionó directamente con mayores valores del índice HOMA (Homeostatic Model Assessment, medida de la resistencia de la insulina), de la tensión arterial sistólica, de la circunferencia de la cintura y del IMC y con valores más bajos de las concentraciones de HDL-c (55). En niños mejicanos de entre 9 a 13 años de edad, también se

observó una asociación directa entre la ingesta de bebidas azucaradas y los niveles plasmáticos de glucosa y de presión arterial diastólica (56). En este mismo estudio, las ingestas elevadas de pan blanco y de grasa añadidas estaban asociadas con mayores concentraciones de insulina y de TG, respectivamente. En el estudio llevado a cabo por Kelishadi et al. (57) en una población joven de entre 6 y 18 años, el riesgo de SM se incrementaba conforme aumentaba el consumo de grasa hidrogenada y de pan hecho con harina refinada. Por el contrario, cuanto mayor era la frecuencia de consumo de frutas, vegetales y productos lácteos, menor era el riesgo de padecer SM.

Tabla 4. Resumen de los estudios que han valorado la asociación entre ingesta de alimentos y síndrome metabólico o factores de riesgo cardiovascular en niños y adolescentes.

Estudio	Población (edad, género, país)	Tamaño de la muestra Diseño del estudio	Resultados
Kelishadi et al., 2008[57]	6-18 años Chicos y chicas Irán	4.811 sujetos Estudio transversal	↑ pan de harina refinada → ↑ SM ↑ fruta y vegetales → ↓ SM ↑ leche, queso, yogur → ↓ SM
Pan & Prat, 2008[58]	12-19 años Chicos y chicas de diferente etnias Estados Unidos	4.450 sujetos Estudio transversal	↑ fruta → ↓ SM ↑ Índice de Ingesta Saludable[a] → ↓ SM
Bremer et al., 2009[55]	12-19 años Chicos y chicas de diferentes etnias Estados Unidos	6.967 sujetos Estudio transversal	↑ bebidas azucaradas → ↑ HOMA, ↑ TAS, ↑ CC, ↑ percentil de IMC, HDL-c
Perichart-Perera et al., 2010[56]	9-13 años Chicos y chicas Méjico	228 sujetos Estudio transversal	↑ bebidas azucaradas → ↑ TAD, ↑ glucosa ↑ pan blanco → ↑ insulina ↑ grasas añadidas → ↑ TG ↑ lácteos de alto contenido graso → ↑ TAD, ↑ HDL-c ↑ fruta → ↑ glucosa ↑ aceites vegetales → ↓ glucosa ↑ carne → ↓ glucosa
Ambrosini et al., 2013[59]	13-17 años Chicos y chicas Australia	1.433 sujetos Estudio longitudinal	↑ bebidas azucaradas → ↑ obesidad, ↑ TG, ↓ HDL-c, ↑ riesgo cardio-metabólico

CC, circunferencia de la cintura; HOMA, homeostatic model assessment; HDL-c, lipoproteína de alta densidad; IMC, índice de masa corporal; SM, síndrome metabólico; TAD, tensión arterial diastólica; TAS, tensión arterial sistólica; TG, triglicéridos.
[a]Índice de Ingesta Saludable (Healthy Eating Index): incluye la ingesta de granos, vegetales, fruta, leche, carne/alternativas a la carne, grasa, grasa saturada, colesterol y sodio.

1.3.2 Actividad física

Es un hecho aceptado que la práctica regular de actividad física supone una medida preventiva efectiva para una gran variedad de factores de riesgo de enfermedad. Sin embargo, la transición que tiene lugar desde la infancia a la edad adulta marca una disminución muy llamativa en la práctica de actividad física que es dependiente de la edad (60-63).

Las recomendaciones de la Academia Americana de Pediatría (AAP) (64) establecen que los niños y los adolescentes deben de realizar una hora de ejercicio físico al día de intensidad moderada-intensa y no deben ver la televisión más de dos horas/día. Sin embargo, estas recomendaciones están muy lejos de la realidad y el aumento de la inactividad se está convirtiendo en un hecho especialmente importante en poblaciones pediátricas (65). Datos obtenidos a través del "*Canadian Health Measures Survey*" (66) ponen de manifiesto que solamente el 7% de los niños y adolescentes de entre 6 y 19 años participan en al menos 60 minutos de actividad física moderada e intensa al día, lo cual alcanzaría las recomendaciones de varios países (67-70) y de la OMS (71).

Pan et al. (58) observaron una menor prevalencia de SM en aquellos adolescentes con mayor práctica de actividad física. Los mismos resultados fueron mostrados por Brage et al. (72) en una muestra de niños daneses de entre 8 y 10 años. Con respecto a los factores de riesgo individuales, niveles elevados de actividad física en adolescentes norteamericanos se han asociado con una disminución de los valores del índice HOMA, de las concentraciones de LDL-C y TG, así como un aumento en la concentración de HDL-c (55). La actividad física total diaria llevada a cabo por niños de entre 7 y 12 años también se relacionó con una mayor concentración de HLC-c (49). Además, en un estudio llevado a cabo por Elekund et al. (73) en niños de 9 y 10 años y en adolescentes de 15 y 16 años, la actividad física fue inversamente relacionada con las concentraciones de insulina, glucosa, y TG y con los valores de tensión sistólica y diastólica, así como con el indicador de riesgo cardio-metabólico de forma

independiente de la adiposidad y de la variable "ver la televisión", como medida del comportamiento sedentario. Resultados similares fueron mostrados por Sardinha et al. (74) al observar que la actividad física estaba asociada a la resistencia a la insulina independientemente de la masa grasa central y total en niños portugueses de entre 9 y 10 años.

1.3.3 Comportamientos sedentarios

No es sólo motivo de alarma la disminución de la práctica de actividad física de la población, sino también el incremento de los comportamientos sedentarios. De hecho, se ha observado que algunos comportamientos sedentarios como ver la televisión o jugar a los videojuegos, entre otros, también están notablemente elevados durante la infancia y la adolescencia (75, 76). La actividad física y los comportamientos sedentarios son dos comportamientos totalmente contrarios; sin embargo, el hecho de que unos sean más sostenibles en el tiempo que otros (por ejemplo, los comportamientos sedentarios son más fáciles de ser sostenidos que la actividad física), hace que a menudo sean analizados de forma conjunta como comportamientos que co-ocurren en lugar de como acciones independientes entre sí (77).

Aparte de las recomendaciones de la AAP para la práctica de actividad física, este organismo también aconseja a los niños y adolescentes no ver la televisión más de dos horas/día (64). Fuentes diversas han señalado que tanto los niños como los adolescentes pasan la mayor parte del tiempo realizando actividades sedentarias (66, 78-83). Los jóvenes canadienses pasan una media de 8.6 horas al día, o el 62% del tiempo que están despiertos siendo sedentarios (66). Tendencias similares se han observado en los jóvenes norteamericanos quienes pasan un media de entre 6 y 8 horas por día siendo sedentarios (78-83).

Elekund et al. (73) observaron en niños (9-10 años) y adolescentes (15-16 años) europeos que la asociación entre el comportamiento sedentario "ver la televisión" y un indicador de riesgo cardio-metabólico estaba mediada por el grado de adiposidad. Según una revisión sistemática reciente, una disminución de tiempo en cualquier tipo de actividad sedentaria está asociada con menor riesgo de enfermedad en jóvenes de entre 5 y 17 años. Es más, pone de manifiesto que el incremento del tiempo de pantalla por día está asociado con un riesgo incrementado para los marcadores de SM y de ECV (65). De hecho, ver la televisión durante más de dos horas al día está asociado con altos niveles de colesterol mientras que 1,2 horas son suficientes para incrementar la tensión arterial sistólica (65). De forma general, datos procedentes de nueve estudios transversales indican que un tiempo de pantalla mayor a 2 horas/día está asociado con mayores niveles de tensión arterial y con un riesgo incrementado de padecer SM (65).

2. Objetivos

Los objetivos generales de la presente Tesis Doctoral son estudiar la validez de dos cuestionarios dietéticos para estimar la dieta de niños europeos, así como ampliar y mejorar el conocimiento científico sobre la relación entre los factores de riesgo cardiovascular con la dieta y los estilos de vida en niños y adolescentes europeos.

Los objetivos específicos de los ocho artículos que componen la Tesis Doctoral son los siguientes:

Artículo I. Evaluar el grado de validez de la información declarada por los padres acerca de la ingesta de energía en comparación con el gasto energético total medido con la técnica del agua doblemente marcada.

Artículo II. Comparar, específicamente por grupo de edad, la ingesta de alimentos declarada por los padres obtenida a través de la sección de frecuencia de consumo de alimentos del *Children's Eating Habits Questionnaire* con la ingesta estimada a través de dos recuerdos dietéticos de 24-horas no consecutivos.

Artículo III. Examinar la asociación entre la ingesta de aminoácidos y el perfil lipídico en adolescentes europeos y valorar si esta asociación es independiente de la ingesta total de grasa.

Artículo IV. Investigar la relación entre la ingesta de macronutrientes y el perfil lipídico en adolescentes europeos y valorar el papel de variables relacionadas con la masa grasa corporal en dicha asociación.

Artículo V. Investigar la relación entre el consumo de alimentos y el riesgo cardiovascular agrupado en niños europeos.

Artículo VI. Identificar los grupos de alimentos que mejor discriminan a aquellos sujetos con alto/bajo riesgo cardiovascular e investigar la relación entre el consumo de lácteos y los factores de riesgo cardiovasculares, tanto individuales como agrupados, en adolescentes.

Artículo VII. Identificar comportamientos relacionados con los estilos de vida agrupados (indicadores de la dieta, la actividad física y los comportamientos sedentarios) y examinar su asociación con factores de riesgo cardiovascular en niños entre 2 y 9 años.

Artículo VIII. Examinar la asociación entre el tiempo dedicado a ver la televisión o jugar con videojuegos y el riesgo cardio-metabólico agrupado en adolescentes.

2. Objectives

The general objectives of the present Doctoral Thesis are to evaluate the validity of two dietary assessment methods aimed to assess the diet of European children and to contribute to the scientific knowledge by addressing research gaps regarding the association of cardiovascular diseases risk factors with diet and lifestyle in European children and adolescents.

Specific objectives of each one of eight manuscripts included in this Doctoral Thesis are:

Manuscript I. To evaluate the validity of proxy-reported energy intake by comparison with total energy expenditure measured by the doubly labelled water technique.

Manuscript II. To compare, specifically by age group, proxy-reported food group estimates obtained from the food frequency section of the Children's Eating Habits questionnaire against the estimates of two non-consecutive 24-hour dietary recalls.

Manuscript III. To examine the relationship between amino acids intake and serum lipid profile in European adolescents, and to assess whether that association was independent of total fat intake.

Manuscript IV. To investigate the relationship between macronutrient intakes and serum lipid profile in European adolescents, and to assess the role of body fat-related variables on this association.

Manuscript V. To investigate food consumption in relation to clustered cardiovascular disease risk among European children.

Manuscript VI. To identify those food groups best discriminating individuals at high/low cardiovascular disease risk and to investigate the relationship between dairy consumption and cardiovascular disease risk factors (individual and scores) in adolescents.

Manuscript VII. To identify clustered lifestyle behaviours (dietary, physical activity and sedentary behaviours indicators) and to examine their association with cardiovascular disease risk factors in children aged 2-9 years.

Manuscript VIII. To examine the association between time engaged in television viewing and/or playing with videogames and a clustered cardio-metabolic risk in adolescents.

3. Materiales y Métodos [Materials and Methods]

La presente Tesis Doctoral está basada en datos procedentes de los estudios IDEFICS (Artículos I, II, IV y VI) y HELENA (Artículos III, V, VII y VIII).

3.1. Comités de Ética

Estudio IDEFICS (Artículos I, II, V, VII)

El protocolo del estudio se desarrolló según la normativa española y siguiendo las consignas éticas establecidas por la Declaración de Helsinki en 1975 (revisión de Edimburgo en 2000). Dicho protocolo fue aprobado por el Comité de Ética de cada centro en el que se llevó a cabo estudio. En el caso de Zaragoza, éste fue aprobado por el Comité Ético de Investigación Clínica de Aragón (CEICA). Finalmente, los padres de los niños que participaron en el estudio entregaron un consentimiento firmado para participar en el mismo.

Estudio HELENA (Artículos III, IV, VI, VIII)

El protocolo del estudio se desarrolló según la normativa española y siguiendo las consignas éticas establecidas por la Declaración de Helsinki en 1975 (revisión de Edimburgo en 2000). Dicho protocolo fue aprobado por el Comité de Ética de cada centro en el que se llevó a cabo estudio. En el caso de Zaragoza, éste fue aprobado por el Comité Ético de Investigación Clínica de Aragón (CEICA). Además, tanto adolescentes como padres entregaron un consentimiento firmado para participar en el estudio.

3.2. Muestra y diseño del estudio

Estudio IDEFICS (Artículos I, II, V, VII)

El estudio IDEFICS es un estudio de cohortes, prospectivo y multicéntrico que se llevó a cabo en ocho países europeos (Italia, Estonia, Chipre, Bélgica, Suecia, Alemania, Hungría y España). En primer lugar, se seleccionaron una zona intervención y una zona control en cada país que fueran comparables en cuanto a sus características sociodemográficas, socioeconómicas y de infraestructura. El contacto con los participantes se realizó a través de las escuelas y las guarderías, para así facilitar su participación en el estudio y la posterior implementación y seguimientos de las actividades relacionadas con la intervención. El tamaño de muestra se estableció inicialmente en 16.000 niños (2.000 por país) distribuidos equitativamente por género, curso escolar y región. Para que un sujeto fuera válido debía haber completado el cuestionario de padres y tener medidas completas de la altura y del peso. La muestra final comprendió a 16.224 niños de entre 2 y 9 años. Para los artículos que se incluyen en esta Tesis Doctoral se han utilizado los datos obtenidos durante el estudio transversal que se llevó a cabo durante el curso académico 2007-2008.

La muestra utilizada en el **artículo I** se obtuvo a través de un estudio de validación específico que se llevó a cabo en el seno del estudio IDEFICS en tres países únicamente: España, Bélgica y Suecia, por ello la muestra es de 36 niños de entre 4 y 10 años en los que se valoró el gasto total energético a través de la técnica del agua doblemente marcada.

Al tratarse el **artículo II** de un estudio de validación de un cuestionario dietético, únicamente se incluyeron a aquellos niños cuyos padres habían completado un CFCA y dos recuerdos dietéticos de 24-horas, con lo que la muestra final fue de 2.508 niños.

La muestra en la que se basan los **artículos V** y **VII** es de 5.448 y 4.619 niños, respectivamente, puesto que se recogieron muestras de sangre en un 79.7% de los niños que participaron en el estudio IDEFICS. Las diferencias en el tamaño de muestra entre el **artículo**

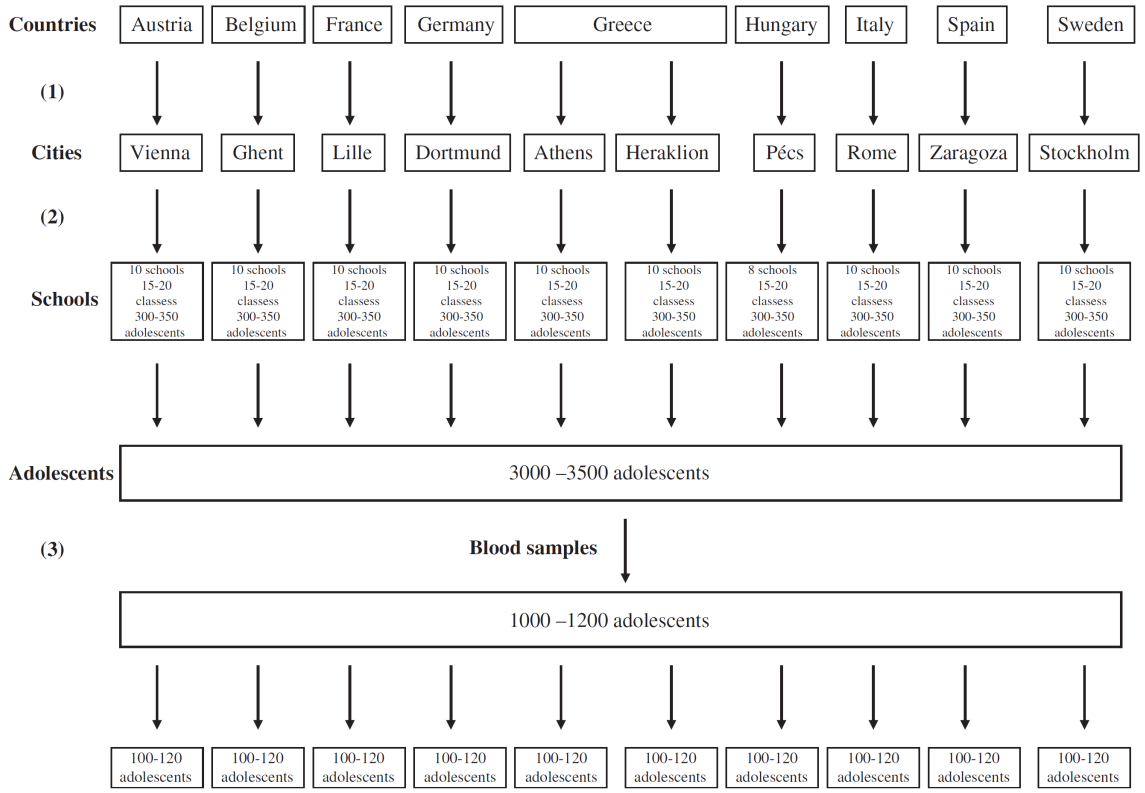
V y el **VII** es debida a la presencia de valores perdidos en alguna de las variables de análisis y/o criterios de inclusión específicos establecidos en cada artículo.

La metodología completa del estudio ya ha sido previamente publicada de forma más detallada (84). Los aspectos más relevantes en relación con la Tesis se describen a continuación.

Estudio HELENA (Artículos III, IV, VI, VIII)

El estudio HELENA es un estudio transversal y multicéntrico que se llevó a cabo en diez ciudades europeas: Dortmund (Alemania), Viena (Austria), Gante (Bélgica), Lille (Francia), Atena y Heraklion (Grecia), Pécs (Hungría), Roma (Italia), Estocolmo (Suecia) y Zaragoza (España) durante los años 2006-2007. Para la selección de la muestra se llevó a cabo un muestreo aleatorio por conglomerados para conseguir una muestra de 3.000 adolescentes de 12,5 a 17,5 años, estratificados según la localización geográfica, la edad y el nivel socioeconómico. Fueron invitados a participar todos los alumnos de entre una selección de clases de entre todas las escuelas presentes en las 10 ciudades europeas, todas ellas mayores de 100.000 habitantes. Los criterios de inclusión del estudio HELENA fueron los siguientes: los participantes no debían de estar participando simultáneamente en otro estudio clínico; haber estado enfermo durante la semana anterior a la toma de medidas; tener entre 12,5 a 17,5 años; haber firmado el consentimiento informado, tener medidas del peso y de la altura y haber completado al menos el 75% del resto de pruebas. Finalmente, la muestra comprendió un total de 3.528 adolescentes de entre 12,5 a 17,5 años.

Figura 2. Esquema sobre el proceso de muestreo utilizado en en estudio HELENA (85).



Según el protocolo del estudio HELENA, se recogieron muestras de sangre en 1/3 de la muestra del estudio, elegida al azar (n=1.089). Tras la obtención de dichas muestras, se analizaron diversos parámetros bioquímicos: glucosa, insulina, TG, colesterol total, HDL-c, lipoproteína de baja densidad (LDL-c), apolipoproteína A1 y apolipoproteína B. Por otro lado, la dieta fue registrada mediante dos recuerdos dietéticos de 24-horas. Lamentablemente, debido a que los datos de ingesta dietética obtenidos en Pécs y en Heraklion eran incompletos, tuvieron que ser excluidos de los análisis. Por ello, la muestra en la que se basan los **artículos III, IV** y **VI** es de 511 adolescentes (si bien puede existir alguna variación debido a la presencia de valores perdidos en alguna de las variables de análisis y/o criterios de inclusión establecidos en cada artículo). La muestra del **artículo VIII** fue mayor (n=769) debido a que no se incluyeron variables relacionadas con la dieta.

Las características generales del estudio ya han sido publicadas previamente con detalle (85). Los aspectos más relevantes en relación con la Tesis se describen a continuación.

3.3. Métodos de medida

Estudio IDEFICS (Artículos I, II, V, VII)

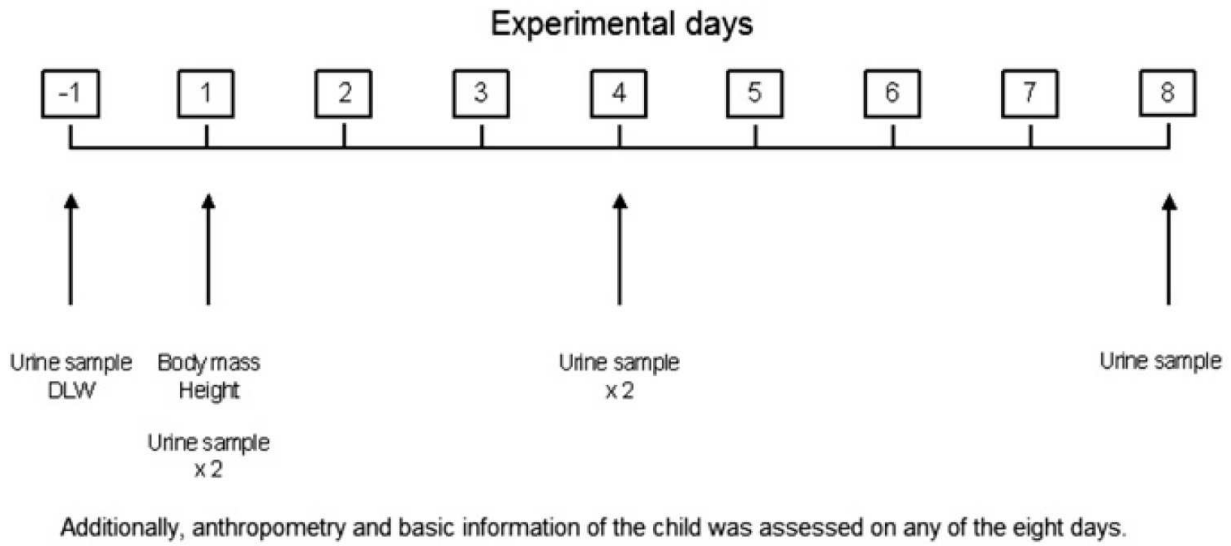
3.3.1 Factores sociodemográficos (Artículos V, VII)

Para el análisis de los factores socio-demográficos se recogieron datos sobre la edad, el género, la educación de los padres y el trabajo de los padres. Para evitar las diferencias que existen en los países a la hora de establecer el nivel socioeconómico, se usó la Clasificación Internacional Normalizada de la Educación (ISCED) (86). Se tuvo en cuenta el mayor nivel educativo alcanzado por cualquiera de los dos padres.

3.3.2 Gasto energético total (Artículo I)

El GET fue medido durante un periodo de 9 días (desde el Día -1 al Día 8) por medio de la técnica del agua doblemente marcada siguiendo el protocolo de Maastricht (87). La **Figura 1** muestra el esquema que se siguió para realizar las mediciones. Cada niño recibió una dosis única de agua doblemente marcada basada en su peso (ml de agua doblemente marcada/kg) compuesta por 250-300 partes por millón (ppm) de Oxígeno-18 y por 125-150 ppm de Deuterio. Se obtuvieron diversas muestras de orina de cada niño. La primera se obtuvo unos minutos antes (máximo 15 minutos) de la ingesta de la dosis de agua doblemente marcada (orina de referencia) y el resto se recogieron en los Días 1, 4 y 8. Cada muestra de orina fue recogida a la misma hora del día (± 1 hora) en todos los niños en función de sus horarios y de sus necesidades fisiológicas. Tras su recogida, las muestras fueron congeladas a -20 ºC y mandadas al laboratorio central situado en Maastricht para su análisis con espectrometría de masas. El GET fue calculado de acuerdo a la fórmula de Schoeller (88).

Figura 1. Esquema del protocolo de medida del estudio de validación de IDEFICS (89).



3.3.3 Dieta e ingesta de energía (Artículos I, II, V, VII)

1. Recuerdo dietético de 24-horas: la ingesta de alimentos y de energía fue estimada a partir de un recuerdo dietético de 24-horas electrónico llamado SACINA (Self Administered Children and Infants Nutrition Assessment), basado en un software previamente diseñado para su uso en adolescentes llamado HELENA-DIAT (Dietary Assessment Tool) (90, 91). Se trata de un programa estructurado de acuerdo a 6 comidas (desayuno, almuerzo, comida, merienda, cena y recena) incorporadas dentro de una serie de preguntas realizadas en orden cronológico sobre actividades diarias (90-93).

Las entrevistas fueron completadas por los padres con la ayuda del personal del estudio y cada entrevista tenía una duración aproximada de 20-30 minutos y debían de registrar todos los alimentos y bebidas que su hijo/a había consumido durante el día anterior. Las ingestas que tenían lugar en el colegio, se registraban por el personal del estudio mediante observación directa. El tamaño de las porciones se estimó principalmente mediante fotos de tamaños de porciones, porciones estándar, tamaños

de envasado habituales y alimentos troceados o en lonchas para así disminuir el error relacionado con la estimación de las cantidades.

La ingesta de energía se obtuvo a partir de tablas de composición de alimentos (TCA) propias de cada país debido a la ausencia de una TCA válida para ser usada en toda Europa. Para minimizar el error, se adoptaron directrices y procedimientos comunes para armonizar las bases de datos de nutrientes de distintos los países.

Tanto en el **artículo I** como en el **II,** únicamente se incluyeron aquellos niños con dos recuerdos dietéticos de 24-horas recogidos en días no consecutivos y distribuidos a lo largo de la semana para recoger información de días de entre semana y de fin de semana. Las ingestas de alimentos y de energía se obtuvieron mediante el cálculo de la ingesta media de los dos días.

2. Cuestionario de frecuencia de consumo de alimentos: se utilizó un CFCA, llamado *Children's Eating Habits Questionnaire-food frequency section* (CEHQ-FFQ), que fue diseñado como un instrumento de medida de lo*s* comportamientos alimentarios de los niños relacionados con el riesgo de sobrepeso, obesidad y con la salud en general. Los padres eran los encargados de rellenar el cuestionario en casa y debían aportar información sobre el número de veces que su hijo/a había consumido los grupos de alimentos incluidos en el cuestionario durante una semana típica del mes anterior. El CEHQ-FFQ consistía en 14 grupos de alimentos: vegetales, frutas, bebidas, cereales de desayuno, leche, yogur, pescado, huevo, carnes y productos cárnicos, productos a base de soja y/o sustitutivos de la carne, queso, productos para untar (mermelada, miel, mantequilla, etc.), cereales (pan, pasta, arroz, etc.) y aperitivos o snacks (frutos secos, dulces, pasteles, chocolate, palomitas de maíz, ganchitos, etc.). Para facilitar las repuestas a los padres, se adoptó una escala utilizada en un estudio previo (94) y que incluía las siguientes categorías de consumo: "nunca/menos de una vez por semana",

"1-3 veces por semana", "4-6 veces por semana", "1 vez al día", "2 veces al día", "3 veces al día", "4 o más veces al día" y "no lo sé". El tamaño de las porciones no fue estimado.

3.3.4 Examen físico (Artículos I, V, VII)

Todas las medias fueron tomadas por personal del estudio entrenado previamente para ello. El peso (kg) se midió en ropa interior con una báscula electrónica (TANITA BC 420 SMA) y la altura (cm) se midió sin zapatos mediante un estadiómetro. Los pliegues cutáneos se midieron por duplicado a través de un lipómetro (Holtain Ltd., Croswell, UK). La tensión arterial se midió a través de un esfingomanómetro electrónico (Welch Allyn 4200B-E2) en el brazo derecho del niños. Se tomaron dos medidas en un intervalo de 2 minutos, y en el caso en el que hubiera una diferencia mayor al 5% entre las dos medidas, se tomaba una tercera medida.

3.3.5 Actividad física (Artículos V, VII)

La información sobre actividad física (AF) fue obtenida mediante un cuestionario auto-administrado a los padres con las siguientes preguntas: "¿Su hijo/a es miembro de algún club deportivo?". A lo que los padres debían de responder "sí" o "no". En el caso de que hubieran contestado "sí", se les preguntaba "¿Cuánto tiempo (en horas) pasa a la semana haciendo ejercicio en un club deportivo?". La respuesta era abierta para que pudieran determinar el número de horas y minutos invertidos en la actividad.

3.3.6 Comportamientos sedentarios (Artículos V, VII)

A través de un cuestionario, se les preguntó a los padres: "¿Cuánto tiempo suele ver su hijo/a la televisión/vídeos/DVDs por día?". Las respuestas estaban divididas para los días de entre semana y los de fin de semana e incluían 5 categorías: "nada en absoluto", "<30 minutos al día", "<1 hora al día", "1-2 horas al día", "2-3 horas al día", y ">3 horas al día". La media de horas al día de televisión/videos/DVDs vistas a la semana se calcularon de la siguiente manera: [(horas/día entre semana × 5) + (horas/día en fin de semana × 2)]/7.

3.3.7 Muestras biológicas (Artículos V, VII)

Las muestras de sangre se obtuvieron después de 8 horas de ayuno. Las concentraciones sanguíneas de TG, colesterol total, HDL-c y glucosa fueron determinadas *in situ*, unos minutos después de que la extracción tuviera lugar con un aparato denomindado Cholestech LDX (Cholestech LDX analyzer, Cholestech Corp., Hayward, CA, USA). Por otro lado, las concentraciones de insulina se valoraron en el laboratorio central situado en Dortmund (Alemania) a través de inmnunoensayo de luminiscencia (Immulite 2000, Siemens, Eschborn, Germany). La resistencia a la insulina se definió a través del índice HOMA (95) y se calculó a través de una fórmula estándar usando los niveles en ayunas de glucosa y de insulina plasmática: HOMA= [insulina (µUI/ml) × glucosa (mg/dl)]/405.

Estudio HELENA (Artículos III, IV, VI, VIII)

3.3.8 Factores socio-demográficos (Artículos II, III, VI, VIII)

Se recogió información relativa al género y a la edad. En cuanto al nivel socieconómico, en el **artículo VI** se valoró a través de la escala de abundancia familiar (Family Affluence Scale, FAS) (96) basada en el concepto de las condiciones materiales de la familia. Para los **artículos III**, **IV** y **VIII** se tuvo en cuenta el máximo nivel educativo obtenido por la madre puesto que esta variable ha sido relacionada fuertemente con algunos factores no saludables como la adiposidad tanto en niños como en adolescentes (97).

3.3.9 Dieta e ingesta de energía y nutrientes (Artículos II, III, VI)

La dieta fue estimada mediante un recuerdo dietético de 24 horas electrónico y autoadministrado llamado HELENA-DIAT, el cual está basado en un programa informático apropiado para valorar la dieta de adolescentes flamencos llamado YANA-C (90, 91). Se obtuvieron dos recuerdos dietéticos de 24 horas no consecutivos durante un periodo de tiempo de dos semanas. Los adolescentes completaron los cuestionarios durante el horario lectivo y con la ayuda de los miembros del estudio. Por esta razón, no hay información disponible de viernes y sábados. El consumo habitual de 31 grupos de alimentos (gramos/día) fue estimado mediante el programa informático *multiple source method* (MSM) para tener en cuenta la variación intra-individual y entre-individuos de la dieta (98). También cabe destacar que los datos procedentes de Pécs (Hungría) y Heraklion (Grecia) fueron excluidos debido a que no estaban completos.

Para calcular las ingestas de energía y nutrientes se usó la tabla de composición de alimentos de Alemania (German Food Code and Nutrition Data Base) como las más completa de toda Europa en cuanto a número de nutrientes y alimentos (99). La ingesta de energía se expresa como kilocalorías por día (kcal) en todos los artículos. En el caso de los nutrientes, en el **artículo II** la ingesta de proteínas y grasa se expresa en gramos/día y la de aminoácidos

como miligramos/día. En el **artículo III**, las ingestas de macronutrientes se expresan como gramos/1000 kilocalorías.

3.3.10 Examen físico (Artículos II, III, VI, VIII)

Todas las medidas se realizaron siguiendo las pautas internacionales en adolescentes (100-103) Los sujetos fueron tallados y pesados descalzos y en ropa interior. El peso (kg) se midió con una báscula electrónica (Seca 861), mientras que la altura se midió con un estadiómetro (Seca 225). Los pliegues cutáneos bicipital, tricipital, subescapular y suprailiaco se tomaron por triplicado en la parte izquierda de los participantes con un lipómetro (Holtain Calliper, Crymmych, UK). La circunferencia de la cintura se midió en el punto medio entre la costilla más baja y la cresta iliaca con una cinta métrica (SECA 200). La presión arterial se midió dos veces, con un intervalo de 10 minutos entre las dos mediciones, con un esfingomanómetro automático (M6, HEM-7001-E, Omron).

3.3.11 Maduración sexual (Artículos II, III, VI, VIII)

En cada centro, un médico designado para tal propósito realizó un breve examen físico para determinar en cual de los cinco estadíos de maduración sexual definidos por Tanner y Whitehouse (104) se encontraba cada adolescente.

3.3.12 Actividad física (Artículos II, III, VI, VIII)

La actividad física se midió de forma objectiva mediante el uso de acelerómetros uniaxiales (Actigraph GT1M, Manufacturing Technology Inc. Pensacola, FL, USA). Los adolescentes debían de llevar el acelerómetro en la zona lumbar y debajo de la ropa durante siete días. Además, fueron informados de que debían de quitarse el acelerómetro para dormir y para realizar actividades acuáticas. Además, debían de rellenar un diario con las horas a las que se quitaban y se ponían el aparato así como el motivo. Todos los acelerómetros se configuraron para registrar datos cada 15 segundos (epoch). Se estableció como criterio de inclusión el que hubiera datos válidos de al menos tres días con un mínimo de ocho horas de

registro. El tiempo invertido en actividad física de intensidad moderada-intensa (> 3 equivalentes metabólicos) se calculó en función de un punto de corte de ≥ 2000 "counts" por minuto.

3.3.13 Comportamientos sedentarios (Artículos II, III, VI, VIII)

El tiempo invertido por los adolescentes en dos comportamientos sedentarios (ver la televisión y jugar a los videojuegos) se utilizó como medida de actividades sedentarias preguntando a los adolescentes: "Durante los días de entre semana/fin de semana, ¿cuántas horas dedicas a ver la televisión o jugando con los videojuegos?". Debían elegir una categoría dentro de las siguientes opciones: 1) 0 minutos, 2) >0-30 minutos, 3) >30-60 minutos, 4) >60-120 minutos, 5) >120-180 minutos, 6) >180-240 minutos, y 7) >240 minutos. Además, los adolescentes registraron si tenían televisión o consolas en su habitación. Este cuestionario mostró buena fiabilidad (105) cuando ésta fue analizada.

3.3.14 Muestras biológicas (Artículos II, III, VI, VIII)

Las muestras de sangre se obtuvieron en ayunas en un tercio de los participantes del estudio seleccionados al azar. El protocolo que se utilizó para procesar y analizar las muestras ha sido descrito previamente (106). Los TG, el colesterol total, HDL-c, LDL-c y la glucosa fueron valorados por medio de métodos enzimáticos (Dade Behring, Schwalbach, Germany). Los niveles de insulina fueron determinados con un analizador Immulite 200 (DPC Bierman GmbH, Bad Nauheim, Germany). Las apolipoproteínas A1 y B se analizaron por medio de reacción inmunoquímica con un analizador BN II (Dade Behring, Schwalbach, Germany). El índice HOMA (95) también se usó como medida de la resistencia a la insulina.

Estudio IDEFICS y estudio HELENA: medidas comunes

3.3.15 Condición Física (Artículos V, VII, VIII)

Como medida de condición física se utilizó la capacidad aeróbica (ml/kg/min) medida con el test llamado *shuttle run test* o *Course Navette*. Durante la prueba, los participantes tenían que correr entre dos líneas separadas por 20 metros de distancia siguiendo unos pitidos cuya frecuencia aumentaba de forma gradual (0.5 km/h per min). El test acababa cuando los participantes paraban debido al cansancio o a que no alcanzaban la línea con el pitido durante dos veces seguidas. La capacidad aeróbica fue estimada mediante la fórmula de Léger et al. (107) usando la velocidad máxima que el participante alcanzó durante el test.

3.3.16 Indicador de riesgo cardiovascular

Como medida del riesgo cardiovascular, se calculó un indicador previamente sugerido por Andersen et al. (48) mediante la agrupación de diversos factores de riesgo cardiovascular: tensión arterial sistólica, TG séricos, índice colesterol total/HDL-c, índice HOMA, y suma de dos pliegues (tricipital y subescapular). En el caso de los adolescentes del estudio HELENA, la suma de pliegues incluyó cuatro pliegues: bicipital, tricipital, subescapular y suprailiaco. Cada factor de riesgo incluido en el indicador fue normalizado según el género y el grupo de edad. Los valores normalizados de todos los factores de riesgo fueron sumados para obtener el indicador de riesgo de enfermedad cardiovascular. La capacidad aeróbica fue multiplicada por -1 para indicar que conforme aumentaban sus valores, aumentaba también el riesgo cardiovascular. Cuanto más bajo era el indicador de riesgo cardiovascular, mejor era el perfil general de salud cardiovascular.

3.4 Análisis estadísticos: consideraciones generales

Las características descriptivas de los sujetos se presentan en forma de porcentajes para variables nominales, como media y desviación estándar para las variables continuas. En las variables continuas que no presentan una distribución normal se muestran la mediana y los percentiles 25 y 75. Las diferencias entre sexos y grupos de edad de variables continuas se analizaron mediante *análisis de la varianza* (ANOVA, con el sexo y la edad como factores fijos) o *test de muestras independientes* (t de Student). En el caso de las variables que carecían de una distribución normal, se usó el *test U de Mann-Whitney* para determinar las diferencias entre sexos. Las variables nominales se analizaron mediante el *test de Chi-cuadrado*.

El *análisis de la covarianza* (ANCOVA) junto con el *test de Bonferroni* **(Artículos IV, VI** y **VII)** se usó para analizar diferencias entre factores de riesgo cardiovascular en función de grupos de edad, género, clusters y terciles de ingesta, controlando los análisis por una serie de covariables.

El *análisis de regresión logística binaria* **(Artículo V)** se utilizó para analizar la asociación entre el indicador agrupado de riesgo cardiovascular con la ingesta de alimentos como variables independientes.

El *análisis de regresión lineal* **(Artículos VI, VII)** se aplicó para analizar la asociación entre los factores de riesgo cardiovascular y el indicador agrupado y la ingesta de productos lácteos **(Artículo VI)** y los clusters de estilo de vida **(Artículo VII)**.

El *análisis de regresión lineal multinivel* se usó para estudiar la asociación entre los lípidos plasmáticos y la ingesta de aminoácidos **(Artículo III)** y la ingesta de macronutrientes **(Artículo IV)** para tener en cuenta la variabilidad de los individuos según su país de procedencia. Por la misma razón se utilizó el *análisis de regresión de Poisson multinivel* **(Artículo VIII)** para valorar la asociación entre el indicador agrupado de riesgo cardio-metabólico y las variables de comportamiento sedentario.

El *análisis discriminante* **(Artículo VI)** se aplicó para averiguar qué alimentos eran capaces de discriminar mejor a aquellos sujetos con alto riesgo cardiovascular de aquellos con bajo riesgo.

El *análisis de conjuntos o clusters* **(Artículo VII)** se utilizó para crear agrupaciones de comportamientos relacionados con los estilos de vida. Para ello se utilizaron cuatro indicadores (consumo de frutas, verduras y bebidas carbonatadas azucaradas, práctica de actividad física y consumo de televisión/vídeo/DVD) cuyas combinaciones dieron lugar a los distintos clusters en función del género y del grupo de edad.

En el **artículo II** se usaron métodos estadísticos específicos y propios de los estudios de validación, como el *coeficiente de correlación de Pearson*, para determinar la intensidad de la relación entre las ingestas de los dos métodos de valoración de la dieta, y *tablas de contingencia* y *kappa ponderada* para valorar el grado de acuerdo entre los cuestionarios. Tanto en el **artículo I** como en el **artículo II** se obtuvieron los gráficos de *Bland-Altman* como medida adicional de concordancia entre los dos métodos de medida de la dieta.

Todos los análisis son mostrados separadamente por sexo, excepto en el **artículo IV** puesto que no se observó una interacción por sexo, y, en el caso de los **artículos II**, **V** y **VII**, también por grupos de edad (2-< 6 años y 6-9 años). Los análisis estadísticos se llevaron a cabo usando los paquetes estadísticos SAS (**Artículo I**), PASW versión 18 (**Artículos III, IV, V, VI, VII**) y STATA versiones 11 y 12 (**Artículos III, IV y VIII**). Como norma general, el nivel de significación se estableció en el 5%. En cada uno de los artículos que componen la presente Tesis Doctoral aparece información mucho más detallada acerca del proceso estadístico empleado.

4. Resultados

Los resultados y discusión de la presente Tesis Doctoral se muestran en forma de artículos científicos.

4. Results

The results and discussion of this Doctoral Thesis are shown as research manuscripts.

Artículo I [Paper I]:

Validity of 24-h recalls in (pre-)school aged children: Comparison of proxy-reported energy intakes with measured energy expenditure

Börnhorst C, Bel-Serrat S, Pigeot I, Huybrechts I, Ottavaere C, Sioen I, De Henauw S, Mouratidou T, Mesana MI, Westerterp K, Bammann K, Lissner L, Eiben G, Pala V, Rayson M, Krogh V, Moreno LA, on behalf of the IDEFICS consortium

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Original article

Validity of 24-h recalls in (pre-)school aged children: Comparison of proxy-reported energy intakes with measured energy expenditure☆,☆☆

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SUMMARY

Background & aims: Little is known about the validity of repeated 24-h dietary recalls (24-HDR) as a measure of total energy intake (EI) in young children. This study aimed to evaluate the validity of proxy-reported EI by comparison with total energy expenditure (TEE) measured by the doubly labeled water (DLW) technique.
Methods: The agreement between EI and TEE was investigated in 36 (47.2% boys) children aged 4–10 years from Belgium and Spain using subgroup analyses and Bland–Altman plots. Low-energy-reporters (LER), adequate-energy-reporters (AER) and high-energy-reporters (HER) were defined from the ratio of EI over TEE by application of age- and sex-specific cut-off values.
Results: There was good agreement between means of EI (1500 kcal/day) and TEE (1523 kcal/day) at group level though in single children, i.e. at the individual level, large differences were observed. Almost perfect agreement between EI and TEE was observed in thin/normal weight children (EI: 1511 kcal/day; TEE: 1513 kcal/day). Even in overweight/obese children the mean difference between EI and TEE was only −86 kcal/day. Among the participants, 28 (78%) were classified as AER, five (14%) as HER and three (8%) as LER.
Conclusion: Two proxy-reported 24-HDRs were found to be a valid instrument to assess EI on group level but not on the individual level.

1. Introduction

Dietary intake has been recognized to be related not only to normal growth, but also to the development and progression of chronic diseases that start early in life.[1] However, accurate assessment of dietary information is problematic - especially among children.[2–5] These lack the ability to report their own intake, so that data in children younger than seven years mainly rely on proxy-reports.[6] Furthermore, children's diets tend to be highly variable from day-to-day and their food habits change rapidly during childhood which makes dietary assessment a challenging issue.[7]

Abbreviations: EI, energy intake; TEE, total energy expenditure; DLW, doubly labelled water; 24-HDR, 24-h dietary recall; LER, low-energy-reporters; HER, high-energy-reporters; AER, adequate energy reporters.

☆ 1. Jahrestagung der Deutschen Gesellschaft für Medizinische Informatik, Biometrie und Epidemiologie (GMDS) und der Deutschen Gesellschaft für Epidemiologie (DGEpi). Mainz (Germany), 2011.

☆☆ 2. 11th European Nutrition Conference (FENS). Madrid (Spain), 2011.

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The validity of a dietary assessment method can be evaluated by comparing reported energy intake (EI) to measured total energy expenditure (TEE)[5] because TEE and EI can be assumed to be equal for individuals in energy balance.[3] This assumption is also justified in children as energy cost of growth and development during childhood is very small, i.e. 1–2%.[4] Doubly labeled water (DLW) is considered the "gold-standard" method to assess TEE.[2]

Validation studies with DLW in children and adolescents have shown self-reported (partly with parental assistance) 24-h dietary recalls (24-HDR) to be a valid measure of EI at least on group level, though misreporting of EI is a common problem especially in overweight/obese study populations.[8] Results concerning misreporting in children and adolescents vary widely among studies (underreporting from 19% to 41%; overreporting from 7% to 11% of reported EI)[9] and yet it is unknown whether the study participants' weight status is predictive for misreporting in data relying on proxy-reports as well. Moreover overweight/obese study subjects may be more likely than thin/normal weight subjects to be on an energy-restricted diet, i.e. to actually eat less than physiologically required (EI < TEE; undereating) which complicates the evaluation of reported EI. Apart from total EI, nutrient intakes such as fat, sugar or micronutrients are commonly misreported which was shown to result in flawed associations between food/nutrient intakes and body weight.[10]

To date, little is known about the validity of 24-HDR data obtained by parents acting as surrogate reporters. Therefore the present study aims to investigate the validity of 24-HDR data in four-to-ten-year-old children by comparison of proxy-reported EI with objectively measured TEE.

2. Materials and methods

2.1. Sample

The present validation study was conducted within the framework of the IDEFICS ("Identification and prevention of dietary- and lifestyle- induced health effects in children and infants") study from October 2008 to July 2009. It is based on a convenience sample of four-to-ten-year-old children from three different centres (Belgium, Sweden, Spain).[11] The burden for participating children and their parents was deemed to be too high to justify a random sample. Belgium and Spain recruited children through schools, newspapers or by asking colleagues or friends. In contrast, a subgroup of ten obese Swedish children was recruited from an obesity clinic which underwent the same protocol. Although these children were weight stable at the time of the validation study, they were excluded from the main analysis presented here as they were selected from a clinical setting and hence this would have limited the generalisability of the results. The Swedish children were considered in a subgroup analysis only.

Furthermore, only children with complete information on age, sex, height, weight, at least two 24-HDR as well as DLW measurements were included in the present study resulting in 36 children (six from Belgium, thirty from Spain). The study was approved by the appropriate local ethics committees in each centre and written informed consent was obtained from the parents before participation.

2.2. Total energy expenditure measurement

TEE was measured over a nine-day period (from Day −1 to Day 8) by means of DLW following the Maastricht protocol.[12] The measurement schedule is summarised in Fig. 1. Each child was given a single oral dose of DLW based on his/her body weight (ml DLW/kg), increasing background levels of Oxygen-18 with

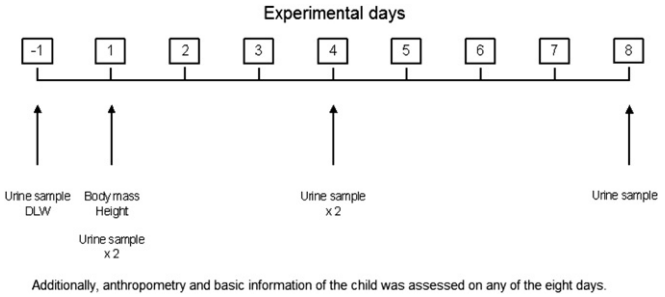

Fig. 1. Measurement schedule of the IDEFICS validation study.[11]

250–300 parts per million (ppm) and background levels of Deuterium with 125–150 ppm. Urine samples were obtained from each child several minutes (maximum 15 min) before ingesting the DLW dose (baseline urine), and on Days 1, 4, and 8 after the DLW dose. Each urine sample was collected at the same time of the day (±1 h) in all children depending on their timetable and physiological needs. Parents were asked to record the collection time for each urine sample. After collection of urine samples, 2 ml from each urine sample were transferred into two individual glass vials and kept frozen at −20 °C at each study centre. Urine samples were sent as one batch directly from all centres to the central laboratory at the end of data collection. Samples were analysed by isotope ratio mass spectrometry with an analytic precision of 0.2 ppm for ^{2}H isotope and 0.4 ppm for ^{18}O isotope. All analytical tests were performed in the Human Biology Department, University of Maastricht (The Netherlands). The value of 0.85 was used as an estimate of the respiratory quotient, based on the consumption of a standard Western diet,[13] and TEE was calculated according to Schoeller et al.[13]

2.3. Energy intake measurement

During the DLW measurement period at least two 24-HDR per child were recorded. EI was assessed using a computerised 24-HDR called SACINA (Self Administered Children and Infants Nutrition Assessment) that is based on the previously designed software YANA-C developed and validated for Flemish adolescents.[14] Within the framework of the IDEFICS study, this 24-HDR was adapted for assessment of proxy-reported dietary intakes in young children.[15] The SACINA is structured according to six meal occasions (breakfast, morning snack, lunch, afternoon snack, dinner, evening snack) embedded within chronological questions related to daily activities aiming to help proxies, mainly the parents, to recall their child's intakes of the previous day.[14] Portion sizes were assessed by photos of serving sizes, standard portions, customary packing size and foods in pieces or slices that were displayed on the screen. For every meal, parents were asked to select all food items their child had eaten at the specific occasion and, for each item, the respondents typed the amount consumed or modified the standard serving size with two command buttons (more/less).

The 24-HDR interviews were completed by proxies under supervision of fieldwork personnel. On weekdays, school meals were additionally assessed either through parents or by means of direct observation ($N = 18$). In the latter case, data was documented by survey personnel using pre-defined recording sheets specifically designed for that purpose. Based on these sheets, the observer indicated the amount eaten by the child. Pre-defined portion sizes ranged from "nothing", "¼ portion", "½ portion", "1 portion", "1 ½ portions", "2 portions" up to "more than 2 portions". Different standard measures like hands-full, slices, table spoons, etc. were given depending on the regarded food item. School meal data

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were merged with the parentally reported 24-HDR data to enhance completeness of dietary intakes. Although up to three repeated 24-HDR were carried out on non-consecutive days, only two recalls per child were used in the current analyses to achieve an equal number of 24-HDR per child. In general, the first and second recall day was used. If both recalls were assessed on a weekday but the third one on a weekend day, the weekend day was chosen to increase the number of children having one weekday and one weekend day. The remaining weekday was selected randomly in such cases.

Energy and nutrient intakes were obtained using country-specific Food Composition Tables (FCTs)[15] due to the lack of a pan-European FCT. Common guidelines and procedures to prevent and minimize bias were adopted to harmonise nutrient databases across countries. Nutrient values were also harmonized using the documentation of the country-specific food components. In detail, the FCTs used were "Centre d'Ensenyament Superior de Nutrició I Dietètica (CESNID). Tablas de composición de alimentos del CESNID, 2nd edn. Madrid: McGraw-Hill Interamericana, 2004" in Spain, "NUBEL. Belgian Food Composition Table, 4th edn. Brussels: Ministry of Public Health, 2004" in Belgium and "Swedish National Food Agency (SNFA). Swedish Food Database (available at http://www.slv.se/en-gb/Group1/Food-and-Nutrition/The-Food-Database/)" in Sweden. EI (kcal/day) and macronutrient intakes (carbohydrates, protein, fat in g/day) were calculated as mean of the two 24-HDR for each child. Macronutrient intakes were additionally expressed as percentage of total EI derived from carbohydrates, proteins and fat.

2.4. Anthropometric measurements

Height was measured to the nearest 0.1 cm with a calibrated stadiometer (Telescopic height measuring instruments SECA 225, Birmingham, UK). Body weight was measured in light underwear on a calibrated scale accurate to 0.1 kg on the first and last day of the measurement period (TANITA BC 420 SMA digital weighing scale, Tanita Europe GmBH, Sindelfingen, Germany). Body mass index (BMI) was calculated by dividing body mass in kg measured at Day 1 (if missing, the measurement of the last day was used) by the squared body height in meters. BMI was categorised according to the International Obesity Task Force (IOTF) criteria[16] and BMI z-scores were calculated according to Cole et al.[17] For most analyses, thin/normal weight and overweight/obese children were each combined into one group.

2.5. Statistical analysis

Analyses were done for all children as well as stratified by study centre, sex, weight status (thin/normal weight vs. overweight/obese) and age group (4 to <6 years vs. 6 to <10 years). Only one of the mentioned variables was considered at the same time due to the small number of children per strata. The Bland–Altman plot[18] was used to assess the agreement between EI and TEE. This method calculates a bias as mean difference between the reference method (TEE) and the reported value (EI). Limits of agreement are calculated as bias ±2 SD of this observed bias. In the following, the term 'bias' always refers to the difference between EI and TEE unless another specification is given.

Following the approach of Sjoberg et al.[19] the ratio of EI over TEE was used to differentiate adequate-energy-reports (AER) from low-energy-reports (LER) and high-energy-reports (HER). Participants were classified as AER, LER or HER based on 95% confidence limits (CL) of the expected ratio EI over TEE, which equals 1.00 under the assumption of energy balance. CLs were calculated according to the following formula[20]:

$$95\%\ CL_{l,u} = \pm 1.96 \cdot \sqrt{\frac{(CV_{EI})^2}{d} + (CV_{TEE})^2}.$$

Child-specific reference values (boys: 22.5%, girls 21.3%) based on previous literature were used for the coefficient of variation for daily EI (CV_{EI}).[21] The number of days (d) was set to two and for the coefficient of variation of TEE (CV_{TEE}) the value 8.2% was chosen.[20]

Children were defined as AER, LER and HER according to the cut-off values calculated by insertion of the reference values in the above formula (LER: boys: EI/TEE<0.65, girls: EI/TEE<0.66; AER: boys: 0.65 ≤ EI/TEE<1.35, girls: 0.66 ≤ EI/TEE<1.34; HER: boys: EI/TEE≥1.35, girls EI/TEE≥1.34).

Macronutrient intakes as well as TEE, EI and study participants' characteristics were compared between groups of AER, LER and HER.

All analyses were performed using the statistical software package SAS (version 9.1; SAS Institute, Cary, NC, USA).

3. Results

Descriptive analyses are presented in Table 1. In total, nine out of the 36 children were overweight or obese. Mean ages differed only

Table 1
Main characteristics of the study population by age group, sex and study centre (mean, SD and total numbers).

	All	4–<6 years	6–<10 years	Boys	Girls	Belgium	Spain
	N	*N*	*N*	*N*	*N*	*N*	*N*
Boys	17	6	11	–	–	2	15
Girls	19	8	11	–	–	4	15
4–<6 years	14	–	–	6	8	3	11
6–<10 years	22	–	–	11	11	3	19
Thin[a]	4	1	3	3	1	1	3
Normal weight[a]	23	11	12	13	10	4	19
Overweight[a]	5	1	4	1	4	1	4
Obese[a]	4	1	3	0	4	0	4
All	36	14	22	17	19	6	30

	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age	6.7	1.4	5.3	0.5	7.7	0.8	6.8	1.4	6.7	1.3	6.2	1.6	6.9	1.3
Weight (kg)	24.4	5.0	20.9	2.8	26.6	4.9	22.8	3.6	25.8	5.7	22.7	4.7	24.7	5.1
Height (cm)	121.1	7.8	114.0	4.0	125.6	6.1	121.4	7.9	120.9	8.0	120.8	11.8	121.2	7.1
BMI *z*-score[b]	0.2	1.2	−0.1	0.9	0.4	1.3	−0.1	0.9	0.4	1.3	−0.2	1.2	0.3	1.6

SD: standard deviation.
Due to the small sample size the authors abstained from the presentation of percentages in the subgroups (sex, age, weight status, study centre).
[a] Cut-offs according to IOTF criteria.[16] Prevalence of overweight not including obesity.
[b] Body mass index *z*-score according to Cole et al.[17]

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Table 2
Mean energy expenditure (TEE), energy intake (EI), ratio of EI over TEE and difference between EI and TEE (bias) by age group, sex, study centre and weight status.

	N	TEE[a] (kcal/day)		EI[a] (kcal/day)		Ratio EI over TEE		Bias[b] (kcal/day)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
4–<6 years	14	1386	217	1273	352	0.95	0.35	−113	460
6–<10 years	22	1610	186	1645	465	1.02	0.27	35	431
Boys	17	1574	219	1535	488	0.99	0.34	−39	485
Girls	19	1477	226	1469	440	1.00	0.28	−8	412
Belgium	6	1491	226	1434	290	0.96	0.11	−57	154
Spain	30	1530	228	1513	487	1.00	0.33	−16	481
Thin/normal weight	27	1513	231	1511	501	1.01	0.33	−2	471
Overweight/obese	9	1554	214	1468	313	0.96	0.23	−86	356
All	36	1523	225	1500	458	1.00	0.30	−23	442

[a] TEE: total energy expenditure; EI: energy intake.
[b] Mean difference between reported energy intake (means of two 24-HDR per child) and total energy expenditure (DLW measurements).

Table 3
Number of low-energy-reports (LER), adequate reports (AER) and high-energy-reports (HER) by age group, sex, study centre and weight status.

	Low-energy-reports[b]	Adequate reports[c]	High-energy-reports[d]
	N	N	N
Total study group	3 (8%)	28 (78%)	5 (14%)
4–<6 years	2	10	2
6–<10 years	1	18	3
Boys	2	13	2
Girls	1	15	3
Belgium	0	6	0
Spain	3	22	5
Thin[a]	1	3	0
Normal weight[a]	2	17	4
Overweight[a]	0	4	1
Obese[a]	0	4	0

Due to the small sample size the authors abstained from the presentation of percentages in the subgroups.
[a] Cut-offs according to IOTF criteria.[23] Prevalence of overweight not including obesity.
[b] Low-energy-reporters defined as: EI/TEE<0.65 (boys), EI/TEE<0.66 (girls).
[c] Adequate reports defined as: 0.65 ≤ EI/TEE<1.35 (boys), 0.66 ≤ EI/TEE<1.34 (girls).
[d] High-energy-reports defined as: EI/TEE≥1.35 (boys), EI/TEE≥1.34 (girls).

slightly between study centres and the sex distribution was almost balanced across the whole study group (17 boys, 19 girls) as well as across study centres.

As expected, TEE was higher in older children, higher in boys compared to girls and higher in overweight/obese compared to thin/normal weight children (Table 2) though differences in TEE were only small between the weight groups (thin/normal weight: 1513 kcal/day; overweight/obese: 1554 kcal/day). EI was again higher in older children as well as in boys but slightly lower in overweight/obese children compared to thin/normal weight children. Mean EI was lower compared to TEE by up to 5% (6–<10 years) and higher by up to 2% (4–<6 years) depending on the study group addressed. Regarding the total study group, the ratio of EI over TEE was 1.00 indicating that reported EI and measured TEE matched almost exactly (EI: 1500 kcal/day; TEE: 1523 kcal/day). The greatest difference between EI and TEE was found in children younger than six years (mean difference: −113 kcal/day), followed by the group of overweight/obese children (mean difference: −86 kcal/day).

The Bland–Altman plot (Fig. 2) revealed only moderate agreement between EI and TEE reflecting in particular the high day-to-day variation in dietary intakes. Individual differences between EI and TEE varied widely, ranging from −836 up to +953 kcal/day. The largest bias was observed in case of very low mean values of EI and TEE (mainly negative bias, EI < TEE), as well as in case of very high mean values of EI and TEE (mainly positive bias, EI > TEE).

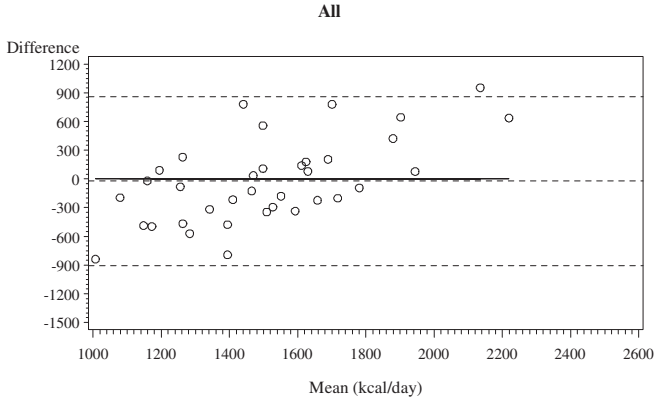

Fig. 2. Bland–Altman plot agreement between measured energy expenditure (TEE; kcal/day) and reported energy intake (EI; kcal/day). The mean of EI and TEE is plotted on the *x*-axis, the difference of both values (bias) on the *y*-axis accordingly. The solid line indicates the line of total agreement (zero differences between EI and TEE). Mean difference and upper/lower limits of agreement (mean difference ± 2 SD) are superimposed by broken line.

The cut-off technique identified five HER (14%) and three LER (8%) (Table 3). Mean TEE was highest in the group of LER whereas, as expected, EI was lowest (Table 4). Fat intakes expressed as percentage of total EI were slightly lower in LER compared to AER and highest in HER whereas the opposite was found for percentages of EI from carbohydrates. Percentages of EI from proteins were lowest in AER. Intakes in g per day were lowest in LER and highest in HER for all macronutrients and water.

In the subgroup analysis of the exclusively obese Swedish children that had previously been treated in an obesity clinic (not included in the tables), a mean difference between EI and TEE of −455 kcal/day was observed and seven out of the ten Swedish children were classified as LER.

4. Discussion

The present study aimed to evaluate the accuracy of EI estimated from two repeated 24-HDR using objective measurements of TEE obtained by the gold-standard technique DLW.[2] Results revealed good agreement between EI and TEE for the total study group as well as in subgroups of age, sex, study centre and weight status; Mean EI was lower compared to mean TEE by up to 5% and higher by up to 2% depending on the study group addressed. Considering the whole sample, the mean ratio of reported EI over TEE equalled one which means that both values agreed almost exactly on group level. Previous studies in children found underreporting of EI relative to TEE ranging from 19% to 41% and overreporting ranging from 7% to 11%.[9] However, findings strongly vary depending on the participants' age and on the dietary assessment method used.[22–24] The good agreement between EI and TEE in our study may be explained by the additional assessment of school meals which may have reduced reporting errors caused by meals not under parental control. Furthermore, the display of pictures with increasing portion sizes on the screen may have improved the estimation of portion sizes. However, also restricting on a convenience study sample may have contributed to these results as it is likely that the participants were highly-motivated.

Even in overweight/obese children, a group that has repeatedly been reported to be strongly influenced by misreporting,[8,25] only a

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Table 4
Mean energy expenditure (TEE), energy intake (EI), difference between EI and TEE (bias) and macronutrient intakes (expressed as g/day and % of total EI/day) by reporting group (low-energy-report, adequate report and high-energy-report).

	Low-energy-reports[c]		Adequate-energy-reports[d]		High-energy-reports[e]		Total study group	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TEE[a] (kcal/day)	1595	183.9	1544	219.0	1364	252.9	1523	225.0
EI[a] (kcal/day)	862	236.4	1460	367.9	2107	336.9	1500	457.6
Bias[b] (kcal/day)	−733	142.7	−84	282.2	743	150.6	−22.9	441.8
Carbohydrate intake (g/day)	89.4	14.1	152.8	50.7	187.1	39.3	152.3	51.8
%EI from carbohydrates	43.3	6.9	42.2	7.4	35.9	3.7	41.4	7.2
Fat intake (g/day)	37.5	13.3	65.0	19.9	99.4	17.7	67.5	24.1
%EI from fat	37.8	3.9	39.4	7.3	41.8	5.0	39.6	6.8
Protein intake (g/day)	41.9	16.7	66.3	17.3	116.8	34.6	71.3	27.8
%EI from proteins	19.3	3.4	18.7	3.4	22.5	5.7	19.3	3.9
Water intake (g/day)	824.8	386.4	1176	398.5	1733	587.7	1224	472.4

[a] TEE, total energy expenditure; EI, energy intake.
[b] Mean difference between reported energy intake (means of two 24-HDR per child) and total energy expenditure (DLW measurements).
[c] Low-energy-reporters defined as: EI/TEE<0.65 (boys), EI/TEE<0.66 (girls).
[d] Adequate reports defined as: 0.65 ≤ EI/TEE<1.35 (boys), 0.66 ≤ EI/TEE<1.34 (girls).
[e] High-energy-reports defined as: EI/TEE≥1.35 (boys), EI/TEE≥1.34 (girls).

mean difference of −84 kcal/day was observed between reported EI and measured TEE in our data. Forrestal[8] stated in a review that higher weight, obesity and BMI had consistently been associated with low-energy-reporting at all ages. In proxy-reports, misreporting (comprising low- and high-energy-reporting) may to a large extent be explained by meals not under parental control and difficulties in estimation of portion sizes, but also by intentional misreporting due to social desirability bias. The lower percentages of EI from fat but higher percentages of EI from carbohydrates in LER may indicate selective omission of certain foods high in fat and protein, although the exact nature of these differences cannot be determined with available methodologies. No study was found investigating socially desirable answer behaviour in proxy-reports in that age group. Social desirability was shown to be a predictor of misreporting among adults,[26] so it can be hypothesised that parents feeling ashamed for their child's unhealthy/energy-dense diet may intentionally misreport in proxy-reports as well. Inconsistently with literature, children classified as LER were either thin or normal weight in our data. It can be assumed that these low-energy-reports rather reflect exceptional days (e.g. child was ill) or reporting errors caused by meals not under parental control than intentional misreporting. In addition, by use of a cut-off approach, underreporting cannot be distinguished from a hypocaloric diet (undereating). Thus, participants classified as LER may not be underreporting but following a specific diet. In these children, EI values may actually be lower than TEE so that the proxy-report may still be valid. However, when investigating diet–disease associations these cases might still bias the true diet–disease association as their current diet (exceptional days) might not be the cause of their current health or weight status.

To the authors' knowledge, child-specific cut-off values to classify 24-HDR in different reporting groups were only applied in a study comparing EI to estimated basal metabolic rates[22] making it difficult to compare proportions of LER, AER and HER to other studies.

Differences between EI and TEE were large at the individual level (regarding single children's values), whereas on group level (mean EI vs. mean TEE) good agreement was observed. For example in thin/normal weight children means of EI and TEE (EI: 1511 kcal/day; TEE: 1513 kcal/day) almost coincide although individual differences between EI and TEE strongly differ from zero (−836 kcal/day up to +953 kcal/day). This may be explained by random errors including day-to-day variation that cancel out on group level. These results agree with other studies reporting biases at the individual level, but better agreement on group level.[23,27–29]

The large difference between reported EI and measured TEE (mean difference: −455 kcal/day) that was observed in the Swedish subgroup may be a consequence of previous treatment in the obesity clinic resulting in changes in lifestyle behaviours including eating habits and leisure time activities. Also intentional or unintentional underreporting of energy intake by the parents of these obese children may have contributed to these findings.

In adults, a combination of methods (two non-consecutive 24-HDR interviews in combination with a food frequency questionnaire (FFQ)) to measure dietary intake has been recommended for use in monitoring and epidemiological surveys (EFCOVAL & IDAMES project: 2 EC 6th FP).[30] However, recommendations for measuring dietary intake among children are still lacking but it can be hypothesised that additional FFQ information may help to improve the estimation of individual intakes in childhood populations as well.

4.1. Limitations and strengths

The relatively small sample size and correspondingly low power is a limitation and was also the reason to abstain from statistical testing for differences between groups. However, the sample size is consistent with numbers in other validation studies using the cost-intense doubly labeled water technique. Furthermore, only approximate agreement between EI and TEE can be expected when assessing only two 24-HDR per child due to the large day-to-day variability in EI. Moreover, the cut-off technique aims only to identify under-/overestimations resulting in physiologically implausible EI[24] and does not allow distinction between various degrees of misreporting or to differentiate between underreporting and undereating. As a convenience sample was chosen, a selection bias of highly motivated parents and children cannot be precluded.

To the authors' knowledge, this is among the first studies documenting accuracy of parental reporting using objective measurements of TEE. A strength is the assessment of EI and TEE within the same time frame,[9] increasing the reliability of the measurements. In addition, the assessment of school meals enhanced the completeness of the 24-HDR and the display of pictures with increasing portion sizes in the SACINA program simplified the estimation of portion sizes for the respondents.

4.2. Conclusions

In summary, good agreement between proxy-reported EI and measured TEE was observed on group level – even in overweight/

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obese children – whereas individual differences between both values varied widely. Two proxy-reported 24-HDR including visual aids and school meal reporting are a valid measure for EI on group level but not sufficient to evaluate individual intakes. Future research is required to improve the precision of proxy-reported EI for individual children.

Ethical statement

The study was approved by the appropriate ethics committees in each centre, specifically by the "Comité Ético de Investigación Clínica de Aragón (CEICA)" in Spain, the Ethical Committee of the Ghent University Hospital in Belgium, and the "Regionala Etikprövningsnämnden i Göteborg" in Sweden.

Conflict of interest

All the authors declare that there are no conflicts of interest.

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Each author has seen and approved the contents of the submitted manuscript. All authors contributed to conception and design, acquisition of data, analysis or interpretation of data. Final approval of the version published was given by all the authors. In detail: C. Börnhorst and S. Bel-Serrat drafted the manuscript; IP, KB, IH, SDH, GE, LL, KW and LAM conceived the study and participated in its design and coordination; IH, IP and LAM helped drafting the manuscript and interpreting the data; all the authors revised the article critically for important intellectual content.

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Artículo II [Paper II]:

Relative validity of the Children's Eating Habits Questionnaire-food frequency section among young European children: the IDEFICS study

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Relative validity of the Children's Eating Habits Questionnaire–food frequency section among young European children: the IDEFICS Study

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Abstract

Objective: To compare, specifically by age group, proxy-reported food group estimates obtained from the food frequency section of the Children's Eating Habits questionnaire (CEHQ-FFQ) against the estimates of two non-consecutive 24 h dietary recalls (24-HDR).

Design: Estimates of food group intakes assessed via the forty-three-food-group CEHQ-FFQ were compared with those obtained by a computerized 24-HDR. Agreement on frequencies of intakes (equal to the number of portions per recall period) between the two instruments was examined using crude and de-attenuated Pearson's correlation coefficients, cross-classification analyses, weighted kappa statistics (κ_w) and Bland–Altman analysis.

Setting: Kindergartens/schools from eight European countries participating in the IDEFICS (Identification and prevention of Dietary- and lifestyle-induced health EFfects In Children and infantS) Study cross-sectional survey (2007–2008).

Subjects: Children aged 2–9 years (n 2508, 50·4 % boys).

Results: The CEHQ-FFQ provided higher intake estimates for most of the food groups than the 24-HDR. De-attenuated Pearson correlation coefficients ranged from 0·01 (sweetened fruit) to 0·48 (sweetened milk) in children aged 2–<6 years (mean = 0·25) and from 0·01 (milled cereal) to 0·44 (water) in children aged 6–9 years (mean = 0·23). An average of 32 % and 31 % of food group intakes were assigned to the same quartile in younger and older children, respectively, and classification into extreme opposite quartiles was ≤12 % for all food groups in both age groups. Mean κ_w was 0·20 for 2–<6-year-olds and 0·17 for 6–9-year-olds.

Conclusions: The strength of association estimates assessed by the CEHQ-FFQ and the 24-HDR varied by food group and by age group. Observed level of agreement and CEHQ-FFQ ability to rank children according to intakes of food groups were considered to be low.

Keywords
Relative validation
FFQ
24 h Dietary recall
Children
Proxy reports

Accurate assessment of food intake in children is essential to conduct epidemiological research on diet–health links[1]. Indeed, the importance of valid methods of diet and food intake assessment in epidemiological studies has increasingly been recognized[2–4] given the increasing prevalence of obesity, cardiovascular risk factors and other diseases with long-term consequences even in young populations[5,6]. Therefore, evidence produced for young population groups could benefit from the use of valid dietary assessment tools in terms of an early identification and primary prevention of diet-related chronic diseases.

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FFQ have often been used in large-scale epidemiological studies, because of their ease of administration and time and cost efficiency[7] compared with other dietary assessment methods[8]. However, all self-reporting methods of food intake and consumption are prone to measurement error leading to bias[9] suggesting that estimates may not represent the 'true' usual intake. More specifically, methods are affected by random and systematic errors leading to erroneous associations between diet and disease[10–12]. Validation studies are therefore necessary to indicate the effect of measurement error and to prevent incorrect estimations[13]. Validity refers to the ability of the instrument to discriminate between individuals with true exposure differences[14], where the test instrument is compared against a 'reference method'[15] when available.

Intervention trials have shown that whole foods rather than individual nutrients may best indicate the potential role of the diet in disease prevention[16], which additionally emphasizes the importance of validating dietary assessment methods in terms of food groups rather than nutrients. However, the ability of FFQ to quantify food intakes is not as well documented as their ability to quantify nutrient intakes[9]. The food frequency section of the Children's Eating Habits Questionnaire (CEHQ-FFQ) was designed to investigate the consumption of foods, not of nutrients, previously shown to be consistently associated with overweight and obesity in children. Therefore, the aim of the present study was to evaluate the ability of the CEHQ-FFQ in estimating, specifically by age group, proxy-reported intakes of obesity-related foods. Food estimates obtained from the CEHQ-FFQ were compared with those obtained from two non-consecutive 24 h dietary recalls (24-HDR) as part of the IDEFICS (Identification and prevention of Dietary- and lifestyle-induced health EFfects In Children and infantS) Study.

Experimental methods

Study design and population

The IDEFICS Study is a prospective cohort study with an embedded intervention study carried out in eight European countries (Italy, Estonia, Cyprus, Belgium, Sweden, Germany, Hungary and Spain) with the aim of investigating the aetiology of diet- and lifestyle-related diseases and disorders in European children[17]. A total of 16 224 children fulfilled the general IDEFICS inclusion criteria: complete information on sex, age, height and weight. The design and methodology of the IDEFICS Study have been described previously[17]. Data for the current analysis were obtained from the baseline survey conducted between September 2007 and June 2008 among children aged 2–9 years. For the purposes of the present analysis, only children with two 24-HDR and a CEHQ-FFQ were included (*n* 2508; 1264 boys, 1244 girls). The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved before the start of the examinations by the appropriate local ethics committees in each of the eight survey centres (Belgium: Ethical Committee of the Ghent University Hospital; Cyprus: Cyprus National Bioethics Committee; Estonia: Tallinn Medical Research Ethics Committee; Germany: Ethics Committee of the University of Bremen; Hungary: Egészségügyi Tudományos Tanács Tudományos és Kutatásetikai Bizottság in Budapest; Italy: Comitato Etico ASL in Avellino; Spain: Comité Ético de Investigación Clínica de Aragón (CEICA); Sweden: Regional Ethics Review Board, University of Gothenburg). Written informed consent was obtained from all children's parents.

Data on age, sex and parental education level were recorded by means of parental report on a questionnaire. Height and weight measurements were also taken by trained fieldworkers[17,18].

Children's Eating Habits Questionnaire–food frequency section (CEHQ-FFQ)

The self-administered CEHQ-FFQ was designed as a screening tool to assess eating behaviours associated with risk of overweight, obesity and general health in children. Children's proxies, mainly the parents, filled it in at home by reporting the number of times the child consumed the food groups included in the questionnaire during a typical week over the previous month.

The whole CEHQ was pre-tested prior to the IDEFICS baseline survey in all involved centres[19]. Country-specific food examples were included to facilitate correct comprehension of the food groups included. The CEHQ-FFQ consisted of forty-three food groups which were clustered into thirty-six according to their nutritional profiles, as similarly done in other studies[20–22]: (i) vegetables (cooked vegetables and legumes); (ii) fried potatoes; (iii) raw vegetables; (iv) fruit; (v) sweetened fruit; (vi) water; (vii) manufactured fruit juices; (viii) soft drinks; (ix) light soft drinks; (x) breakfast cereals; (xi) sweetened breakfast cereals; (xii) milk; (xiii) sweetened milk; (xiv) yoghurt; (xv) sweetened yoghurt; (xvi) fish; (xvii) fried fish; (xviii) fried eggs; (xix) eggs; (xx) mayonnaise; (xxi) cold cults; (xxii) meat (raw and cooked meat); (xxiii) cheese (sliced, spreadable and grated cheese); (xxiv) jam & honey; (xxv) chocolate/nut-based spread; (xxvi) butter & margarine; (xxvii) ketchup; (xxviii) white bread; (xxix) wholemeal bread; (xxx) pasta & rice; (xxxi) milled cereal; (xxxii) pizza; (xxxiii) fast food (hamburgers, hot dogs, kebabs, etc.); (xxxiv) nuts; (xxxv) snacks (crisps, popcorn, savoury pastries and fritters, etc.); and (xxxvi) sweets (chocolates, candy bars, biscuits, cakes, puddings, ice creams, etc.). To facilitate the proxy's responses, a frequency scale was adopted from the US Department of Agriculture eating habits questionnaire of the Early Childhood Longitudinal Survey[23], consisting

of the following categories of consumption: 'never/less than once a week', '1–3 times a week', '4–6 times a week', '1 time per day', '2 times per day', '3 times per day', '4 or more times per day' and 'I have no idea'. These were converted into times per week ranging from 0 up to 30 and thereafter into daily. No portion size estimates were obtained. The CEHQ-FFQ showed acceptable reproducibility comparable to others[24]. Furthermore, previous findings evaluating the CEHQ-FFQ[25] found a positive correlation between milk consumption frequencies and respectively K and Ca urinary excretion ratios.

24 h Dietary recall

The 'reference' method was a computerized version of a 24-HDR, namely SACINA (Self Administered Children and Infants Nutrition Assessment). Two recalls were collected in 2508 participants. Children's proxies were interviewed by trained survey personnel. Each interview lasted an average of 20–30 min. Non-consecutive dietary recalls were conveniently distributed across weekdays and weekend days in an effort to capture intakes spread throughout the week. For the present analysis, average food intakes were computed as the mean of the two 24-HDR. Both the CEHQ-FFQ and the two 24-HDR were administered during the same time span.

The SACINA software is based on the HELENA-DIAT Dietary Assessment Tool software developed for European adolescents within the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) project[26,27]. The program consists of a single 24-HDR structured according to six meal occasions (breakfast, mid-morning snack, lunch, afternoon snack, dinner and evening snack) embedded in questions related to a range of chronological daily activities[26–29]. Proxies were asked to recall all food and drinks consumed the previous day by their child. Survey personnel registered school meals data by direct observation. Portion size estimation was assessed mainly by photos of serving sizes, standard portions, customary packing size and foods in pieces or slices in order to reduce reporting bias. When a specific food was not available within the software it was entered manually specifying the total amount consumed. In Hungary, the procedure of collecting 24-HDR was different from that of the rest countries. Proxies were asked to complete a self-administered 24-HDR at home. This information was thereafter entered in the SACINA software by fieldwork personnel when received. Considering these methodological differences in the application of the 24-HDR, results excluding Hungary (n 1418) are presented as Supplementary Materials. The validity of SACINA was previously tested by means of the doubly labelled water method. Findings indicated the 24-HDR to be a valid instrument in assessing energy intake at group level (total energy intake – total energy expenditure = −0·23; C Börnhorst, S Bel-Serrat, I Pigeot *et al.*, unpublished results).

To relate CEHQ-FFQ estimates of food consumption to those of the 24-HDR and to enable comparisons, it was assumed that 'number of times per day' as reported in the CEHQ-FFQ could be equated to 'number of portions per day'[30]. Each reported 24-HDR food item was mapped and subsequently matched to one of the forty-three food groups initially included in the CEHQ-FFQ.

Statistical methods

Statistical analyses included all countries and were performed by age group (2–<6 years, 6–9 years) using the Predictive Analytics SoftWare (PASW) version 18. Means, medians and standard deviations were calculated for food group estimates obtained from the CEHQ-FFQ and 24-HDR. Crude data were log-transformed ($\log_{10}$) to improve normality for all thirty-six food groups. Food groups rarely consumed (<5 %) were excluded (i.e. meat replacement & soya products) from subsequent comparisons. Participants exceeding 25 % of missing values in the CEHQ-FFQ (n 43) were also excluded from the analysis[21].

Pearson's and Spearman's rank correlation coefficients were calculated for all participants. Results were similar between Pearson and Spearman correlation coefficients, therefore only Pearson coefficients are shown. Correlation coefficients were corrected for attenuation due to random error in the 24-HDR by taking into account the ratio of within-person variance to between-person variance (variance ratio). De-attenuation of crude correlation coefficients was computed according to the equation from Willett[31]:

$$r_{\text{adjusted}} = r_{\text{observed}}\sqrt{1+\lambda_x/n_x}$$

where λ_x is the variance ratio for x and n_x is the number of replicates for the x variables (here $n=2$).

Agreement of the CEHQ-FFQ and 24-HDR in ranking individuals was examined by the construction of quartiles for each food group (non-adapted food groups). An alternative approach was used to address the issue of zero consumption observed for >25 % of the participants[21]. Non-consumers were considered as one group and the remaining participants were grouped into tertiles (adapted food groups)[32]. Cross-classification analyses were finally applied in fourteen (fifteen in younger children) out of the thirty-six food groups: vegetables, fruit, milk, cold cuts, meat, cheese, white bread and sweets (non-adapted food groups) and, on the other hand, raw vegetables, fruit juices, soft drinks, breakfast cereals (only for younger children), sweetened milk, butter & margarine and wholemeal bread (as the adapted food groups). The proportion of individuals who fell into the same (correct classification) or into the extreme category (misclassification) was examined. The weighted kappa statistic (κ_w) was calculated with a linear set of weights[33] as a measurement of agreement. Bland–Altman limits of agreement (LOA)[35] were calculated

for frequently consumed foods to examine the agreement between the CEHQ-FFQ and the two 24-HDR. The mean differences (bias) between the two measurements ($CEHQ\text{-}FFQ_{log} - 24\text{-}HDR_{log}$) were plotted *v.* their means ($(CEHQ\text{-}FFQ_{log} + 24\text{-}HDR_{log})/2$). The LOA define the limits within which 95 % of the differences are expected to fall (mean ± 2 SD of the difference).

Results

The general characteristics of the 2508 participants are shown in Table 1. Included participants were older, taller and heavier compared with the rest of the IDEFICS participants not included in the present analysis (data not shown).

At the group level, the CEHQ-FFQ provided higher estimates of number of portions than the 24-HDR for the majority of the food groups in both younger and older children (Tables 2 and 3). Significant differences across means were found for all food groups except for fried potatoes, sweetened fruit, milled cereal and fast food in children aged 2–<6 years (Table 2) and breakfast cereals and pizza in children aged 6–9 years (Table 3). Pearson correlation coefficients ranged from 0·01 for sweetened fruit to 0·45 for sweetened milk in younger children (Table 4) and from 0·01 for milled cereal to 0·42 for water in older children (Table 5) in absolute values. After correction for within-person variation, the de-attenuated Pearson correlation coefficients were slightly higher than the crude values (0·01 for sweetened fruit to 0·48 for sweetened milk in younger children and 0·01 for milled cereals to 0·44 for water in older children). The average de-attenuated coefficient for all food groups was 0·25 and 0·23 for younger and older children, respectively. Low de-attenuated coefficients values (<0·20) were observed in thirteen and fourteen out of the thirty-six food groups, respectively, for 2–<6-year-olds and 6–9-year-olds. A higher association (>0·40), however, was observed for fruit, water, breakfast cereals and sugared milk in young children and for raw vegetables, butter & margarine and water in older children. The average de-attenuated correlation coefficient was 0·25 and 0·23, respectively, for younger and older children.

Cross-classification agreement and κ_w values are presented in Tables 6 and 7, showing the ability of the CEHQ-FFQ to classify individuals into the same quartile of intake estimated by the 24-HDR. Among the non-adapted groups, the proportion classified in the same quartile varied from 26 % for sweets to 39 % for milk in children aged 2–<6 years (mean = 32 %) and from 28 % for meat to 34 % for fruit in children aged 6–9 years

Table 1 Baseline characteristics of study participants: children aged 2–9 years from eight European countries participating in the IDEFICS Study (2007–2008)

	All (*n* 2508)		Boys (*n* 1264)		Girls (*n* 1244)	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	6·3	1·8	6·3	1·8	6·3	1·8
Height (cm)	119·2	7·5	119·9	12·6	118·6	12·7
Weight (kg)	24·0	7·5	24·4	7·6	23·6	7·4
BMI (kg/m^2)	16·5	2·7	16·6	2·7	16·4	2·6
	n	%	*n*	%	*n*	%
BMI†						
Thinness (BMI < 18·5 kg/m^2)	305	12·2	164	13·0	141	11·3
Normal weight (18·5 ≤ BMI < 25·0 kg/m^2)	1689	67·3	840	66·5	849	68·2
Overweight (25·0 ≤ BMI < 30·0 kg/m^2)	333	13·3	163	12·9	170	13·7
Obesity (BMI ≥ 30·0 kg/m^2)	181	7·2	97	7·7	84	6·8
Age group						
2–<6 years	993	39·6	492	38·9	501	40·3
6–9 years	1515	60·4	772	61·1	743	59·7
Parental education level‡						
Low	213	10·9	112	11·5	101	10·3
Medium	1012	51·8	505	52·0	507	51·7
High	727	37·2	355	36·5	372	38·0
Country						
Italy	398	15·9	217	17·2	181	14·5
Estonia	15	0·6	5	0·4	10	0·8
Cyprus	28	1·1	12	0·9	16	1·3
Belgium	11	0·4	5	0·4	6	0·5
Sweden	97	3·9	47	3·7	50	4·0
Germany	366	14·6	180	14·2	186	15·0
Hungary	1418	56·5	699	55·3	719	57·8
Spain	175	7·0	99	7·8	76	6·1

ISCED, International Standard Classification of Education.
†According to Cole *et al.*'s categories[45]. Thinness includes: thinness grade III, thinness grade II and thinness grade I.
‡Low = ISCED Levels 1 and 2; medium = ISCED Levels 3 and 4; high = ISCED Level 5. ISCED is an indicator of socio-economic level[46].

Table 2 Food group intakes (daily number of portions) from the CEHQ-FFQ and 24-HDR: younger children aged 2–<6 years from eight European countries participating in the IDEFICS Study (2007–2008)

		CEHQ-FFQ			24-HDR (SACINA)				
Food group (portions/d)	*n*	Mean	Median	SD	Mean	Median	SD	Mean Δ	*P* value
Vegetables	983	0·57	0·29	0·47	0·91	1·00	0·68	−0·34	0·000*
Fried potatoes	976	0·11	0·00	0·19	0·09	0·00	0·22	0·02	0·101
Raw vegetables	979	0·67	0·29	0·66	0·55	0·50	0·70	0·12	0·000*
Fruit	971	1·07	1·00	0·76	0·87	0·50	0·79	0·20	0·000*
Sweetened fruit	914	0·19	0·00	0·46	0·04	0·00	0·14	0·15	0·648
Water	955	3·06	4·29	1·51	1·72	1·50	1·12	1·34	0·000*
Fruit juices	979	1·08	0·71	1·57	0·60	0·50	0·76	0·48	0·000*
Soft drinks	978	0·37	0·00	0·81	0·53	0·50	0·72	−0·16	0·000*
Light soft drinks	959	0·15	0·00	0·61	0·01	0·00	0·09	0·14	0·000*
Sweetened breakfast cereals	976	0·33	0·29	0·42	0·17	0·00	0·33	0·16	0·000*
Breakfast cereals	936	0·20	0·00	0·46	0·17	0·00	0·43	0·03	0·000*
Milk	940	0·88	0·71	0·93	0·79	0·50	0·77	0·09	0·000*
Sweetened milk	969	0·70	0·29	0·79	0·41	0·00	0·62	0·29	0·000*
Yoghurt	940	0·20	0·00	0·39	0·07	0·00	0·23	0·13	0·000*
Sweetened yoghurt	971	0·51	0·25	0·50	0·30	0·00	0·45	0·21	0·000*
Fish	941	0·13	0·00	0·18	0·07	0·00	0·20	0·06	0·000*
Fried fish	956	0·14	0·00	0·24	0·07	0·00	0·21	0·07	0·000*
Cold cuts	982	0·60	0·29	0·54	0·88	1·00	0·72	−0·28	0·000*
Meat	989	0·70	0·57	0·59	0·71	0·50	0·56	−0·01	0·047*
Fried eggs	976	0·15	0·00	0·20	0·07	0·00	0·20	0·08	0·000*
Eggs	959	0·11	0·00	0·15	0·07	0·00	0·19	0·04	0·000*
Mayonnaise	967	0·08	0·00	0·18	0·03	0·00	0·12	0·05	0·000*
Cheese	986	0·83	0·61	0·70	0·47	0·50	0·56	0·36	0·000*
Jam & honey	966	0·26	0·29	0·36	0·16	0·00	0·34	0·10	0·000*
Chocolate/nut-based spread	977	0·26	0·00	0·39	0·14	0·00	0·31	0·12	0·000*
Butter & margarine	966	0·61	0·29	0·74	0·42	0·00	0·61	0·19	0·000*
Ketchup	971	0·21	0·29	0·10	0·11	0·00	0·25	0·10	0·000*
White bread	978	1·07	1·00	0·94	1·37	1·50	0·84	−0·30	0·000*
Wholemeal bread	960	0·47	0·29	0·65	0·39	0·00	0·61	0·08	0·000*
Pasta & rice	974	0·43	0·29	0·35	0·66	0·50	0·50	−0·23	0·000*
Milled cereal	959	0·07	0·00	0·18	0·00	0·00	0·02	0·07	0·674
Pizza	963	0·06	0·00	0·13	0·09	0·00	0·23	−0·03	0·000*
Fast food	982	0·26	0·00	0·41	0·04	0·00	0·16	0·22	0·239
Nuts	978	0·16	0·00	0·25	0·04	0·00	0·18	0·12	0·000*
Snacks	988	0·23	0·29	0·34	0·09	0·00	0·24	0·14	0·001*
Sweets	992	1·17	1·00	0·95	1·39	1·50	0·97	−0·22	0·000*

CEHQ-FFQ, Children's Eating Habits Questionnaire–food frequency section; 24-HDR, 24 h dietary recall; SACINA, Self Administered Children and Infants Nutrition Assessment; Δ, difference.
*$P < 0·05$.

(mean = 31 %). Extreme misclassification was about or even lower than 12 % for all food groups in both younger and older children; the highest values were observed for white bread (12 %) in young children and cheese (11 %) in older children. Mean κ_w was 0·20 for 2–<6-year-old children and 0·17 for 6–9-year-old children. The κ_w values showed an acceptable agreement for fruit, milk, cold cuts, cheese and white bread, whereas low agreement (<0·20) was seen for vegetables, meat and sweets in both age groups. Results changed when examining the adapted food groups, since the proportion of correct classification ranged from 38 % for wholemeal bread to 49 % for sweetened milk in younger children (mean = 40 %) and from 32 % for wholemeal bread to 52 % for sweetened milk in older children (mean = 38 %). The mean proportion of individuals classified into the opposite tertile, however, was 22 % in both age groups, varying from 10 % and 6 % for sweetened milk to 29 % and 28 % for soft drinks in younger and older children, respectively. Mean κ_w for the adapted food groups was 0·20 for children aged 2–<6 years and 0·17 for those aged 6–9 years. Poor agreement was found except for sweetened milk, which showed acceptable agreement in both younger and older children (κ_w = 0·30 and 0·36, respectively). Among younger children, breakfast cereals and butter & margarine also showed acceptable agreement (>0·20).

Following exclusion of the Hungarian data, de-attenuated correlation coefficients were slightly higher compared with the crude coefficients, with an average of 0·31 in 2–<6-year-old children and 0·28 in 6–9-year-old children (Supplementary Materials, Tables 3 and 4). Average variance ratio increased to 0·64. Supplementary Materials, Tables 5 and 6 show the results of the cross-classification analysis excluding the Hungarian data. The mean percentage of correctly classified subjects increased to 35 % in younger children and to 32 % in older children. Mean extreme misclassification was considerably lower for both younger (5 %) and older children (7 %). The κ_w values

Table 3 Food group intakes (daily number of portions) from the CEHQ-FFQ and the 24-HDR: older children aged 6–9 years from eight European countries participating in the IDEFICS Study (2007–2008)

		CEHQ-FFQ			24-HDR (SACINA)				
Food group (portions/d)	*n*	Mean	Median	SD	Mean	Median	SD	Mean Δ	*P* value
Vegetables	1499	0·52	0·29	0·43	0·86	1·00	0·69	−0·34	0·000*
Fried potatoes	1485	0·15	0·00	0·20	0·09	0·00	0·23	0·06	0·000*
Raw vegetables	1501	0·65	0·29	0·62	0·56	0·50	0·72	0·09	0·000*
Fruit	1498	0·97	1·00	0·81	0·76	0·50	0·77	0·21	0·000*
Sweetened fruit	1377	0·23	0·00	0·56	0·03	0·00	0·12	0·20	0·000*
Water	1455	3·05	4·29	1·54	1·86	2·00	1·10	1·19	0·000*
Fruit juices	1484	1·01	0·71	1·20	0·57	0·50	0·74	0·44	0·000*
Soft drinks	1480	0·48	0·00	0·96	0·61	0·50	0·76	−0·13	0·000*
Light soft drinks	1472	0·15	0·00	0·56	0·01	0·00	0·13	0·14	0·000*
Sweetened breakfast cereals	1495	0·44	0·29	0·45	0·19	0·00	0·36	0·25	0·000*
Breakfast cereals	1411	0·13	0·00	0·29	0·12	0·00	0·30	0·01	0·086
Milk	1422	0·76	0·71	0·83	0·66	0·50	0·64	0·10	0·020*
Sweetened milk	1464	0·64	0·50	0·71	0·32	0·00	0·53	0·32	0·000*
Yoghurt	1426	0·19	0·00	0·37	0·05	0·00	0·18	0·14	0·000*
Sweetened yoghurt	1485	0·45	0·29	0·48	0·23	0·00	0·41	0·22	0·000*
Fish	1432	0·11	0·00	0·17	0·06	0·00	0·19	0·05	0·000*
Fried fish	1446	0·12	0·00	0·16	0·05	0·00	0·17	0·07	0·000*
Cold cuts	1500	0·67	0·71	0·60	0·99	1·00	0·76	−0·32	0·000*
Meat	1505	0·73	0·57	0·54	0·81	0·50	0·62	−0·08	0·002*
Fried eggs	1481	0·16	0·00	0·18	0·07	0·00	0·18	0·09	0·000*
Eggs	1482	0·10	0·00	0·17	0·04	0·00	0·15	0·06	0·000*
Mayonnaise	1475	0·09	0·00	0·20	0·04	0·00	0·17	0·05	0·000*
Cheese	1510	0·89	0·71	0·83	0·46	0·50	0·54	0·43	0·000*
Jam & honey	1486	0·26	0·29	0·37	0·16	0·00	0·34	0·10	0·000*
Chocolate/nut-based spread	1490	0·27	0·29	0·37	0·13	0·00	0·29	0·14	0·000*
Butter & margarine	1490	0·64	0·29	0·71	0·46	0·00	0·64	0·18	0·000*
Ketchup	1490	0·25	0·29	0·34	0·07	0·00	0·22	0·18	0·000*
White bread	1503	1·27	1·00	1·01	1·61	1·50	0·92	−0·34	0·000*
Wholemeal bread	1467	0·40	0·29	0·63	0·24	0·00	0·51	0·16	0·000*
Pasta & rice	1489	0·37	0·29	0·30	0·64	0·50	0·48	−0·27	0·000*
Milled cereal	1471	0·06	0·00	0·20	0·00	0·00	0·01	0·06	0·000*
Pizza	1477	0·07	0·00	0·17	0·06	0·00	0·18	0·01	0·057
Fast food	1500	0·39	0·29	0·52	0·03	0·00	0·14	0·36	0·000*
Nuts	1488	0·14	0·00	0·26	0·04	0·00	0·17	0·10	0·000*
Snacks	1505	0·24	0·29	0·35	0·11	0·00	0·25	0·13	0·000*
Sweets	1511	1·07	0·86	0·93	1·38	1·00	0·94	−0·31	0·000*

CEHQ-FFQ, Children's Eating Habits Questionnaire–food frequency section; 24-HDR, 24 h dietary recall; SACINA, Self Administered Children and Infants Nutrition Assessment; Δ, difference.
*$P < 0·05$.

showed acceptable agreement except for sweets and fruit, for which it was poor (<0·20) and moderate (>0·40), respectively, in both younger and older children. Vegetables, milk and meat also showed poor agreement among 6–9-year-old children. Regarding the adapted food groups, the mean proportion of individuals classified into the same tertile increased in both age groups compared with the non-adapted food groups. Average misclassification of individuals into the opposite tertiles decreased, being 16 % and 20 %, respectively, for 2–<6-year-old and 6–9-year-old children. Higher mean κ_w values were obtained: 0·20 in younger children and 0·14 in older children.

Figure 1 (Supplementary Materials) illustrates findings of the Bland–Altman analysis representative of the observed trends. For most food groups (vegetables, raw vegetables, breakfast cereals, sweetened milk, cold cuts, meat, cheese, butter and sweets), a systematic increase in difference between the two methods with increasing intake was observed indicating worse agreement at higher intakes. For fruit, fruit juices, soft drinks, milk, white bread and wholemeal bread, however, a double interpretation is possible. When considering intakes within the LOA only, it was observed that the agreement between methods was similar regardless of the average intake. On the other hand, beyond the LOA, it seemed that when mean intake increased the bias also increased up to a certain value, after which it started decreasing.

Discussion

The aim of the present study was to evaluate the ability of the CEHQ-FFQ in estimating age group-specific proxy-reported intakes of obesity-related foods compared with two 24-HDR (SACINA). To the authors' knowledge, the present study is the largest one carried out in children in which relative validity has been evaluated through food group intakes. Results showed wide differences in relative

Table 4 Pearson correlation coefficients between food group intakes (daily number of portions) from the CEHQ-FFQ and the 24-HDR: younger children aged 2–<6 years from eight European countries participating in the IDEFICS Study (2007–2008)

Food group	Pearson correlation coefficient	Variance ratio	De-attenuated correlation coefficient
Vegetables	0·14	0·80	0·17
Fried potatoes	0·05	0·92	0·06
Raw vegetables	0·33	0·58	0·37
Fruit	0·36	0·53	0·40
Sweetened fruit	−0·01	0·95	−0·01
Water	−0·41	0·25	−0·44
Fruit juices	0·32	0·50	0·36
Soft drinks	0·14	0·42	0·15
Light soft drinks	0·17	0·87	0·20
Sweetened breakfast cereals	0·28	0·66	0·32
Breakfast cereals	0·41	0·33	0·44
Milk	0·32	0·33	0·35
Sweetened milk	0·45	0·30	0·48
Yoghurt	0·20	0·63	0·23
Sweetened yoghurt	0·35	0·54	0·39
Fish	0·24	0·72	0·28
Fried fish	0·12	0·86	0·14
Cold cuts	0·27	0·53	0·30
Meat	0·06	0·79	0·07
Fried eggs	0·17	0·81	0·20
Eggs	0·13	0·95	0·16
Mayonnaise	0·11	0·88	0·13
Cheese	0·25	0·52	0·28
Jam & honey	0·29	0·54	0·33
Chocolate/nut-based spread	0·30	0·49	0·33
Butter & margarine	0·35	0·49	0·39
Ketchup	0·22	0·80	0·26
White bread	0·26	0·60	0·30
Wholemeal bread	0·35	0·34	0·38
Pasta & rice	0·24	0·78	0·28
Milled cereal	−0·01	1·00	−0·01
Pizza	0·11	0·85	0·13
Fast food	0·11	0·71	0·13
Nuts	0·18	0·71	0·21
Snacks	0·11	0·83	0·13
Sweets	0·17	0·57	0·19

CEHQ-FFQ, Children's Eating Habits Questionnaire–food frequency section; 24-HDR, 24 h dietary recall.

validity across the different food groups, emphasizing the importance of validating dietary assessment methods in terms of food groups rather than nutrients. It should also be considered that comparison of findings among validation studies is compromised by differences among the type of FFQ administered, sample size, food groups examined, unit of estimates, use of reference method, recall period or number of recorded days[35].

As expected, the CEHQ-FFQ gave higher mean intakes as opposed to the 24-HDR, a tendency also observed in previous studies carried out in adults and/or children[14,22,35–37]. Our findings suggest that episodically consumed food groups such as milled cereal, light soft drinks, fast food and sweetened fruit tended to be over-reported by the CEHQ-FFQ in this population group. This can partly be explained by the difficulty of the 24-HDR to capture infrequently consumed products, especially in children with highly varying diets and rapidly changing food habits[8].

More specifically, the low crude correlations observed increased slightly following correction for attenuation effect in the 24-HDR. Correlations tended to be stronger for foods with higher frequency of consumption, again indicating current problems in the assessment of episodically consumed foods. Respectively for younger and older children, fifteen and ten out of the thirty-six foods groups had correlation coefficients within the range of 0·3–0·8 as shown by others[9,14,22,36,38]. Coefficients for fruit (younger children), water, fish, cheese or white bread were comparable to or even higher (raw vegetables, sweetened milk (younger children), chocolate/nut-based spread, wholemeal bread and pasta & rice) than those found in a validation study conducted with Belgian adolescents[39]. Similarly, low coefficients for cooked vegetables (0·17 in younger children and 0·13 in older children) and for fried potatoes in older children (0·14) were comparable to those of an American validation study in 8–9-year-old students[40].

Correlations from food frequency instruments have generally been shown to be lower in child and adolescent populations than among adults[8]. Such observations

Table 5 Pearson correlation coefficients between food group intakes (daily number of portions) from the CEHQ-FFQ and the 24-HDR: older children aged 6–9 years from eight European countries participating in the IDEFICS Study (2007–2008)

Food group	Pearson correlation coefficient	Variance ratio	De-attenuated correlation coefficient
Vegetables	0·11	0·80	0·13
Fried potatoes	0·12	0·79	0·14
Raw vegetables	0·36	0·55	0·41
Fruit	0·30	0·51	0·34
Sweetened fruit	−0·02	0·93	−0·02
Water	−0·42	0·24	−0·44
Fruit juices	0·28	0·48	0·31
Soft drinks	0·21	0·43	0·23
Light soft drinks	0·08	0·47	0·09
Sweetened breakfast cereals	0·23	0·56	0·26
Breakfast cereals	0·18	0·47	0·20
Milk	0·24	0·39	0·26
Sweetened milk	0·33	0·38	0·36
Yoghurt	0·10	0·62	0·11
Sweetened yoghurt	0·32	0·46	0·35
Fish	0·25	0·71	0·29
Fried fish	0·12	0·89	0·14
Cold cuts	0·26	0·56	0·29
Meat	0·15	0·82	0·18
Fried eggs	0·10	0·92	0·12
Eggs	0·08	0·97	0·10
Mayonnaise	0·18	0·77	0·21
Cheese	0·24	0·59	0·27
Jam & honey	0·32	0·53	0·36
Chocolate/nut-based spread	0·31	0·54	0·35
Butter & margarine	0·40	0·47	0·44
Ketchup	0·20	0·85	0·24
White bread	0·23	0·47	0·26
Wholemeal bread	0·35	0·31	0·38
Pasta & rice	0·18	0·76	0·21
Milled cereal	−0·01	1·00	−0·01
Pizza	0·10	0·80	0·12
Fast food	0·12	0·91	0·14
Nuts	0·14	0·63	0·16
Snacks	0·10	0·84	0·12
Sweets	0·18	0·58	0·20

CEHQ-FFQ, Children's Eating Habits Questionnaire–food frequency section; 24-HDR, 24 h dietary recall.

could be partly attributed to the effect of proxy reporting, as proxies are conditioned by their ability to accurately recall their children's food intake[41]. Additionally, parents as proxies seem to be reliable reporters in the home setting[41] but the opposite is true for food intake out of home[41]. This limits parents' suitability as the sole informants of their children's intake.

Findings from the cross-classification analyses varied by food group and at times demonstrated the rather limited ability of the questionnaire to discriminate between quartiles of food groups. A third of the participants were allocated into the same category by both methods and on average only 7% and 8% of younger and older children, respectively, were likely to be classified into the opposite quartile. Although among the adapted food groups the proportion of misclassified individuals increased, higher agreement between the methods was found in terms of classification. Percentage agreement and misclassification were within the ranges reported by other authors[14,21,38] for the non-adapted food groups. However, the degree of misclassification observed among the adapted groups was remarkably higher compared with previous studies[14,21,38]. Findings from the κ_w analysis also confirmed fair agreement between the CEHQ-FFQ and 24-HDR.

In general, no great differences were observed by age group in terms of correlation coefficients and agreement between the CEHQ-FFQ and 24-HDR, since values were similar for most of the food groups. It is noteworthy, however, that correlation coefficients for some highly consumed food groups – i.e. fruit, breakfast cereals, milk, sweetened milk and yoghurt – were considerably higher in younger children compared with those obtained among their older peers. Similarly, κ_w values were also higher for milk, white bread, sweetened milk and butter & margarine in 2–<6-year-old children. This can be explained by the fact that younger children are less likely to be unsupervised during in-home and out-of-home eating than older children[1,41]. Consequently, parents become more reliable reporters and more capable of reporting their children's intake in an accurate way.

Table 6 Cross-classification by quartile of food group intakes from the CEHQ-FFQ and the 24-HDR: younger children aged 2–<6 years from eight European countries participating in the IDEFICS Study (2007–2008)

	CEHQ-FFQ *v.* two 24-HDR		
Food group	Correctly classified (%)	Grossly misclassified (%)	κ_w
Vegetables	30·2	3·6	0·13
Fruit	35·5	4·7	0·34
Milk	38·6	7·8	0·36
Cold cuts	32·0	4·8	0·27
Meat	27·0	10·0	0·10
Cheese	33·7	10·5	0·30
White bread	35·8	12·3	0·29
Sweets	25·9	9·9	0·17
Adapted food groups†			
Raw vegetables	42·1	22·6	0·14
Fruit juices	41·2	17·7	0·17
Soft drinks	42·0	29·2	0·10
Breakfast cereals	45·9	19·4	0·26
Sweetened milk	49·3	10·1	0·30
Butter & margarine	47·0	16·2	0·24
Wholemeal bread	38·2	18·6	0·12

CEHQ-FFQ, Children's Eating Habits Questionnaire–food frequency section; 24-HDR, 24 h dietary recall; κ_w, weighted kappa statistic.
For fried potatoes, sweetened fruit, water, light soft drinks, sweetened breakfast cereals, yoghurt, sweetened yoghurt, fish, fried fish, fried eggs, eggs, mayonnaise, jam & honey, chocolate/nut-based spread, ketchup, pasta & rice, milled cereal, pizza, fast food, nuts and snacks, ranking into quartiles or tertiles was not possible since >25 % of the participants did not consume these foods on each recall day.
†Within that food groups, zero consumers were considered as one group and tertiles were constructed for the remaining participants.

Table 7 Cross-classification by quartile of food group intakes from the CEHQ-FFQ and the 24-HDR: older children aged 6–9 years from eight European countries participating in the IDEFICS Study (2007–2008)

	CEHQ-FFQ *v.* two 24-HDR		
Food group	Correctly classified (%)	Grossly misclassified (%)	κ_w
Vegetables	28·7	3·9	0·10
Fruit	34·5	7·3	0·31
Milk	33·3	7·4	0·24
Cold cuts	33·9	4·7	0·26
Meat	27·7	7·0	0·14
Cheese	32·6	11·5	0·31
White bread	29·3	7·5	0·23
Sweets	30·4	10·2	0·18
Adapted food groups†			
Raw vegetables	39·6	21·0	0·15
Fruit juices	41·9	18·2	0·16
Soft drinks	38·8	28·0	0·10
Sweetened milk	52·5	5·8	0·36
Butter & margarine	38·0	12·3	0·10
Wholemeal bread	32·3	17·0	0·05

CEHQ-FFQ, Children's Eating Habits Questionnaire–food frequency section; 24-HDR, 24 h dietary recall; κ_w, weighted kappa statistic.
For fried potatoes, sweetened fruit, water, light soft drinks, sweetened breakfast cereals, breakfast cereals, yoghurt, sweetened yoghurt, fish, fried fish, fried eggs, eggs, mayonnaise, jam & honey, chocolate/nut-based spread, ketchup, pasta & rice, milled cereal, pizza, fast food, nuts and snacks, ranking into quartiles or tertiles was not possible since >25 % of the participants did not consume these foods on each recall day.
†Within that food groups, zero consumers were considered as one group and tertiles were constructed for the remaining participants.

The lack of agreement between methods of assessment observed in children has often been attributed to a number of factors[41], including the use of proxy reporting as discussed earlier, the nature of the diet of young age groups and the lack of a gold standard for directly assessing the validity or relative validity of FFQ, among others[13]. Moreover, FFQ validity is highly conditioned by the reference method, which is also subject to instrument-specific limitations. In addition, proxies reported the 24-HDR, who tend to under-report intake[8]. It should be noted that the European Food Consumption Survey Method (EFCOSUM) has recommended the use of two or more non-consecutive 24 h recalls as the best method to assess food consumption in individuals aged 10 years and above in different European countries[42].

Dietary information is affected by high day-to-day variability in children's diets[8], which could explain the lack of agreement between methods. This influence could be minimized by an increase in the number of recording days, but long recording periods reduce the accuracy of recording owing to increasing fatigue and boredom, potential alterations of dietary habits and increasing likelihood of drop-outs[43]. Additionally, the large sample size included in the present study makes up for the small number of replicates to keep the same precision of the corrected correlation coefficient[13]. Moreover, the fact that portion sizes were not assessed in the CEHQ-FFQ might also affect the agreement between both methods; i.e. overestimation of foods consumed in small quantities and underestimation of those consumed in higher quantities. Considering the increased respondent burden however, no attempts were done to capture portion sizes in the current study[13]. Our sample differed from the IDEFICS whole sample in terms of baseline characteristics, which means that these results might not be generalized to all participating children. However, no differences were found for BMI which is considered to be an indicator of misreporting[44].

As stated before, Hungary collected the 24-HDR information differently from the other survey centres and this is considered as one of the study limitations influencing the generalizability of its results. Our findings suggest that when Hungarian data were excluded, the strength of the associations between the CEHQ-FFQ and the 24-HDR increased. In fact, the number of food groups showing moderate correlation coefficients increased and the number of slight correlations decreased. Furthermore, when cross-classification analyses were applied, without considering Hungarian data the degree of agreement in both non-adapted and adapted food groups increased. Indeed, the proportions of correctly classified individuals as well as κ_w values improved towards higher values, whereas the percentages of grossly misclassified individuals decreased.

To our knowledge, the present study is the first one performed in a large sample of European children of

(pre)school age in which proxy-reported data obtained from an FFQ were compared with those from two 24-HDR. Another important strength of the study is standardized procedures followed during the data collection of the IDEFICS fieldwork[17]. High-quality control procedures were applied during the different stages of the project, including checks for plausibility already implemented in the database and performed during data entry. In addition, the reference method used in the study was previously validated with the doubly labelled water method considered as the 'gold standard' method for this purpose. Furthermore, portions/d were used instead of g/d offering newer approaches and insights into validation studies using FFQ despite associated limitations.

Conclusions

Findings of the present study suggest that the strength of association estimates assessed by the CEHQ-FFQ and the 24-HDR varied by food group intakes and by age group. In addition, the ability of the CEHQ-FFQ to rank children according to intakes of food groups was lower than expected but in line with other studies. Overall, these results suggest low agreement for the majority of food groups examined by a proxy-estimated FFQ and two 24-HDR in a large sample of 2–9-year-old European children. However, one should consider that both instruments are subject to measurement errors affecting the strength of the association. In that sense, the CEHQ-FFQ could provide acceptable food estimates at group level. It is of great importance to detect true diet–disease relationships with the aim to develop public health strategies to prevent children from suffering chronic diseases. For that reason, validation studies are indispensable to test the validity and appropriateness of dietary assessment methods used within epidemiological surveys to accurately assess food intake.

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Supplementary Materials

For Supplementary Materials for this article, please visit http://dx.doi.org/10.1017/S1368980012005368

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Public Health Nutrition

Artículo III [Paper III]:

The role of dietary fat on the association between dietary amino acids and serum lipid profile in European adolescents participating in the HELENA Study

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Abstract

Background/Objectives: The role of dietary amino acid (AA) on serum lipid profile remains unclear. Our aim was to examine the relationship between AA intake and serum lipid profile in European adolescents from eight European cities participating in the cross-sectional (2006-2007) HELENA study, and to assess whether this association was independent of total fat intake.

Subjects/Methods: Diet, skinfold thicknesses, triglycerides, total cholesterol (TC), high density lipoprotein-cholesterol (HDL-c), TC/HDL-c ratio, low density lipoprotein-cholesterol (LDL-c), apolipoprotein B, apolipoprotein A1 and apolipoprotein B/apolipoprotein A1 ratio were measured in 454 12.5-to-17.5-year-old adolescents (44% boys). Intake was assessed via two non-consecutive 24-hour dietary recalls. Data on maternal education and sedentary behaviours was obtained via questionnaires. Physical activity was objectively measured by accelerometry.

Results: Alanine, arginine, asparaginic acid, glycine, histidine, lysine and serine intakes were inversely associated with serum triglyceride concentrations in both boys and girls. Intake of other AAs such as alanine and/or arginine was also inversely associated with serum TC, LDL-c and apolipoprotein B/apolipoprotein A1 ratio in girls, but not in boys. In girls, an inverse association was observed between intakes of alanine, isoleucine, leucine, methionine, serine, tryptophan, tyrosine and valine and TC/HDL-c ratio. Similar results were found in males but only for serine and tryptophan intakes. It is noteworthy, however, that associations were no longer significant in both genders when total fat intake was considered as a confounding factor.

Conclusions: In this sample of adolescents, the association between AA intakes and serum lipid profile did not persist when dietary fat was considered. Therefore, dietary interventions

and health promotion activities should focus on fat intake to improve lipid profile and potentially prevent CVD.

Keywords: amino acids, lipoproteins, dietary fats, adolescents.

Introduction

Cardiovascular diseases (CVD) are the number one cause of death worldwide, equally affecting men and women, not only in high-income countries, but also across those of low- and middle-income (1). Furthermore, the presence of CVD in childhood shows that the onset of atherosclerosis occurs in early stages of life (2). The development and clustering of CVD risk factors seems to be influenced by several characteristics including heritable traits, prenatal and infantile influences, diet, physical activity and socioeconomic status (2). Abnormal lipid metabolism has previously been associated with lifestyle-related diseases (3); indeed, hypertriglyceridemia and hypercholesterolemia are well established as significant risk factors for CVD (4, 5). In addition, serum lipids and lipoprotein levels seem to track from early childhood into young adulthood i.e. adverse levels of low density lipoprotein-cholesterol (LDL-c) in childhood appeared to persist over time and progress to adult dyslipidemias (6) emphasizing the need of rapid identification and treatment of individuals at risk early in life.

Plasma lipid levels are controlled not only by dietary fat and carbohydrates but also by dietary protein (3). Vegetable protein has been shown to reduce cholesterol plasma levels compared with animal protein (3), but some authors have suggested amino acids (AA) *per se* may be more important than the protein source (3). The role that dietary AA may exert on individual CVD risk factors, however, remains unclear coupled by the lack of human studies. Dietary protein and, more specifically, intakes of specific AA are inversely associated with obesity (7, 8). Contradictory findings have been reported, however, with elevated serum concentrations of branched-chain AA (BCAA) and aromatic AA (e.g. phenylalanine and tyrosine) being markers of insulin resistance in young normoglycemic adults (9). No association was found between glutamic acid, arginine, lysine, tyrosine and cysteine intakes and blood pressure or risk of hypertension in a Dutch older population(10). Available literature between the association of AA intake and serum lipid profile is scarce in humans,

especially in young populations. Studies carried out in elderly people have reported a decrease in plasma cholesterol and triglycerides (TG) when essential AA plus arginine or only arginine was supplemented (11, 12). Essential AA in elderly people and sulfur-containing AA in animal models have previously been related to serum lipid profile (3, 10, 11). The associations, however, between other AA and blood lipids have not been examined yet in humans or animals in spite of the fact that several AA like tyrosine or glutamic acid, among others, have been found to be associated with other CVD risk factors such as insulin resistance or blood pressure, respectively (9, 13). To confirm previous findings during adolescence and explore new associations, the relationship between serum lipid profile and the following AA were examined: alanine, arginine, asparaginic acid, cysteine, glycine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

Total fat intake, specifically saturated fat, is observed to be positively associated to CVD (14); however, this relationship may depend on the dietary context in which saturated fat is consumed (15). Therefore, the current study aimed to investigate the relationship between AA intake and serum lipid profile, and to assess whether this association was independent of total fat intake in a sample of European adolescents.

Materials and Methods

Data for this study were obtained from the cross-sectional multi-centre HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) study (n=3 528) conducted between 2006 and 2007 in ten European cities (Athens in Greece, Dortmund in Germany, Ghent in Belgium, Heraklion in Greece, Lille in France, Pecs in Hungary, Rome in Italy, Stockholm in Sweden, Vienna in Austria and Zaragoza in Spain). General procedures, characteristics and inclusion criteria of the HELENA study have been previously described (16, 17). The study was performed following the ethical guidelines of the Declaration of Helsinki 1964 and ethical

approval was obtained from the local Ethical Committee at each study center (18). Both adolescents and their parents signed a written informed consent.

Blood samples were drawn randomly in one third of the HELENA participants (n=1,089) after an overnight fast. As such, adolescents aged 12.5-17.5 years with complete measurements on TG, total cholesterol (TC), high density lipoprotein-cholesterol (HDL-c), LDL-c, apolipoprotein B (Apo B), apolipoprotein A1 (Apo A1) and two 24-hour dietary recalls (24-HDR) were included in this analysis (n=454, 44.6% boys). It should be noted that eight out of the ten study centers were included in the 24-HDR analyses; Heraklion and Pécs were excluded due to logistical reasons. Adolescents form the entire HELENA cohort were significantly ($p<0.05$) older, weighed more and had higher mean body mass index (BMI) (data not shown) than those included in this study.

Maternal education. Maternal educational attainment, accessed via a self-administered questionnaire, was considered as an indicator of socioeconomic status with four included categories: 1) lower education, 2) lower secondary education, 3) higher secondary education, and 4) higher education/university degree.

Sedentary behaviors. The average time engaging in two separate sedentary behaviors (TV viewing and playing with videogames) was estimated by means of a self-administered questionnaire previously reported to demonstrate good reliability (19).

Physical activity. Uni-axial accelerometers (Actigraph MTI, model GT1M, Manufacturing Technology Inc., Fort Walton Beach, FL, USA) were used to objectively measure physical activity (20). At least three days of recording, with a minimum of 8 hours registration per day, was set as an inclusion criterion. The time sampling interval was set at 15 seconds. The time spent at moderate to vigorous physical activity (MVPA) (>3 metabolic equivalents) was calculated on the basis of the following cut-off points: ≥ 2000 counts per minute for moderate PA and ≥ 4000 counts per minute for vigorous PA (20, 21).

Amino acids intake. Dietary intake was assessed by the HELENA-DIAT (Dietary Assessment Tool), a self-administered computer-based tool shown to accurately assess dietary information of European adolescents (22). The software consists of a single 24-HDR structured according to six meal occasions. Adolescents were asked to recall all food and drinks consumed the previous day. Two non-consecutive 24-HDR within a time span of two weeks were obtained from each participant. One of the 24-HDR was collected the same day that blood drawing took place. Questionnaires were completed during school time and assisted by fieldworkers; therefore no information on Fridays and Saturdays was available.

The German Food Code and Nutrition Date Base (Bundeslebensmittelschlüssel, BLS Version II.3.1) (23) was used to calculate energy and nutrient intakes. The usual food and nutrients intake was estimated by the multiple source method (MSM) which takes into account the within-person variability of the dietary data (24). Energy intake was estimated in kilocalories per day (kcal/d), protein and fat intake in (g/d) and AA intake in milligrams per day (mg/d).

The following AAs were included in the analysis: alanine, arginine, asparaginic acid, cysteine, glycine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

Physical examinations. Weight and height were measured in underwear and barefoot with an electronic scale (SECA 861, Seca Ltd., Birmingham, UK) and a stadiometer (SECA 225, Seca Ltd., Birmingham, UK). Body mass index (BMI) was calculated as body weight in kilograms divided by the square of height in meters. Skinfold thickness was measured with a Holtain Caliper (Holtain Ltd., Crymmych, UK) in triplicate on the left side at biceps, triceps, subscapular and suprailiac sites. All anthropometric measures were taken following a standardized protocol (25).

Blood sampling. Blood sampling procedures have been previously described in detail (26). Briefly, blood samples were drawn after an overnight fast and analyzed in centralized

laboratories. Serum TG, TC, HDL-c, and LDL-c were measured on a Dimension RxL clinical chemistry system (Dade Behring, Schwalbach, Germany) using enzymatic methods. Apo B and Apo A1 were measured in an immunochemical reaction with a BN II analyzer (Dade Behring, Schwalbach, Germany). The TC/HDL-c and the Apo B/Apo A1 ratio were computed.

Statistical analysis. The normality of all variables was checked and non-normally distributed variables (TG, TC, HDL-c, LDL-c, TC/HDL-c ratio, Apo B/Apo A1 ratio and AA intake) were log-transformed prior to the analysis. Gender differences were tested by means of the independent samples T-test for normally distributed variables and the Mann-Whitney U test for non-normally distributed variables. In case of categorical variables, the Chi-squared test was applied.

The association between AA intakes (independent variables) and plasma lipids concentrations (dependent variables) was examined separately by gender by performing multilevel linear regression analysis. Study center was included as random intercept. Age, maternal education, sum of four skinfolds, MVPA, sedentary behaviors and total daily energy intake were entered as covariates in model 1. Model 2 included covariates from model 1 plus total fat intake. The statistical software packages Stata version 11.0 (Stata Corp., college Station, TX, USA) and Predictive Analytics SoftWare (PASW, version 18; SPSS Inc., Chicago, IL, USA) were used to perform the analyses. Statistical significance was set at $p<0.05$.

Results

Descriptive data is provided in **Table 1** and **Table 2**. Boys were significantly taller and weighed more than their female peers ($p<0.001$) but no significant differences in terms of BMI were observed. Additionally, girls showed significantly higher concentrations of TG, TC, HDL-c, LDL-c, Apo A1 and Apo B than boys ($p<0.001$), whereas AA intake was significantly higher in adolescent males ($p<0.001$).

Results obtained by multilevel linear regression analysis are displayed in **Tables 3-5**. In girls and considering model 1, alanine, arginine, asparaginic acid, cysteine, glycine,

histidine, lysine, threonine and valine were inversely associated with TC (Table 3). A negative association was also observed between alanine, isoleucine, leucine, methionine, serine, tryptophan, tyrosine and valine and TC/HDL-c ratio in girls (Table 3). Intakes of all AAs were inversely associated with TG in girls (Table 4). A negative association was also observed between alanine and arginine intakes and LDL-c (Table 4) and between alanine intake and Apo B/Apo A1 ratio (Table 5). These associations were no longer significant after adjustment for total fat intake (model 2).

In boys, serine and tryptophan were inversely associated with TC/HDL-c ratio (Table 3) in model 1. An inverse association was also observed between alanine, arginine, asparaginic acid, glycine, histidine, lysine and serine intakes and TG (Table 4). Similarly to girls, these associations did not persist in model 2 when total fat intake was considered as a confounder. No associations were observed between AA intake and HDL-c and Apo A1 in any of both genders (data not shown).

Discussion

This study examined the relationship between AA intake and serum lipid profile in European adolescents participating in the HELENA study. Our initial analysis suggested an inverse association of AA intake with TG, TC, LDL-c, TC/HDL-c ratio, Apo B and Apo B/Apo A1 ratio in girls and with TG and TC/HDL-c ratio in boys; these associations did not remain significant following adjustments for the effect of total fat intake. To the authors' knowledge, this is the first study addressing this topic in adolescents.

Apart from their structural function in proteins, AAs are precursors of many essential biological compounds (27) and are involved in a large amount of metabolic pathways in the human organism. Previous studies have focused on the association of AA with diverse CVD risk factors such as obesity, insulin resistance or blood pressure (7, 8, 10, 28) among others, but the available literature on the association between AA intake and serum lipid profile in humans is scarce and remains unclear. For instance, diet supplementation with essential AAs

has been shown to lower plasma TG, TC and very low density lipoprotein cholesterol (VLDL-c) concentrations in elderly people (11). Similarly, Hurson et al. (12) also observed a decrease in TC and LDL-c in elderly people supplemented with arginine. Our results are in agreement with these previous findings (only in model 1). In these studies, however, data were not adjusted for potential confounders such as total fat intake and addressed older population. The mechanisms underlying the effect of AA supplementation on serum lipid profile are not known (11). Sulfur AA, i.e. cysteine and methionine, have been recognized as potent modulators of lipid metabolism (29) by increasing plasma HDL-c and lowering VLDL-c (3). Although we did not observe such associations, an inverse association was observed in female adolescents between cysteine and methionine intake and Apo B in model 1, the main protein constituent of LDL-c (30), shown to play a beneficial role on CVD (3).

We have also hypothesized that the observed results could be due to an indirect association, i.e. AA intake might be associated with one factor, in this instance body fat, which in turn is associated with serum lipids. Indeed, it is known that obesity enhances metabolic syndrome features such as dyslipemia by increasing LDL-c and TG and lowering HDL-c plasma concentrations (31). This suggests that a decrease on fat mass induced by AA consumption could result in a better lipid profile. Qin et al. (8) observed inverse associations between intakes of BCAA, including leucine, isoleucine and valine, and prevalence of overweight status among apparently healthy middle-aged adults in those from East Asian, i.e. China and Japan, and Western, i.e. UK and USA, countries and with prevalence of obesity in adults from Western countries. Arginine and lysine are also inversely associated with fat mass among 6-year-old (32) and 8-10-year-old (7) European girls. Mechanisms that explain the beneficial role of dietary arginine are not completely known but it seems, however, that arginine alters the balance of energy intake and expenditure in favour of fat loss or reduced growth of white adipose tissue by stimulating mitochondrial biogenesis signaling and brown adipose tissue development (33). Furthermore, the somatotropic effects of arginine and lysine

(34) may result in a decrease in serum levels of TG and fat mass (7). In concordance with this, our described associations were found to be significant mainly in girls. This might be explained by gender-differences in body composition e.g. greater percentage of body fat in girls compared to their male peers (35, 36) also seen in our findings, i.e. adolescent females had significantly higher ($p<0.001$) sum of four skinfolds than boys.

Protein-rich diets have been tested in many occasions in order to identify the role of high protein intake in the body. In a cross-over study carried out in adults, Appel et al. (37) found that following the administration of a healthy diet, rich in protein and low in saturated fat, TG concentrations significantly decreased compared to a carbohydrate-rich diet and a diet rich in unsaturated fat. This fact led authors to suggest that protein could have a direct lowering effect on TG, beyond that of replacing carbohydrates, which usually increase serum TG (11). A review carried out by Clifton (38) suggested that high protein diets were associated with greater weight loss, lower plasma TG and blood pressure and, in times, increased lean mass compared to high carbohydrate diets. The Food and Nutrition Board report (39) states that high levels of protein intake may have detrimental effects but no documented adverse effects of high rich protein diets exists.

Fat intake, specifically saturated fat, has been observed to be positively associated with CVD by increasing plasma LDL-c and TC concentrations (14); indeed, adolescent boys participating in a dietary intervention focused on decreasing fat intake showed a more favourable lipid profile than those in the control group (40). A recent meta-analysis of prospective epidemiologic studies, however, has shown no significant evidence to conclude that that dietary saturated fat is associated with an increased risk of CVD (14). On the other hand, it has been reported that the saturated fat content of red meat, which is one of the richest sources of protein and, consequently of AA, may be partly responsible for its positive association with CVD risk (41). Our results showed that the initial association observed between AA intakes and blood lipids in model 1 disappeared after adjustment for total fat

intake (model 2). This leads authors to hypothesize that the positive role that AAs might play on blood lipids is neutralized when AA, or proteins, are consumed together with fat, mainly saturated fat. High protein intake, however, is not necessarily related to high red meat intake but to other protein-rich foods like fish, poultry, milk and dairy products, nuts, egg or plant-based products among others. Indeed, poultry and dairy intake have been shown to have neutral effects on CVD risk (42). Furthermore, Mangravite et al. (15) reported that on carbohydrate-restricted subjects the source of dietary protein may modify the effects of saturated fat on atherogenic lipoproteins and proposed that the relationship between saturated fat and CVD risk may vary according to the dietary context in which saturated fat is consumed.

A limitation of our study is its cross-sectional design that does not allow the determination of any causal associations. Diet was assessed by means of two self-administered, computer-assisted, non-consecutive 24-HDR. Like any other self-reporting method, our self-administered 24-HDR is also prone to measurement error (43). An increase in the number of recording days would have been advisable to compensate for day-to-day variability (44); however, this method has been shown to be an appropriate measure to collect detailed dietary data in adolescents (22, 45). Furthermore, dietary information was corrected for within-person variability (24). The fact that AA intake might be estimated less accurately than other nutrients is another limitation of the study. It is noteworthy to mention that blood samples were collected following a specific methodology and transport system to a centralized laboratory in order to assure samples viability and stability (26) as study strengths. Additionally, fieldworkers were trained and a manual of operation was developed to guarantee good clinical practice (26).

In conclusion, our findings suggest that the association between AA intakes and serum lipid profile does not persist when dietary fat is considered. Whether AA intakes could be associated with a healthier lipid profile in adolescents and, therefore, with CVD risk remains

unclear, at least based on the evidence from this sample of European adolescents. Furthermore, our findings add new evidence to the lack of studies addressing the association of AA intakes with plasma lipids concentration during adolescence, and emphasize the importance of considering dietary fat as a possible confounder of this relationship. More prospective and intervention studies are needed to confirm our findings and to obtain robust conclusions on the effect of AA intakes on plasma lipid concentrations and, consequently, on CVD risk in adolescents.

Table 1. Main characteristics of the study participants stratified by sex.

	All (n= 454)		Boys (n= 200)		Girls (n= 254)		P
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	
Age (years)	14.6	1.15	14.6	1.21	14.6	0.07	0.085
Weight (kg)	57.4	12.13	59.4	13.3	55.9	10.92	<0.001*
Height (cm)	165.3	9.44	169.6	9.84	161.9	7.53	<0.001*
BMI (kg/m²)	20.9	3.48	20.5	3.41	21.2	3.50	0.097
Underweight (%)[1]		7.3		8.2		6.6	-
Normal weight (%)		74.0		74.9		73.4	-
Overweight (%)		13.2		10.3		15.6	-
Obese (%)		5.5		6.7		4.5	-
Maternal education (%)							0.035‡
Lower education		8.4		6.2		10.2	-
Lower secondary education		20.7		17.9		23.0	-
Higher secondary education		32.3		30.3		34.0	-
Higher education/University degree		38.5		45.6		32.8	-
Apo A1 (g/L)	1.52	0.01	1.48	0.01	1.55	0.01	<0.001*
Apo B (g/L)	0.65	0.01	0.61	0.01	0.67	0.01	<0.001*
	Median	***25th-75thpercentile***	***Median***	***25th-75thpercentile***	***Median***	***25th-75thpercentile***	
Sum four skinfolds (mm)	45.8	31.6-65.3	132.9	77.1-208.9	107.1	65.3-167.1	<0.001†
MVPA (min/day)	53.8	40.5-70.7	64.5	50.0-81.8	48.0	34.3-60.0	<0.001†
Sedentary behaviors (min/day)	111.4	75.0-184.3	33.7	26.1-51.9	54.5	39.9-75.4	<0.001†
TG (mg/dl)	60.0	46.0-80.0	57.0	43.0-77.0	63.0	48.0-84.5	<0.001†
TC (mg/dl)	161.6	143.0-178.0	154.0	138.0-168.0	167.0	148.5-184.0	<0.001†
HDL-c (mg/dl)	55.0	49.0-63.0	54.1	47.5-59.5	56.0	50.0-64.5	<0.001†
LDL-c (mg/dl)	94.0	79.0-108.0	90.0	77.0-100.0	97.0	80.0-114.0	<0.001†
TC/HDL-c ratio	2.88	2.52-3.29	2.81	2.53-3.20	2.89	2.52-3.37	0.340
Apo B/Apo A1 ratio	0.43	0.35-0.43	0.43	0.33-0.48	0.42	0.35-0.50	0.169

Apo A1, apolipoprotein A1; Apo B, apolipoprotein B; BMI, body mass index; HDL-c, high densitity lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol; MVPA, moderate to vigorous physical activity; TC, total cholesterol; TG, triglycerides.

[1]BMI categories according to Cole et al. (46).

*p<0.05 by means of independent samples T-Test; †p<0.05 by means of Mann-Whitney U test; ‡p<0.05 by means of Chi-squared test.

Table 2. Dietary characteristics of the studied participants stratified by sex.

	All (n= 454)		Boys (n= 200)		Girls (n= 254)		P
	Median	*25th-75thpercentile*	*Median*	*25th-75thpercentile*	*Median*	*25th-75thpercentile*	
Energy intake (kcal/day)	2080.3	1675.3-2577.4	2453.4	2032.7-3002.2	1832.5	1513.2-2208.8	<0.001†
Total fat intake (g/day)	86.7	65.4-113.2	100.3	79.2-131.5	77.3	57.9-96.2	<0.001†
Total fat intake (% energy)	37.2	31.3-43.1	36.7	32.0-42.3	37.4	31.0-43.6	0.594
Total fat intake (g/kg/day)	1.47	1.13-1.96	1.73	1.30-2.18	1.29	1.04-1.69	<0.001†
Total protein intake (g/day)	83.9	65.5-103.9	99.6	80.6-120.7	71.4	60.1-87.5	<0.001†
Total protein intake (% energy)	16.0	13.9-18.0	15.9	13.9-17.9	16.0	13.9-18.3	0.881
Total protein intake (g/kg/day)	1.58	1.13-2.10	1.77	1.34-2.34	1.41	0.97-1.83	<0.001†
Amino acids intake (mg/day)							
Alanine	3962.3	3051.5-4969.9	4745.6	3778.6-5652.8	3381.0	2708.2-4420.6	<0.001†
Arginine	4501.0	3437.5-4501.0	5471.4	4171.5-6332.0	3837.02	3096.6-4972.6	<0.001†
Asparaginic acid	7258.2	5553.4-9062.4	8479.4	6856.4-10248.9	6206.7	5028.0-7998.9	<0.001†
Cysteine	1071.8	848.9-1071.8	1298.0	1035.1-1528.2	934.6	759.7-1166.0	<0.001†
Glutamic acid	16304.5	12859.0-20420.6	19573.6	15869.5-23337.1	14180.2	11654.0-17491.1	<0.001†
Glycine	3364.9	2613.1-4259.0	4022.5	3168.1-4775.7	2887.2	2345.1-3708.3	<0.001†
Histidine	2275.6	1731.0-2792.7	2707.7	2189.8-3241-2	1904.3	1548.5-2470.0	<0.001†
Isoleucine	3993.1	3087.5-4982.4	4728.3	3746.7-5712.7	3360.6	2783.6-4324.5	<0.001†
Leucine	6503.2	5046.9-8089.6	7700.8	6101.0-9291.7	5458.4	4504.1-7041.8	<0.001†
Lysine	5572.6	4280.6-7038.5	6560.4	5255.7-8014.5	4782.51	3752.9-6213.1	<0.001†
Methionine	1795.9	1400.3-2272.4	2139.4	1711.8-2610.6	1540.3	1273.7-2020.3	<0.001†
Phenilalanine	3677.0	2885.8-4563.1	4378.2	3528.3-5183.8	3115.0	2520.8-3891.3	<0.001†
Proline	5780.1	4466.8-7235.0	6902.6	5729.4-8200.4	5034.4	4114.4-6115.5	<0.001†
Serine	3962.2	3106.5-4991.7	4788.5	3858.9-5662.9	3403.5	2750.3-4202.9	<0.001†
Threonine	3290.6	2535.36-4103.5	3968.7	3163.5-4672.0	2810.2	2322.9-3601.1	<0.001†
Tryptophan	944.3	743.8-1172.6	1138.2	880.2-1352.5	810.0	661.3-1018.7	<0.001†
Tyrosine	2953.4	2277.0-3684.7	3528.6	2779.8-4243.5	2502.8	2047.4-3185.6	<0.001†
Valine	4489.83	3500.1-5592.4	5397.7	4322.9-6470.2	3819.4	3128.8-4840.7	<0.001†

†p<0.05 by means of Mann-Whitney U test.

Table 3. Multilevel regression analysis addressing the association between amino acids (AA) intakes and total cholesterol (TC) and total cholesterol/high density lipoprotein cholesterol (TC/HDL-c) ratio and in the participating adolescents.

Aminoacids intake(mg/d)[a]	TC								TC/HDL-c ratio							
	Boys (n=200)				Girls (n=254)				Boys (n=200)				Girls (n=254)			
	β	95% CI	P[1]	P[2]	β	95% CI	P[1]	P[2]	β	95% CI	P[1]	P[2]	β	95% CI	P[1]	P[2]
Aliphatic side chains																
Alanine	-0.025	-0.115,0.065	0.589	0.540	-0.101	-0.186,-0.016	0.020	0.184	-0.094	-0.207,0.018	0.101	0.207	-0.103	-0.202,-0.004	0.041	0.445
Glycine	-0.016	-0.107,0.076	0.735	0.705	-0.097	-0.184,-0.010	0.030	0.244	-0.103	-0.217,0.011	0.075	0.154	-0.089	-0.190,0.012	0.083	0.680
Isoleucine	-0.045	-0.139,0.049	0.352	0.283	-0.090	-0.183,0.003	0.057	0.407	-0.107	-0.224,0.010	0.074	0.161	-0.115	-0.222,-0.009	0.033	0.446
Leucine	-0.045	-0.141,0.050	0.351	0.276	-0.092	-0.186,0.003	0.058	0.397	-0.104	-0.223,0.015	0.088	0.192	-0.115	-0.224,-0.006	0.038	0.467
Valine	-0.050	-0.147,0.048	0.318	0.237	-0.098	-0.194,-0.003	0.044	0.365	-0.112	-0.233,0.010	0.072	0.159	-0.122	-0.232,-0.013	0.029	0.453
Aromatic side chains																
Phenilalanine	-0.050	-0.151.0.051	0.330	0.258	-0.094	-0.194,0.006	0.065	0.469	-0.125	-0.250,0.000	0.050	0.114	-0.114	-0.229,0.001	0.051	0.615
Tryptophan	-0.054	-0.153,0.044	0.281	0.205	-0.086	-0.184,0.012	0.086	0.543	-0.124	-0.247,-0.002	0.047	0.105	-0.113	-0.225,-0.000	0.049	0.579
Tyrosine	-0.047	-0.141,0.048	0.331	0.255	-0.083	-0.176,0.011	0.083	0.506	-0.111	-0.229,0.007	0.064	0.142	-0.109	-0.216,-0.001	0.047	0.527
Basic side chains																
Arginine	-0.018	-0.110,0.074	0.699	0.658	-0.113	-0.200,-0.025	0.011	0.131	-0.106	-0.221,0.010	0.072	0.153	-0.099	-0.200,0.002	0.055	0.587
Histidine	-0.028	-0.119,0.064	0.551	0.495	-0.092	-0.180,-0.004	0.041	0.312	-0.114	-0.228,0.000	0.050	0.111	-0.094	-0.196,0.007	0.069	0.624
Lysine	-0.033	-0.117,0.050	0.430	0.376	-0.080	-0.159,-0.001	0.047	0.322	-0.099	-0.203,0.006	0.062	0.131	-0.088	-0.179,0.002	0.057	0.525
Acidic side chains																
Asparaginic acid	-0.038	-0.130,0.053	0.409	0.351	-0.098	-0.185,-0.010	0.028	0.235	-0.098	-0.213,0.016	0.092	0.187	-0.098	-0.199,0.002	0.056	0.549
Glutamic acid	-0.041	-0.143,0.062	0.440	0.376	-0.087	-0.194,0.019	0.109	0.609	-0.105	-0.234,0.023	0.108	0.233	-0.115	-0.238,0.007	0.066	0.649
Hydroxyl side chains																
Serine	-0.057	-0.160,0.046	0.277	0.202	-0.089	-0.190,0.013	0.088	0.585	-0.129	-0.257,-0.002	0.047	0.106	-0.120	-0.237,-0.003	0.044	0.605
Threonine	-0.039	-0.132,0.055	0.417	0.346	-0.096	-0.187,-0.006	0.037	0.300	-0.110	-0.227,0.007	0.066	0.143	-0.111	-0.215,-0.007	0.036	0.457
Sulfur-containing side chains																
Cysteine	-0.023	-0.126,0.079	0.659	0.619	-0.110	-0.215,-0.004	0.042	0.347	-0.116	-0.244,0.012	0.076	0.162	-0.116	-0.238,0.006	0.062	0.664
Methionine	-0.036	-0.126,0.053	0.428	0.364	-0.084	-0.170.0.003	0.057	0.393	-0.105	-0.216,0.007	0.065	0.142	-0.107	-0.206,-0.007	0.035	0.434
Cyclic side chain																
Proline	-0.051	-0.152,0.050	0.323	0.263	-0.051	-0.157,0.055	0.348	0.950	-0.087	-0.213,0.039	0.176	0.353	-0.092	-0.214,0.030	0.138	0.811

[1]Model 1: adjusted by center, maternal education, age, sum of four skinfolds, physical activity, sedentary behaviors, and energy intake.

[2]Model 2: adjusted by model 1 plus total fat intake

[a]Log-transformed data.

Table 4. Multilevel regression analysis addressing the association between amino acids (AA) intakes and triglycerides (TG) and low density lipoprotein cholesterol (LDL-c) in the participating adolescents.

Aminoacids intake(mg/d)[a]	TG								LDL-c							
	Boys (n=200)				Girls (n=254)				Boys (n=200)				Girls (n=254)			
	β	95% CI	P[1]	P[2]	β	95% CI	P[1]	P[2]	β	95% CI	P[1]	P[2]	β	95% CI	P[1]	P[2]
Aliphatic side chains																
Alanine	-0.258	-0.513,-0.004	0.046	0.159	-0.229	-0.434,-0.025	0.028	0.471	-0.075	-0.219,0.068	0.305	0.349	-0.137	-0.267,-0.008	0.038	0.312
Glycine	-0.293	-0.548,-0.037	0.025	0.089	-0.226	-0.436,-0.017	0.034	0.529	-0.067	-0.213,0.079	0.371	0.430	-0.126	-0.259,0.006	0.062	0.470
Isoleucine	-0.239	-0.503,0.024	0.075	0.250	-0.301	-0.521,-0.081	0.007	0.236	-0.106	-0.256,0.044	0.168	0.180	-0.127	-0.268,0.014	0.078	0.563
Leucine	-0.227	-0.496,0.041	0.097	0.319	-0.294	-0.520,-0.069	0.011	0.286	-0.102	-0.255,0.050	0.189	0.203	-0.127	-0.272,0.018	0.085	0.582
Valine	-0.266	-0.540,0.008	0.057	0.214	-0.304	-0.531,-0.076	0.009	0.301	-0.115	-0.270,0.041	0.148	0.150	-0.135	-0.281,0.011	0.070	0.563
Aromatic side chains																
Phenylalanine	-0.276	-0.557,0.005	0.054	0.211	-0.301	-0.539,-0.062	0.013	0.376	-0.114	-0.275,0.046	0.164	0.173	-0.123	-0.276,0.030	0.114	0.748
Tryptophan	-0.259	-0.536,0.018	0.066	0.240	-0.286	-0.519,-0.082	0.016	0.397	-0.127	-0.284,0.030	0.112	0.110	-0.119	-0.268,0.030	0.119	0.741
Tyrosine	-0.243	-0.508,0.021	0.072	0.250	-0.300	-0.522,-0.077	0.008	0.243	-0.114	-0.264,0.037	0.139	0.142	-0.115	-0.258,0.025	0.116	0.692
Basic side chains																
Arginine	-0.291	-0.551,-0.030	0.029	0.114	-0.267	-0.475,-0.058	0.012	0.328	-0.072	-0.220,0.076	0.339	0.391	-0.139	-0.271,-0.006	0.041	0.381
Histidine	-0.281	-0.538,-0.024	0.032	0.123	-0.252	-0.462,-0.041	0.019	0.385	-0.092	-0.238,0.054	0.217	0.242	-0.121	-0.256,0.013	0.076	0.533
Lysine	-0.259	-0.493,-0.26	0.030	0.107	-0.227	-0.415,-0.039	0.018	0.346	-0.095	-0.228, 0.038	0.161	0.177	-0.111	-0.231,0.009	0.069	0.478
Acidic side chains																
Asparaginic acid	-0.286	-0.544,-0.027	0.030	0.109	-0.232	-0.441,-0.024	0.029	0.486	-0.093	-0.239,0.052	0.209	0.232	-0.127	-0.260,0.006	0.061	0.458
Glutamic acid	-0.223	-0.512,0.066	0.131	0.400	-0.307	-0.561,-0.052	0.018	0.404	-0.052	-0.246,0.083	0.331	0.382	-0.114	-0.278,0.050	0.172	0.885
Hydroxyl side chains																
Serine	-0.302	-0.588,-0.015	0.039	0.170	-0.314	-0.556,-0.072	0.011	0.368	-0.127	-0.291,0.038	0.131	0.131	-0.123	-0.279,0.033	0.123	0.805
Threonine	-0.257	-0.521,0.006	0.056	0.197	-0.268	-0.484,-0.082	0.015	0.356	-0.105	-0.255,0.044	0.167	0.179	-0.135	-0.273,0.003	0.055	0.447
Sulfur-containing side chains																
Cysteine	-0.249	-0.539,0.041	0.093	0.281	-0.286	-0.540,-0.033	0.027	0.540	-0.063	-0.228,0.101	0.448	0.524	-0.142	-0.303,0.020	0.086	0.634
Methionine	-0.246	-0.497,0.005	0.055	0.186	-0.265	-0.471,-0.059	0.012	0.293	-0.097	-0.240,0.045	0.181	0.198	-0.122	-0.253,0.009	0.068	0.503
Cyclic side chain																
Proline	-0.186	-0.471,0.098	0.200	0.530	-0.287	-0.541,-0.033	0.027	0.378	-0.087	-0.249,0.074	0.290	0.331	-0.054	-0.219,0.110	0.518	0.649

[1]Model 1: adjusted by center, maternal education, age, sum of four skinfolds, physical activity, sedentary behaviors, and energy intake.

[2]Model 2: adjusted by model 1 plus total fat intake

[a]Log-transformed data.

Table 5. Multilevel regression analysis addressing the association between amino acids (AA) intakes and apolipoprotein B (Apo B) and apolipoprotein B/ apolipoprotein A1 (Apo B/Apo A1) ratio in the participating adolescents.

Aminoacids intake(mg/d)[a]	Apo B								Apo B/Apo A1 ratio							
	Boys (n=200)				Girls (n=254)				Boys (n=200)				Girls (n=254)			
	β	95% CI	P[1]	P[2]	β	95% CI	P[1]	P[2]	β	95% CI	P[1]	P[2]	β	95% CI	P[1]	P[2]
Aliphatic side chains																
Alanine	-0.045	-0.127,0.036	0.278	0.369	-0.097	-0.178,-0.016	0.019	0.179	-0.088	-0.243,0.066	0.263	0.550	-0.147	-0.291, -0.022	0.046	0.280
Glycine	-0.043	-0.126,0.039	0.304	0.401	-0.091	-0.174,-0.009	0.030	0.257	-0.095	-0.251,0.062	0.236	0.478	-0.133	-0.281,0.014	0.076	0.402
Isoleucine	-0.056	-0.141,0.029	0.197	0.260	-0.097	-0.184,-0.009	0.030	0.277	-0.101	-0.262,0.059	0.217	0.491	-0.152	-0.307,0.003	0.054	0.350
Leucine	-0.056	-0.143,0.030	0.201	0.266	-0.095	-0.185,-0.006	0.036	0.308	-0.102	-0.266,0.061	0.220	0.511	-0.152	-0.310,0.007	0.060	0.369
Valine	-0.061	-0.150,0.027	0.177	0.230	-0.102	-0.193,-0.012	0.026	0.271	-0.106	-0.273,0.061	0.213	0.507	-0.157	-0.317,0.002	0.054	0.374
Aromatic side chains																
Phenilalanine	-0.064	-0.155,0.026	0.165	0.217	-0.096	-0.190,-0.002	0.046	0.399	-0.121	-0.293,0.051	0.168	0.412	-0.146	-0.313,0.021	0.086	0.506
Tryptophan	-0.067	-0.156, 0.022	0.138	0.177	-0.090	-0.183,0.002	0.055	0.430	-0.125	-0.294,0.043	0.146	0.365	-0.148	-0.311,0.016	0.076	0.453
Tyrosine	-0.060	-0.146,0.024	0.163	0.212	-0.090	-0.178,-0.001	0.046	0.361	-0.113	-0.274,0.048	0.170	0.411	-0.141	-0.297,0.016	0.078	0.440
Basic side chains																
Arginine	-0.043	-0.127,0.040	0.313	0.417	-0.101	-0.184,-0.019	0.016	0.177	-0.095	-0.254,0.064	0.240	0.519	-0.132	-0.280,0.015	0.079	0.445
Histidine	-0.056	-0.138,0.027	0.186	0.248	-0.087	-0.170,-0.003	0.041	0.321	-0.121	-0.278,0.035	0.129	0.312	-0.125	-0.273,0.023	0.098	0.488
Lysine	-0.050	-0.125,0.025	0.194	0.256	-0.081	-0.155,-0.006	0.034	0.265	-0.096	-0.239,0.047	0.187	0.406	-0.122	-0.255,0.010	0.071	0.372
Acidic side chains																
Asparaginic acid	-0.049	-0.132,0.033	0.240	0.317	-0.091	-0.174,-0.008	0.031	0.258	-0.090	-0.248,0.068	0.264	0.550	-0.135	-0.282,0.012	0.072	0.391
Glutamic acid	-0.058	-0.151,0.034	0.218	0.293	-0.090	-0.191,0.011	0.080	0.532	-0.111	-0.287,0.064	0.215	0.500	-0.152	-0.330,0.026	0.093	0.505
Hydroxyl side chains																
Serine	-0.068	-0.161,0.025	0.152	0.197	-0.099	-0.195,-0.003	0.043	0.396	-0.121	-0.297,0.055	0.178	0.441	-0.155	-0.325,0.014	0.073	0.478
Threonine	-0.057	-0.141,0.028	0.189	0.250	-0.098	-0.184,-0.013	0.024	0.231	-0.109	-0.269,0.052	0.184	0.431	-0.151	-0.303,0.000	0.050	0.325
Sulfur-containing side chains																
Cysteine	-0.052	-0.145,0.041	0.272	0.364	-0.111	-0.211,-0.011	0.029	0.291	-0.108	-0.284,0.069	0.231	0.498	-0.191	-0.338,0.017	0.075	0.451
Methionine	-0.055	-0.136,0.026	0.181	0.240	-0.092	-0.173,-0.010	0.027	0.249	-0.104	-0.257,0.049	0.184	0.420	-0.144	-0.289,0.000	0.051	0.322
Cyclic side chain																
Proline	-0.054	-0.145,0.037	0.244	0.326	-0.064	-0.165,0.036	0.211	0.814	-0.087	-0.260,0.086	0.322	0.672	-0.116	-0.292,0.060	0.197	0.717

[1]Model 1: adjusted by center, maternal education, age, sum of four skinfolds, physical activity, sedentary behaviors, and energy intake.

[2]Model 2: adjusted by model 1 plus total fat intake

[a]Log-transformed data.

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Artículo IV [Paper IV]:

Associations between macronutrient intakes and serum lipid profile depend on body fat in European adolescents: the HELENA study

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Abstract

Objectives: To investigate the relationships between macronutrient intakes and serum lipid profile in European adolescents from eight European cities participating in the HELENA cross-sectional study (2006-2007), and to assess the role of body fat-related variables on these associations.

Methods: Weight, height, waist circumference, skinfolds thicknesses, total cholesterol (TC), high density lipoprotein-cholesterol (HDL-c), low density lipoprotein-cholesterol (LDL-c), triglycerides, apolipoprotein B and A1 were measured in 454 adolescents (44% boys) aged 12.5-17.5 years. TC/HDL-c, apolipoprotein B/apolipoprotein A1 and waist-to-height ratios were calculated. Macronutrient intakes (g/1000 kcal/day) were assessed by two 24-hour dietary recalls. Associations were evaluated by multilevel analysis clustered by high and low body fat indicators (z-BMI, sum of four skinfolds and waist-to-height ratio) and adjusted for a wide range of confounders.

Results: An inverse association was observed between protein intake and triglycerides (β=-0.242, $p<0.05$). Carbohydrate intake was also inversely associated with HDL-c (β=-0.189, $p<0.001$) and apolipoprotein A1 (β=-0,177, $p<0.01$) whereas a positive association was observed with TC/HDL-c ratio (β=0.153, $p<0.05$). Inverse associations were found between fat intake and triglycerides (β=-0.319, $p<0.001$), LDL-c (β=-0.131, $p<0.05$), apolipoprotein B (β=-0.068, $p<0.05$), TC/HDL-c (β=-0.118, $p<0.01$) and apolipoprotein B/apolipoprotein A1 (β=-0.117, $p<0.05$) ratios; a positive association, however, was observed with HDL-c (β=0.086, $p<0.05$). Associations between macronutrients and serum lipids differed between high/low body fat, i.e. protein and fat intake were inversely associated with triglycerides whereas a positive association was observed between carbohydrates intake and triglycerides only in those adolescents with higher adiposity levels.

Conclusions: Our results showed that observed associations of the three macronutrients intakes with serum lipid profile which varied according to adiposity levels. As serum lipids and excess body fat are major markers of cardiovascular diseases, these findings should be considered when developing strategies to prevent cardiovascular disease risk among adolescents.

Keywords: protein intake, carbohydrates intake, fat intake, serum lipids, body fat, adolescents.

Introduction

High levels of low density lipoprotein-cholesterol (LDL-c) in childhood and the onset of atherosclerosis in early life (1) could result into adult dyslipidemias (2) which are important public health threats. Therefore, evidence regarding factors influencing lipid profiles is necessary for public health protection and promotion.

Dietary modifications that lower atherogenic lipids and lipoproteins are effective in the prevention and treatment of cardiovascular diseases (CVD) risk (3). Nonetheless, the optimal dietary pattern(s) to restrain atherosclerosis progression is still to be identified (4). For instance, high intake of total carbohydrates is suggested to lower high density lipoprotein cholesterol (HDL-c) and to increase triglycerides (TG) in adults (5), and a protein-rich diet low in saturated fat significantly decrease LDL-c, TG and total cholesterol (TC) concentrations in comparison to a carbohydrate-rich diet and a diet rich in unsaturated fat (6). However, findings about the role of dietary fat, mainly those of saturated fat, on CVD risk are still controversial (7) because of individual variability in serum lipid response to changes in dietary saturated fat and cholesterol (3). Additionally, obesity is said to strongly affect serum lipid response to diet (3), making obese individuals less responsive to dietary interventions aimed to improve serum lipid profile.

Adolescence is a key period in life because of the growth spurt and sexual maturation that take place; therefore, having a healthy diet is essential to achieve optimal development and to prevent the appearance of chronic diseases later in life (8). Furthermore, associations between macronutrient intakes and serum lipids have mainly been investigated among adults and there is a lack of literature addressing this topic in adolescents. Therefore, the aims of this study were 1) to investigate the relationship between macronutrient intakes and serum lipid profile in European adolescents and 2) to assess the role of body fat-related variables on these associations.

Materials and Methods

The present study sample was derived from the cross-sectional multi-centre HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) study (n=3,528) carried out in adolescents (12.5-17.5 years) between 2006 and 2007 in ten European cities (Athens and Heraklion in Greece, Dortmund in Germany, Ghent in Belgium, Lille in France, Pecs in Hungary, Rome in Italy, Stockholm in Sweden, Vienna in Austria and Zaragoza in Spain). General HELENA procedures, characteristics and inclusion criteria can be found elsewhere (9, 10). Adolescents and their parents gave written informed consent, and the study was performed following the ethical guidelines of the Declaration of Helsinki 1964. Ethical approval was obtained by the local Ethical Committee at each study centre (11).

Participants with complete data on TG, TC, HDL-c, LDL-c, apolipoprotein A1 (Apo A1), apolipoprotein B (Apo B) and two 24-hour dietary recalls (24-HDR) were included (n=454; 44.0% boys). Decreases from the original sample size are partially explained by the fact that blood samples were randomly drawn only in one third of the HELENA participants and partially from the fact that Heraklion and Pecs were excluded from 24-HDR analyses due to logistical reasons; therefore, eight out of the ten study centres were included in the 24-HDR analyses resulting in a sample size decrease. Excluded participants (n=3,074) were significantly ($p<0.05$) older, heavier and had higher mean body mass index (BMI) than those included in this study (data not shown).

Macronutrient intakes. Dietary intakes were assessed by a self-administered computer-based tool called HELENA-DIAT (Dietary Assessment Tool), based on the previously developed software Young Adolescents' Nutrition Assessment on Computer (YANA-C) shown to be appropriate in assessing dietary information of European adolescents (12, 13). The software consists of a single structured 24-HDR according to six meal occasions. Adolescents were asked to recall all food and drinks consumed the previous day. Two non-consecutive 24-HDR within a time span of two weeks were obtained from each participant during school time

and assisted by fieldworkers. Therefore, no information on Fridays and Saturdays was collected.

The German Food Code and Nutrition Date Base (Bundeslebensmittelschlüssel, BLS Version II.3.1) (14)(99) was used to calculate energy and nutrient intakes. Usual food and nutrient intakes were estimated by the multiple source method (MSM) in order to account for within-person variability (15). Energy intake was estimated in kilocalories per day (kcal/d) and macronutrients intake (fat, protein and carbohydrates) in grams/day (g/d). Subsequently, macronutrients intakes were divided by energy intake and were expressed as g/1000 kcal to account for total energy intake (16). Additionally, monosaturated-to-saturated fat ratio (M/S), polyunsaturated-to-saturated fat (P/S) ratio, and Cholesterol-Saturated Fat Index (CSI = [(1.01 × g of saturated fat) + (0.05 × mg of cholesterol)]) (17) were computed.

Diet Quality Index for Adolescents. A previously validated Diet Quality Index for Adolescents (DQI-A) (18) was used to adjust for all dietary factors simultaneously. The technical aspects regarding the development of the DQI-A are published elsewhere (18). Briefly, the DQI-A was calculated on food intake data in order to assess adolescents adherence to food-based dietary guidelines. Therefore, daily diet was divided into nine recommended food groups: 1) water, 2) bread and cereals, 3) grains and potatoes, 4) vegetables, 5) fruit, 6) milk products, 7) cheese, 8) meat, fish, eggs and substitutes, and 9) fat and oils. The DQI-A consisted of three components: quality (optimal food quality choices within a food group), diversity (degree of variation of the diet) and equilibrium (difference between the adequacy and the excess to the recommendations). Furthermore, a meal index was also taken into consideration since the number of meals consumed per day is related to a healthier diet. The DQI-A was obtained by computing the mean of the four components (dietary quality, diversity, equilibrium and meal index), resulting in scores ranging from -33 to +100. Higher scores reflected a higher diet quality.

Physical examinations. All anthropometric measures were taken following a standardized protocol described elsewhere (19). Weight and height were measured in underwear and barefoot using an electronic scale (Type SECA 861) and a stadiometer (Type SECA 225). BMI was calculated as body weight in kilograms divided by the square of height in meters and was categorized according to Cole et al. (20, 21). Skinfolds thicknesses were measured with a Holtain Calliper (Crymmych, UK) in triplicate on the left side at biceps, triceps, subscapular and suprailiac sites. Waist circumference was taken at the midpoint between the lowest rib and the iliac crest with an anthropometric tape (SECA 200). The waist-to-height ratio (WHeR) was calculated.

Blood sampling. Blood sampling procedures have been described elsewhere (22). Briefly, blood samples were drawn after an overnight fast and analyzed in centralized laboratories. Serum TG, TC, HDL-c, TG, and LDL-c were measured on a Dimension RxL clinical chemistry system (Dade Behring, Schwalbach, Germany) using enzymatic methods. Apo B and Apo A1 were measured in an immunochemical reaction with a BN II analyzer (Dade Behring, Schwalbach, Germany). The TC/HDL-c and the Apo B/Apo A1 ratios were computed.

Education. Maternal education was used as a proxy of socioeconomic status and was assessed via questionnaire according to the following four categories: 1) lower education, 2) lower secondary education, 3) higher secondary education, and 4) higher education/university degree.

Sedentary behaviours. Average time engaged in two sedentary behaviours (TV viewing and playing with videogames) was estimated by means of a self-administered questionnaire previously found to demonstrate good reliability (23).

Physical activity (PA). PA was objectively measured by uni-axial accelerometers during 7 consecutive days (Actigraph MTI, model GT1M, Manufacturing Technology Inc., Fort Walton Beach, FL, USA) (24). At least three days of recording, with a minimum of 8 hours registration per day, was set as an inclusion criterion. The time sampling interval was set at 15 seconds.

The time spent at moderate-to-vigorous PA (MVPA) (>3 metabolic equivalents) was calculated on the basis of the following cut-off point: ≥ 2000 counts per minute for moderate-to-vigorous PA (24, 25).

Statistical analysis. The normality of all variables was checked and non-normally distributed variables (TG, TC, HDL-c, LDL-c, TC/HDL-c ratio, Apo B/Apo A1 ratio, fat intake and P/S ratio) were log-transformed prior to the analysis. Normality for CSI was reached by comprising to the power of two. M/S ratio was converted as 1/(M/S). Differences across groups were tested by means of the independent samples T-test for normally distributed variables and the Mann-Whitney U test for non-normally distributed variables. Chi-squared test was applied for categorical variables.

Multilevel linear regression analyses were performed to investigate the association between macronutrients intakes (independent variables) and plasma lipids concentrations (dependent variables). As no interaction by gender was found the analyses were conducted with boys and girls combined. Study centre was included as random intercept. Gender, age, maternal education, sum of four skinfolds, MVPA, sedentary behaviors, and DQI-A were entered as covariates.

Since serum lipid profile has previously been associated with body fat in the HELENA adolescents (26), participants were categorized into low and high body fat content according to three body fat indicators, i.e. z-score of BMI (z-BMI), sum of four skinfolds and WHeR. These cut-offs were calculated specifically by gender and by 1-year groups (12.5-13.49, 13.5-14.49, 14.5-15.49, 15.5-16.49, and 16.5-17.5) based on the median of each subgroup. Multilevel linear regression analyses were performed separately for each group of low/high body fat indicator and by tertiles of protein, carbohydrates and fat intake adjusted for potential confounders, i.e. gender, age, maternal education, sum of four skinfolds, MVPA, sedentary behaviors, and DQI-A. Study center was entered as random intercept. Collinearity tests showed no collinearity among covariates. The statistical software packages Stata version 12.0 (Stata Corp., college Station, TX,

USA) and Predictive Analytics SoftWare (PASW, version 18; SPSS Inc., Chicago, IL, USA) were used to perform the analyses. Statistical significance was set at $p<0.05$.

Results

Main characteristics of the study sample are shown in **Table 1**. **Table 2** displays means and medians of dietary intake and blood lipids levels according to high/low body fat-related variables, i.e. above or below gender- and age-specific median-based cut-offs of z-BMI, sum of four skinfolds and WHeR. Adolescents in the high z-BMI group had significantly higher protein intake, TG serum concentrations and TC/HDL-c values and lower total energy intake and HDL-c concentrations than those with low z-BMI ($p<0.05$). Adolescents with high sum of skinfolds also showed significantly lower HDL-c serum levels and higher fat intake compared to their peers in the low sum of skinfolds group ($p<0.05$). Those in the high WHeR group showed significantly higher protein intake, M/S ratio, TG, TC/HDL-c and Apo B/Apo A1 ratios and lower total energy intake than adolescents in the low WHeR group.

Associations between macronutrient intakes (g/1000 kcal/day)and serum lipid profile are shown in **Table 3**. An inverse association was observed between protein intake and TG. Carbohydrates intake was also inversely associated with HDL-c and Apo A1 whereas a positive association was observed for TC/HDL-c ratio. Inverse associations were found between fat intake and TG, LDL-c, Apo B, TC/HDL-c and Apo B/Apo A1 ratios; a positive association, however, was observed with HDL-c. CSI was also positively associated with HDL-c.

Table 4 shows the associations of macronutrients intake and blood lipid profile across adiposity status categories. We observed that protein intake was inversely associated with TG in the high z-BMI group and in the high WHeR group ($p<0.05$). Carbohydrates intake showed an inverse association with HDL-c in all body fat groups ($p<0.05$ in low z-BMI and low sum of skinfolds; $p<0.01$ in high z-BMI and high sum of skinfolds, and $p<0.001$ in high WHeR), except in those with low WHeR. Carbohydrates intake was also inversely associated with Apo A1 in

all low body fat groups and in the high WHeR group ($p<0.05$). A positive association was observed between the intake of carbohydrates and TG concentrations in those adolescents within the high fat groups ($p<0.05$). Fat intake was inversely associated with TG ($p<0.01$ in high z-BMI and in high WHeR, $p<0.001$ in high sum of skinfolds). The M/S ratio was inversely associated with TC in those adolescents within the low z-BMI ($p<0.01$) and the low WHeR ($p<0.05$) and with Apo B in all the low body fat groups ($p<0.05$). CSI was positively associated with HDL-c and negatively associated with TC/HDL-c ratio in both the low sum of skinfolds and the low WHeR groups ($p<0.05$).

Figure 1 and **Figure 2** display means (SE) of TG, TC, HDL-c and LDL-c by tertiles of fat intake for high and low sum of skinfolds and for high and low WHeR, respectively. Overall, adolescents within the high sum skinfolds or high WHeR in the lower tertile of fat intake showed clinically adverse values of serum lipids concentrations, i.e. higher TG, TC, and LDL-c; lower HDL-c, compared to those in the higher tertile of fat intake. Within the upper tertile of fat intake blood parameters acquired better values in both adolescents within the high sum of skinfolds and the high WHeR adolescents whereas slight differences were observed across tertiles of fat intake in adolescents with lower adiposity levels. Indeed, adolescents with high z-BMI (data not shown), high sum of skinfolds (Figure 1A) and high WHeR (Figure 1B) within the lowest tertile of fat intake showed significantly higher TG concentrations than those within the upper tertile ($p<0.05$). Contrarily, those with high adiposity levels, i.e. high z-BMI, sum of skinfolds and WHeR, in the upper tertile of carbohydrates intake showed significantly higher TG levels than those with lower intakes ($p<0.05$, data not shown). No significant differences were observed across tertiles of protein intake (data not shown).

Discussion

The present study examined the associations between macronutrient intakes and serum lipid profile, as well as the potential role that body adiposity may exert on these associations among healthy European adolescents. Overall, results suggested dietary fat to

have a beneficial role on serum lipids levels by lowering serum levels of TG, LDL-c, Apo B and TC/HDL-c and Apo B/Apo A1 ratios and rising HLD-c concentrations, whereas carbohydrates were adversely associated with lipid profile by decreasing HDL-c and Apo 1 serum concentrations and increasing TC/HDL-ratio. Above mentioned associations varied according to adolescents' body fat status, i.e. associations between fat and carbohydrates intake and blood lipids were observed mainly among adolescents within the high body fat group. To the best of our knowledge, this is the first study addressing such relationships among adolescents.

The associations between carbohydrates intake and HDL-c and Apo A1 add further evidence to the complex and adverse role that dietary carbohydrates appear to play on lipid profile (5). Indeed, high intake of total carbohydrates is also related to lower HDL-c and higher TG in adults (5) and children (27). It is known, however, that serum lipid levels are controlled not only by dietary carbohydrates but also by dietary proteins (28). Although available data addressing the associations between dietary proteins and serum lipids is still limited, vegetal protein *per se* is observed to lower plasma cholesterol (29). Furthermore, Appel et al. (6) observed that a healthy diet, rich in protein and low in saturated fat, significantly decreased LDL-c, TG and TC concentrations among adults compared to a carbohydrate-rich diet and a diet rich in unsaturated fat. The inverse association we found between protein intake and TG is partially in concordance with these findings.

The role that fat intake exerts on serum lipids differs according to the type of fat addressed. Indeed, the fatty acid profile of the diet seems to be the major determinant of serum cholesterol concentrations (30). Findings from a follow-up study (31) suggested that replacing dietary saturated fat with polyunsaturated fat rather than monounsaturated fat or carbohydrates prevented middle-aged and older men and women from coronary heart disease. Furthermore, other studies observed that replacement of saturated fat with polyunsaturated fat, monounsaturated fat and/or carbohydrates reduced LDL-c (32, 33).

Moreover, further analysis carried out in our sample showed a positive association between monounsaturated fat and HDL-c and Apo A1.

Total fat intake was inversely related to all serum lipids, except to TC and Apo A1, which could be partially explained by the presence of polyunsaturated and monounsaturated fat. In addition, Lyu et al. (34) found a positive association between total fat and Apo 1 in both men and women. Such findings could denote a positive role of dietary fat on different fractions of serum lipids and are consistent with several experimental studies that observed unfavourable effects of low fat diets on HDL-c, TC/HDL-c ratio and postprandial TG concentrations in women when compared with men (35-38). Mozaffarian et al. (4) observed that a greater saturated fat intake was associated with higher HDL-c and Apo A1, and lower TG and TC/HDL-c ratio in postmenopausal women. This might be true as not all saturated fatty acids have the same effects on serum cholesterol levels (30). In addition, a meta-analysis of prospective cohort studies (7) did not find significant evidence to conclude that dietary saturated fat was associated with increased risk of CVD, however, that should be interpreted with caution as that conclusion was later questioned by Kromhout et al. (39).

Variability in lipid response to diet is affected by numerous factors, but excess adiposity seems to be one of the strongest factors (3). Our findings have shown that associations between macronutrients and serum lipids varied by body fat status in this sample of healthy adolescents. It is noteworthy that we observed significant associations between dietary macronutrients and serum lipids only in adolescents with high body fat or only in those with low body fat, but were not seen in high and low body fat simultaneously, except the associations between carbohydrates intake and HDL-c and Apo A1. This highlights the role that carbohydrates may have on these serum lipids regardless of adiposity. Our findings suggest that associations between macronutrient intakes and serum lipids depend on the initial body fat status, regardless of the definition employed i.e. general body fatness (z-BMI and sum of skinfolds) or central body fat (WHeR). These observations are in agreement

with previous reports in which adiposity seems to play a key role on these associations among adults (34, 40). Lyu et al. (34) observed that general body fatness, but also body fat distribution, might exert different effects on HDL-c subclasses.

Obese individuals have shown lower responses to dietary interventions focused on improving their serum lipid profile (3). Indeed, Denke et al. (41) found that the response to a LDL-c lowering diet was lower in obese participants compared to those with a lower BMI (<21kg/m^2). Our findings, however, showed significant associations between macronutrient intakes and blood lipids in the high adiposity groups mainly, which initially would be in contrast with previous literature. Nevertheless, that should be interpreted with caution since our sample was composed of healthy adolescents and those within the high adiposity groups had higher body fat compared to their peers in the low adiposity groups, but this does not necessarily imply that they were obese.

There were several limitations to our findings. Due to the cross-sectional nature of the study, we cannot determine causality. Although the self-administered 24-HDR used to assess diet is subject to measurement errors as happens with other self-reporting methods, it has been shown to be appropriated to collect detailed dietary data in adolescents (12, 13). Collection of dietary data for more than two days would have been desirable to compensate for day-to-day variability (42). Nevertheless, dietary information was corrected for within-person variability to partially mitigate such limitation (43). Also, the used gender- and age-specific median-based cut-offs used are sample specific, and therefore, limits its comparability with other studies. Although the BLS food composition table was used to make sure that all countries used the same food composition data (obtained via the same definitions and analytical methods) this also included some limitations as not all country specific recipes and foods could be found in this German food composition table. However, recipes were generated to calculate the nutrient intakes for those particular recipes.

Our study has several strengths. Blood samples were collected following a standardised methodology and transport system to a centralized laboratory in order to assure samples viability and stability (22). Fieldworkers were trained and a manual of operation was developed to guarantee good clinical practice (22). Additionally, all measurements were taken following the same methodology across survey centres. It is important to bear in mind that after adjusting for multiple testing (Bonferroni method, $p \leq 0.006$) several associations would still remain statistically significant.

In conclusion, this study showed associations between macronutrient intakes and serum lipid profile in adolescents; fat intakes were related to a better serum lipid profile while carbohydrates intake was observed to be associated with an adverse lipid profile. It is noteworthy that these associations differed according to body fat status and were consistent across the obesity definitions used. General and central body fatness seemed to play a similar role on serum lipids and on its association with diet. These findings emphasize the importance of considering body fat status when developing strategies to prevent CVD risk among adolescents since serum lipids and obesity are major markers of CVD risk. More research is needed, preferably with a longitudinal design, to confirm these findings.

Table 1. Descriptive characteristics of the study sample stratified by sex.

	Boys (n= 200)		Girls (n= 254)		P
	Mean	*SD*	*Mean*	*SD*	
Age (years)	14	1.2	14	1.2	0.085
Weight (kg)	59	13	56	11	<0.001*
Height (cm)	170	9.8	162	7.5	<0.001*
BMI (kg/m²)	20	3.4	21	3.5	0.097
Underweight (%)[a]	8.2		6.6		-
Normal weight (%)	75		73		-
Overweight (%)	10		16		-
Obese (%)	6.7		4.5		-
Maternal education (%)					0.035‡
Lower education	6.2		10		-
Lower secondary education	18		23		-
Higher secondary education	30		34		-
Higher education/University degree	46		33		-
Apo A1 (g/L)	1.48	0.01	1.55	0.01	<0.001*
Apo B (g/L)	0.61	0.01	0.67	0.01	<0.001*
	Median	***25th-75thpercentile***	***Median***	***25th-75thpercentile***	
Sum four skinfolds (mm)	133	77-209	107	65-167	<0.001†
WHeR	0.42	0.40-0.44	0.43	0.40-0.47	0.005†
MVPA (min/day)	64	50-82	48	34-60	<0.001†
Sedentary behaviors (min/day)	34	26-52	54	40-75	<0.001†
DQI-A	51	37-62	55	44-65	<0.001†
Energy intake (kcal/day)	2453	2033-3002	1832	1513-2209	<0.001†
Protein intake (g/1000 kcal)	40	35-45	40	35-46	0.881
Carbohydrates intake (g/1000 kcal)	41	36-47	42	34-48	0.594
Fat intake (g/1000 kcal)	120	112-132	123	112-135	0.193
Monounsaturated/saturated ratio	0.86	0.78-0.99	0.86	0.77-0.99	0.966
Polyunsaturated/saturated ratio	0.32	0.26-0.41	0.33	0.27-0.42	0.468
Cholesterol-saturated fat index	23	21-26	24	21-27	0.267
Triglycerides (mg/dL)	57	43-77	63	48-84	<0.001†
TC (mg/dL)	154	138-168	167	148-184	<0.001†
HDL-c (mg/dL)	54	47-59	56	50-64	<0.001†
LDL-c (mg/dL)	90	77-100	97	80-114	<0.001†
TC/HDL-c	2.8	2.5-3.2	2.9	2.5-3.4	0.340
Apo B/ Apo A1	0.43	0.33-0.48	0.42	0.35-0.50	0.169

Apo A1, apolipoprotein A1; Apo B, apolipoprotein B; BMI, body mass index; DQI-A; diet quality index for adolescents; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol; MVPA, moderate to vigorous physical activity; TC, total cholesterol; WHeR, waist-to-height ratio.

[a]BMI categories according to Cole et al. (20, 21).

*p<0.05 by means of independent samples T-Test; †p<0.05 by means of Mann-Whitney U test; ‡p<0.05 by means of Chi-squared test.

Table 2. Dietary characteristics and serum lipid parameters stratified by body fat groups.

	z-BMI				Sum of skinfolds				WHeR			
	High (n=216)		Low (n=219)		High (n=242)		Low (n=196)		High (n=217)		Low (n=218)	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Apo A1 (g/L)	1.50[a]	0.21	1.54[b]	0.23	1.50[a]	0.22	1.55[b]	0.20	1.49[a]	0.21	1.55[b]	0.22
Apo B (g/L)	0.65	0.16	0.64	0.15	0.65	0.17	0.64	0.14	0.65	0.16	0.64	0.15
	Median	*25th-75th p*	*Median*	*25th-75th p*	*Median*	*25th-75th p*	*Median*	*25th-75th p*	*Median*	*25th-75th p*	*Median*	*25th-75th p*
Energy intake (kcal/day)	1904[a]	1530-2400	2245[b]	1871-2721	2018	1569-2557	2168	1783-2577	1920[a]	1552-2432	2223[b]	1860-2728
Protein intake (g/1000 kcal)	41[a]	35-46	38.7[b]	34-43	40	35-46	39	34-44	41[a]	36-46	38[b]	34-43
Carbohydrates intake (g/1000 kcal)	122	112-135	123	112-133	121	111-134	124	113-134	121	112-135	124	112-134
Fat intake (g/1000 kcal)	42	35-48	41	35-47	42[a]	36-49	40[b]	34-46	42	36-49	41	34-47
Monounsaturated/saturated ratio	0.87	0.78-1.00	0.86	0.75-0.91	0.87	0.78-1.00	0.86	0.75-0.98	0.89[a]	0.80-1.01	0.84[b]	0.74-0.97
Polyunsaturated/saturated ratio	0.33	0.27-0.40	0.32	0.26-0.41	0.33	0.27-0.41	0.33	0.26-0.41	0.33	0.28-0.41	0.33	0.26-0.42
Cholesterol-saturated fat index	24	21-26	23	21-26	24	21-26	23	20-26	24	21-26	23	21-26
Triglycerides (mg/dL)	63[a]	46-88	57[b]	46-77	62	47-85	58	45-78	63[a]	46-91	58[b]	46-76
TC (mg/dL)	160	141-177	163	144-178	161	141-176	162	144-179	162	141-177	161	143-178
HDL-c (mg/dL)	54[a]	48-60	56[b]	50-65	54[a]	48-60	56[b]	50-65	54	48-60	56	50-65
LDL-c (mg/dL)	93	78-110	94	79-107	93	79-109	93	77-107	94	80-109	93	79-107
TC/HDL-c	2.9[a]	2.6-3.4[b]	2.8	2.4-3.2	2.9	2.6-3.3	2.8	2.4-3.2	3.0[a]	2.6-3.4	2.8[b]	2.4-3.2
Apo B/Apo A1	0.43	0.36-0.50	0.41	0.33-0.50	0.43	0.35-0.50	0.41	0.33-0.50	0.44[a]	0.36-0.50	0.41[b]	0.33-0.50

Apo A1, apolipoprotein A1; Apo B, apolipoprotein B; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol; TC, total cholesterol; WHeR, waist-to-height ratio; z-BMI, z-score body mass index.

Means and medians with different superscripts letters are significant different ($p<0.05$) by means of independent samples T-Test and Mann-Whitney U test, respectively.

High and low body fat groups were defined by means of gender- and age-specific medians.

Table 3. Multilevel regression analysis addressing the association between intake of protein, carbohydrates, fat, monounsaturated/saturated fat ratio, polyounsaturated/saturated fat ratio and cholesterol/saturated fat index and serum lipid profile (estimated values and 95% confidence intervals).

	Protein (g/1000 kcal)		Carbohydrates (g/1000 kcal)		Fat (g/1000 kcal)		Monounsaturated/ saturated fat ratio		Polyunsaturated/ saturated fat ratio		Cholesterol/ saturated fat index	
	β[a]	95% CI	β[a]	95% CI	β[a]	95% CI	β[a]	95% CI	β[a]	95% CI	β[a]	95% CI
Triglycerides	**-0.242**	**-0.456,-0.028***	0.230	-0.033,0.493	**-0.319**	**-0.494,-0.143*****	0.042	-0.145,0.230	0.025	-0.097,0.146	-0.053	-0.143,0.038
TC	-0.056	-0.142,0.030	-0.048	0.153,0.056	-0.073	-0.147,-0.000	-0.071	-0.147,0.005	0.027	-0.018,0.76	0.003	-0.032,0.038
HDL-c	0.014	-0.080,0.109	**-0.189**	**-0.302,-0.076*****	**0.086**	**0.001,0.172***	-0.034	-0.118,0.049	0.003	-0.048,0.055	**0.041**	**0.003,0.080***
TC/HDL-c	-0.080	-0.179,0.018	**0.153**	**0.032,0.274***	**-0.118**	**-0.200,-0.035****	-0.005	-0.091,0.080	0.023	-0.033,0.080	-0.039	-0.081,0.003
LDL-c	-0.120	-0.251,0.010	0.077	-0.084,0.238	**-0.131**	**-0.240,-0.022***	-0.061	-0.174,0.051	0.049	-0.025,0.124	-0.035	-0.091,0.020
Apo A1	-0.011	-0.123,0.102	**-0.177**	**-0.311,-0.042****	0.031	-0.638,0.130	-0.075	-0.172,0.023	0.012	-0.050,0.075	0.011	-0.036,0.057
Apo B	-0.065	-0.143,0.011	0.022	-0.073,0.118	**-0.068**	**-0.133,-0.003***	-0.062	-0.129,0.004	0.038	-0.006,0.082	-0.022	-0.055,0.011
Apo B/Apo A1	-0.084	-0.223,0.055	0.136	-0.035,0.307	**-0.117**	**-0.234,-0.001***	-0.047	-0.167,0.073	0.035	-0.045,0.114	-0.034	-0.093,0.025

*p<0.05, **p<0.01

[a]Adjusted for gender, age, study centre, maternal education, sum of four skinfolds, moderate-to-vigorous physical activity, sedentary behaviours, dietary quality index.

Apo A1, apolipoprotein A1; Apo B, apolipoprotein B; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol; TC, total cholesterol.

Table 4. Multilevel regression analysis addressing the association between macronutrients intakes and serum lipid profile categorized by high vs. low z-score body mass index, sum of four skinfolds and waist-to-height ratio (estimated values and 95% confidence intervals).

	High z-BMI (n=216)		Low z-BMI (n=219)		High sum skinfolds (n=242)		Low sum skinfolds (n=196)		High WHeR (n=217)		Low WHeR (n=218)	
	β[a]	95% CI	β[a]	95% CI	β[a]	95% CI	β[a]	95% CI	β[a]	95% CI	β[a]	95% CI
	Protein (g/1000 kcal)				**Protein (g/1000 kcal)**				**Protein (g/1000 kcal)**			
Triglycerides	**-0.335**	**-0.650,-0.021***	-0.063	-0.349,0.223	-0.234	-0.253,0.054	-0.172	-0.481,0.137	**-0.400**	**-0.738,-0.061***	-0.040	-0.301,0.221
TC	**-0.137**	**-0.263,-0.011***	-0.010	-0.102,0.121	-0.096	-0.208,0.016	0.016	-0.106,0.137	-0.118	-0.248,0.012	-0.008	-0.116,0.099
HDL-c	-0.043	-0.169,0.082	0.050	-0.083,0.183	-0.040	-0.161,0.081	0.055	-0.092,0.203	0.028	-0.106,0.161	0.012	-0.116,0.141
TC/HDL-c	-0.101	-0.234,0.032	-0.042	-0.188,0.104	-0.062	-0.196,0.073	-0.068	-0.215,0.079	-0.145	-0.292,0.002	-0.021	-0.155,0.114
LDL-c	-0.177	-0.364,0.010	-0.041	-0.225,0.142	-0.149	-0.325,0.026	-0.050	-0.244,0.144	**-0.198**	**-0.395,-0.001***	-0.042	-0.217,0.134
Apo A1	-0.117	-0.262,0.028	0.083	-0.082,0.249	-0.080	-0.228,0.067	0.075	-0.091,0.242	-0.030	-0.185,0.124	0.036	-0.120,0.193
Apo B	-0.094	-0.205,0.015	-0.017	-0.126,0.093	-0.073	-0.181,0.034	-0.019	-0.129,0.091	-0.108	-0.221,0.005	-0.019	-0.125,0.088
Apo B/Apo A1	-0.057	-0.241,0.127	-0.081	-0.289,0.126	-0.040	-0.225,0.146	-0.095	-0.307,0.117	-0.128	-0.333,0.076	-0.059	-0.251,0.133
	Carbohydrates (g/1000 kcal)				**Carbohydrates (g/1000 kcal)**				**Carbohydrates (g/1000 kcal)**			
Triglycerides	**0.504**	**0.107,0.900***	-0.082	-0.418,0.253	**0.487**	**0.099,0.875***	-0.028	-0.371,0.315	**0.573**	**0.130,1.016***	-0.065	-0.368,0.238
TC	-0.093	-0.252,0.066	-0.040	-0.171,0.090	-0.060	-0.212,0.092	-0.039	-0.170,0.091	-0.114	-0.284,0.056	-0.009	-0.3-134,0.115
HDL-c	**-0.220**	**-0.374,-0.066****	**-0.192**	**-0.345,-0.038***	**-0.246**	**-0.404,-0.089****	**-0.174**	**-0.333,-0.017***	**-0.286**	**-0.455,-0.116*****	-0.128	-0.277,0.020
TC/HDL-c	0.126	-0.042,0.295	0.151	-0.019,0.321	**0.186**	**0.005,0.367***	0.133	-0.028,0.294	0.181	-0.011,0.374	0.119	-0.036,0.274
LDL-c	-0.054	-0.293,0.185	0.156	-0.058,0.371	0.061	-0.177,0.298	0.109	-0.103,0.321	-0.029	-0.289,0.232	0.141	-0.061,0.344
Apo A1	-0.145	-0.325,0.036	**-0.247**	**-0.437,-0.057***	-0.191	-0.387,0.005	**-0.210**	**-0.389,0.031***	**-0.205**	**-0.403,-0.006***	**-0.186**	**-0.366,-0.006***
Apo B	-0.017	-0.157,0.124	0.033	-0.095,0.161	0.036	-0.110,0.182	0.017	-0.103,0.138	0.012	-0.137,0.162	0.025	-0.099,0.148
Apo B/Apo A1	-0.014	-0.219,0.248	0.213	-0.029,0.455	0.127	-0.123,0.377	0.157	-0.076,0.391	0.083	-0.185,0.352	0.175	-0.047,0.397
	Fat (g/1000 kcal)				**Fat (g/1000 kcal)**				**Fat (g/1000 kcal)**			
Triglycerides	**-0.410**	**-0.670,-0.150****	-0.140	-0.374,0.094	**-0.419**	**-0.672,-0.166*****	-0.136	-0.376,0.104	**-0.379**	**-0.659,-0.099****	-0.172	-0.389,0.044
TC	-0.101	-0.205,0.003	-0.079	-0.170,0.011	-0.081	-0.182,0.019	-0.086	-0.179,0.007	-0.068	-0.181,0.044	-0.088	-0.177,0.001
HDL-c	0.085	-0.027,0.197	-0.011	-0.119,0.098	0.038	-0.073,0.150	0.062	-0.058,0.183	0.110	-0.009,0.229	-0.007	-0.115,0.100
TC/HDL-c	**-0.139**	**-0.249,-0.028***	-0.069	-0.188,0.050	-0.106	-0.226,0.014	-0.112	-0.225,0.002	**-0.125**	**-0.247,-0.003***	-0.081	-0.193,0.031
LDL-c	-0.112	-0.269,0.046	-0.131	-0.280,0.019	-0.091	-0.248,0.066	**-0.161**	**-0.309,-0.013***	-0.124	-0.288,0.040	-0.131	-0.276,0.015
Apo A1	0.020	-0.106,0.147	0.011	-0.124,0.146	0.016	-0.117,0.150	0.025	-0.106,0.156	0.026	-0.107,0.161	0.016	-0.114,0.147
Apo B	-0.083	-0.176,0.008	-0.039	-0.129,0.051	-0.075	-0.171,0.021	-0.044	-0.131,0.042	-0.081	-0.174,0.013	-0.050	-0.138,0.039
Apo B/Apo A1	-0.141	-0.294,0.013	-0.073	-0.244,0.096	-0.126	-0.291,0.039	-0.092	-0.259,0.075	-0.115	-0.284,0.054	-0.102	-0.262,0.058

Table 4 . *Continued.*

	High z-BMI (n=216)		Low z-BMI (n=219)		High sum skinfolds (n=242)		Low sum skinfolds (n=196)		High WHeR (n=217)		Low WHeR (n=218)	
	β^a	95% CI	β^a	95% CI	β^a	95% CI	β^a	95% CI	β^a	95% CI	β^a	95% CI
	Monounsaturated/saturated fat ratio				**Monounsaturated/saturated fat ratio**				**Monounsaturated/saturated fat ratio**			
Triglycerides	0.256	-0.032,0.544	-0.190	-0.416,0.036	0.050	-0.216,0.316	-0.046	-0.295,0.201	**0.349**	**0.052,0.647***	**-0.239**	**-0.461,-0.017***
TC	0.005	-0.111,0.122	**-0.134**	**-0.224,-0.043****	-0.047	-0.149,0.054	-0.079	-0.176,0.018	-0.025	-0.143,0.093	**-0.092**	**-0.183,-0.000***
HDL-c	0.007	-0.108,0.123	-0.080	-0.186,0.025	-0.031	-0.140,0.078	-0.036	-0.154,0.082	-0.084	-0.202,0.035	-0.009	-0.120,0.101
TC/HDL-c	0.013	-0.109,0.134	-0.039	-0.155,0.077	-0.012	-0.133,0.110	-0.026	-0.144,0.091	0.081	-0.048,0.211	-0.083	-0.198,0.032
LDL-c	-0.031	-0.203,0.140	-0.110	-0.256,0.035	-0.073	-0.231,0.086	-0.071	-0.227,0.085	0.007	-0.168,0.182	-0.135	-0.284,0.015
Apo A1	-0.010	-0.143,0.123	**-0.144**	**-0.275,-0.012***	-0.055	-0.187,0.078	-0.094	-0.226,0.038	-0.100	-0.236,0.036	-0.077	-0.211,0.057
Apo B	-0.042	-0.143,0.058	**-0.099**	**-0.185,-0.013***	-0.050	-0.148,0.047	**-0.093**	**-0.181,-0.006***	-0.002	0.103,0.098	**-0.116**	**-0.206,-0.026***
Apo B/Apo A1	-0.070	-0.238,0.097	-0.055	-0.220,0.110	-0.044	-0.211,0.123	-0.076	-0.247,0.095	0.060	-0.120,0.240	-0.122	-0.286,0.042
	Polyunsaturated/saturated fat ratio				**Polyunsaturated/saturated fat ratio**				**Polyunsaturated/saturated fat ratio**			
Triglycerides	-0.028	-0.221,0.165	0.047	-0.110,0.204	0.102	29-0.526,0	-0.158	-0.732,0.415	-0.018	-0.227,0.190	0.036	-0.105,0.178
TC	0.019	-0.058,0.095	0.053	-0.008,0.114	-0.119	-0.361,0.122	-0.184	-0.402,0.033	0.043	-0.034,0.120	0.015	-0.043,0.074
HDL-c	0.002	-0.073,0.077	0.014	-0.059,0.087	-0.137	-0.393,0.119	-0.020	-0.289,0.248	0.047	-0.031,0.126	-0.038	-0.107,0.032
TC/HDL-c	0.013	-0.069,0.095	0.038	-0.042,0.118	0.021	-0.269,0.311	-0.146	-0.417,0.126	-0.007	-0.097,0.083	0.053	-0.019,0.126
LDL-c	-0.047	-0.069,0.162	0.087	-0.013,0.187	-0.145	-0.523,0.233	-0.254	-0.609,0.101	0.046	-0.075,0.167	0.062	-0.032,0.157
Apo A1	0.004	-0.083,0.091	0.043	-0.047,0.133	-0.202	-0.515,0.111	-0.125	-0.429,0.180	0.066	-0.026,0.157	-0.029	-0.113,0.057
Apo B	0.035	-0.032,0.103	**0.062**	**0.002,0.121***	-0.114	-0.346,0.118	**-0.241**	**-0.440,-0.042***	0.030	-0.040,0.099	0.046	-0.012,0.103
Apo B/Apo A1	0.037	-0.076,0.150	0.063	-0.051,0.177	-0.007	-0.406,0.391	-0.293	-0.685,0.099	-0.021	-0.146,0.104	0.084	-0.019,0.188
	Cholesterol-saturated fat index				**Cholesterol-saturated fat index**				**Cholesterol-saturated fat index**			
Triglycerides	-0.037	-0.173,0.098	-0.035	-0.155,0.084	-0.055	-0.192,0.082	-0.030	-0.146,0.087	-0.039	-0.185,0.108	-0.051	-0.162,0.060
TC	0.005	-0.049,0.059	-0.012	-0.059,0.034	-0.023	-0.077,0.030	0.018	-0.025,0.062	0.001	-0.053,0.056	-0.002	-0.048,0.044
HDL-c	0.037	-0.016,0.089	0.024	-0.031,0.080	-0.005	-0.061,0.052	**0.071**	**0.018,0.124****	0.004	-0.051,0.060	**0.066**	**0.012,0.121***
TC/HDL-c	-0.030	-0.088,0.028	-0.037	-0.097,0.024	-0.018	-0.083,0.046	**-0.057**	**-0.111,-0.002***	-0.006	-0.069,0.057	**-0.069**	**-0.125,-0.012***
LDL-c	-0.024	-0.105,0.058	-0.050	-0.126,0.026	-0.044	-0.128,0.039	-0.031	-0.103,0.040	-0.013	-0.098,0.071	-0.053	-0.128,0.021
Apo A1	0.010	-0.051,0.072	-0.003	-0.071,0.066	-0.034	-0.103,0.036	0.050	-0.011,0.111	-0.034	-0.099,0.030	0.051	-0.015,0.117
Apo B	-0.028	-0.076,0.020	-0.015	-0.061,0.030	-0.033	-0.085,0.018	-0.013	-0.053,0.028	-0.012	-0.061,0.137	-0.027	-0.072,0.018
Apo B/Apo A1	-0.044	-0.123,0.036	-0.017	-0.104,0.069	-0.015	-0.103,0.073	-0.054	-0.133,0.026	0.015	-0.073,0.102	-0.074	-0.156,0.007

*p<0.05, **p<0.01, ***p<0.001

[a]Adjusted by gender, age, study centre, maternal education, sum of four skinfolds, moderate-to-vigorous physical activity, sedentary behaviours, diet quality index for adolescents.

Apo A1, apolipoprotein A1; Apo B, apolipoprotein B; LDL-c, low density lipoprotein cholesterol; WHeR, waist-to-height ratio; z-BMI, z-score body mass index.

High and low body fat groups were defined by means of gender- and age-specific medians.

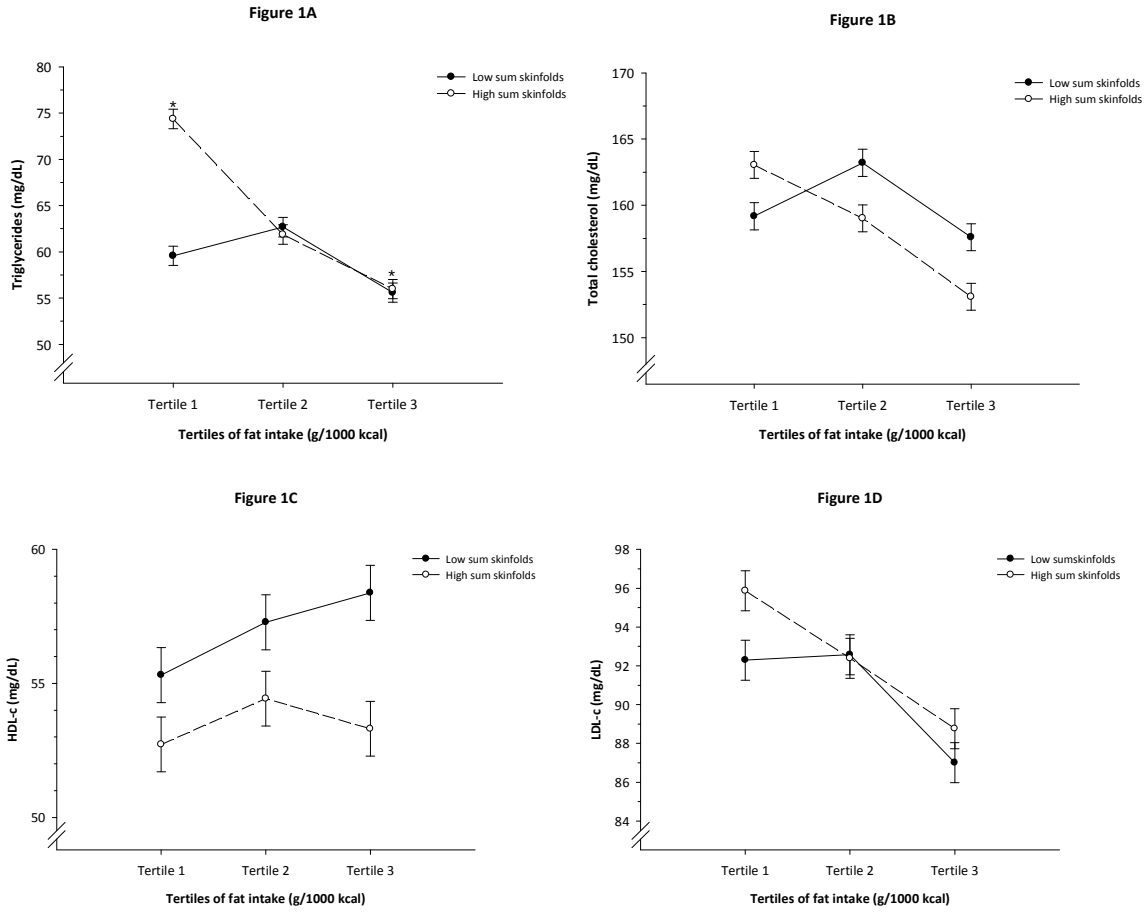

Figure 1. Mean (SE) triglycerides (1A), total cholesterol (1B), HDL-c (1C) and LDL-c (1D) by tertiles of fat intake for high and low sum of skinfolds after adjustment for covariates: age, sex, study centre, socioeconomic status, moderate-to-vigorous physical activity, sedentary behaviours and diet quality index for adolescents.

*p<0.05 across tertiles of fat intake after Bonferroni correction for post-hoc multiple comparisons.

Unlogged values for easier interpretability.

Median fat intake:

Tertile 1: 32.1 g/1000 kcal (low sum skinfolds), 33.6 g/1000 kcal (high sum skinfolds)

Tertile 2: 40.4 g/1000 kcal (low sum skinfolds), 42.2 g/1000 kcal (high sum skinfolds)

Tertile 3: 49.7 g/1000 kcal (low sum skinfolds), 51.4 g/1000 kcal (high sum skinfolds)

HDL-c, high density lipoprotein-cholesterol; LDL-c, low density lipoprotein-cholesterol.

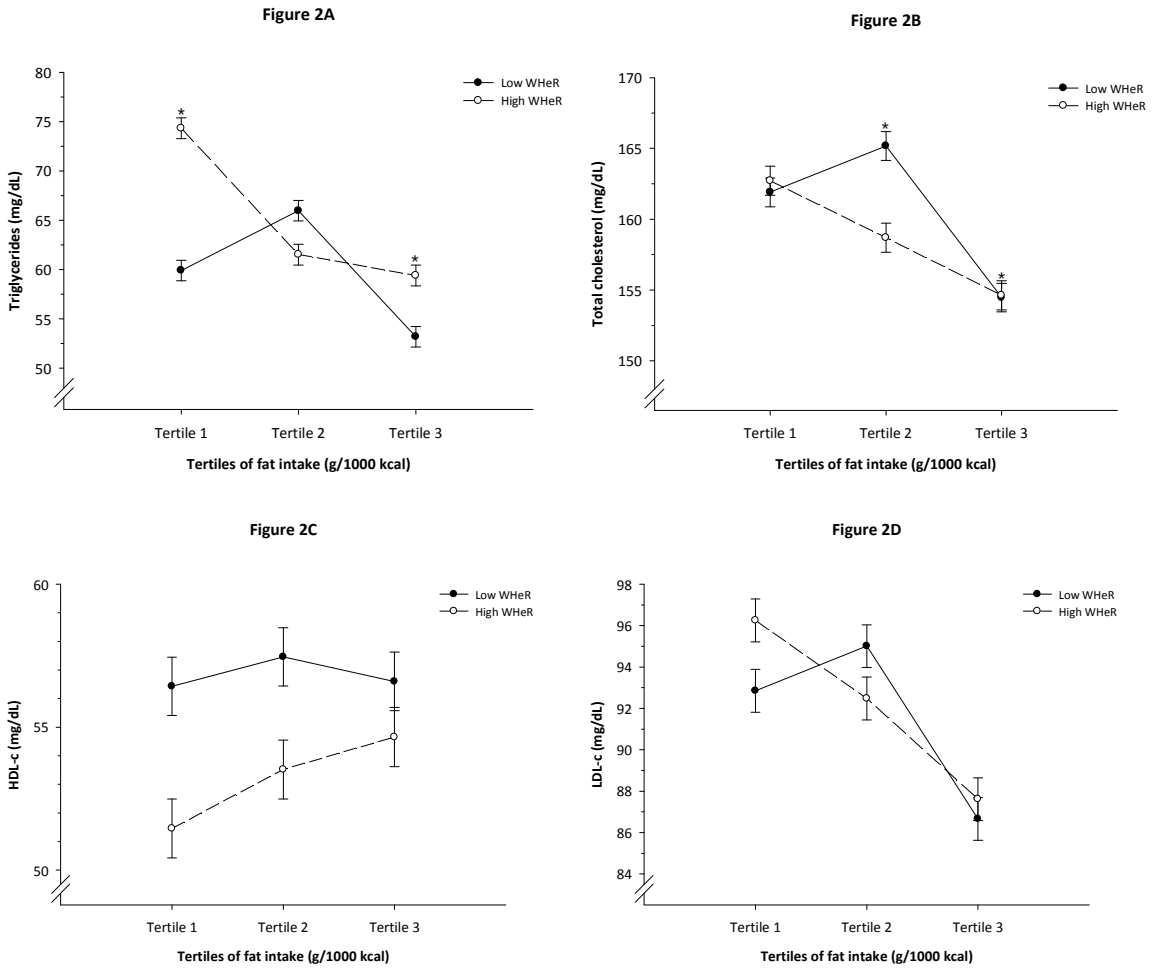

Figure 2. Mean (SE) triglycerides (2A), total cholesterol (2B), HDL-c (2C) and LDL-c (2D) by tertiles of fat intake for high and low waist-to-height ratio after adjustment for covariates: age, sex, study centre, socioeconomic status, moderate-to-vigorous physical activity, sedentary behaviours and diet quality index for adolescents.

*p<0.05 across tertiles of fat intake after Bonferroni correction for post-hoc multiple comparisons.

Unlogged values for easier interpretability.

Median fat intake:

Tertile 1: 32.1 g/1000 kcal (low WHeR), 33.6 g/1000 kcal (high WHeR)

Tertile 2: 40.8 g/1000 kcal (low WHeR), 41.9 g/1000 kcal (high WHeR)

Tertile 3: 50.0 g/1000 kcal (low WHeR), 51.4 g/1000 kcal (high WHeR)

HDL-c, high density lipoprotein-cholesterol; LDL-c, low density lipoprotein-cholesterol; WHeR, waist-to-height ratio.

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Artículo V [Paper V]:

Food consumption and cardiovascular risk factors in European children: the IDEFICS study

Bel-Serrat S, Mouratidou T, Börnhorst C, Peplies J, De Henauw S, Marild S, Molnár D, Siani A, Tornaritis M, Veidebaum T, Krogh V, Moreno LA.

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Food consumption and cardiovascular risk factors in European children: the IDEFICS study

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What is already known about this subject

- Few studies addressing the relationship between food consumption and cardiovascular disease or metabolic risk have been conducted in children. Previous findings have indicated greater metabolic risk in children with high intakes of solid hydrogenated fat and white bread, and low consumption of fruits, vegetables and dairy products.

What this study adds

- In a large multinational sample of 2 to 9 years old children, high consumption of sweetened beverages and low intake of nuts and seeds, sweets, breakfast cereals, jam and honey and chocolate and nut-based spreads were directly associated with increased clustered cardiovascular disease risk. These findings add new evidence to the limited literature available in young populations on the role that diet may play on cardiovascular health.

Summary

Objective: To investigate food consumption in relation to clustered cardiovascular disease (CVD) risk.

Methods: Children (n = 5548, 51.6% boys) from eight European countries participated in the IDEFICS study baseline survey (2007–2008). Z-scores of individual CVD risk factors were summed to compute sex- and age-specific (2–<6 years/6–9 years) clustered CVD risk scores A (all components, except cardiorespiratory fitness) and B (all components). The association of clustered CVD risk and tertiles of food group consumption was examined.

Results: Odds ratio (OR) of having clustered CVD risk A increased in older children with higher consumption of chocolate and nut-based spreads (boys: OR = 0.46; 95% CI = 0.32–0.69; girls: OR = 0.60; 95% CI = 0.42–0.86), jam and honey (girls: OR = 0.45; 95% CI = 0.26–0.78) and sweets (boys: OR = 0.69; 95% CI = 0.48–0.98). OR of being at risk significantly increased with the highest consumption of soft drinks (younger boys) and manufactured juices (older girls). Concerning CVD risk score B, older boys and girls in the highest tertile of consumption of breakfast cereals were 0.41 (95% CI = 0.21–0.79) and 0.45 (95% CI = 0.22–0.93) times, respectively, less likely to be at risk than those in tertile 1.

Conclusions: High consumption of sugar-sweetened beverages and low intake of breakfast cereals, jam and honey, sweets and chocolate and nut-based spreads seem to adversely affect clustered CVD risk.

Keywords: Cardiovascular risk factors, children, European cohort, food consumption.

Abbreviations: BMI, body mass index; HDL-c, high-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; HOMA-IR, homeostatic assessment model; SBP, systolic blood pressure; DBP, diastolic blood pressure; CFR, cardiorespiratory fitness; VO_{2max}, maximum oxygen uptake; CVD, cardiovascular disease.

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Introduction

Increased risk of cardiovascular diseases (CVD) is characterized by a cluster of metabolic abnormalities including abdominal obesity, atherogenic dyslipemia, hypertension, insulin resistance and glucose intolerance (1). During the last decades, clustering of CVD and metabolic risk factors have frequently been observed among children and adolescents (2). In children, criteria still have to be established reflecting a universally accepted paediatric definition for metabolic syndrome, strongly depending on the age group assessed. This is of concern given that the onset of related risk factors occurring in early childhood may persist during adulthood (3), making therefore an early diagnosis essential.

CVD and metabolic risk factors in children have been related to lifestyle factors such as diet (4–6) and physical activity (7). Most of the dietary studies carried out in adults and children have focused on intakes of nutrients, not on food consumption (5,6,8,9). Addressing potential associations between foods or food groups consumption might help to capture some of the complexity of nutrient-based analysis taking into consideration however, existing problems of food-based analysis (9). An independent association between low fruit and vegetable consumption and high sweetened beverage consumption with rising prevalence of metabolic syndrome has been observed among adults (10). In children, results have indicated greater metabolic risk with high intakes of solid hydrogenated fat and white bread, and low consumption of fruits, vegetables and dairy products (4). Despite this, research in young age population groups is scarce. The aim of the present study was to examine the association between food consumption and clustered CVD risk factors in (pre)school children.

Methods

Data used in this report were obtained from the baseline survey (2007–2008) of the IDEFICS (Identification and prevention of Dietary- and lifestyle-induced health EFfects in children and InfantS) study carried out in eight European countries: Italy, Estonia, Cyprus, Belgium, Sweden, Germany, Hungary and Spain. A total of 16 224 children aged 2–9 years were measured. More details about the study procedures have been previously published (11).

Only those subjects with complete data on gender, weight, height, triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), glucose, insulin systolic blood pressure (SBP), and diet were included ($n = 5548$). No differences were observed between individuals with valid measurements and those excluded when considering mean body mass index (BMI). The study sample differed with respect to mean age, height and weight from the total IDEFICS sample since they were older, taller and heavier ($P < 0.05$). The study protocol was approved by the ethics committee at each study centre and written informed consent signed by the parents was obtained from each participating child.

Socioeconomic status

The International Standard Classification of Education (ISCED) (12) was used as indicator for socioeconomic status (SES). The maximum ISCED level of both parents was considered.

Food consumption

The food frequency section of the Children's Eating Habits Questionnaire (CEHQ-FFQ) was completed by the parents, querying on the number of times the child had consumed the food item at home or at other people's homes during a typical week over the previous month. The CEHQ-FFQ included 43 food groups, which were clustered in 25 food groups according to their nutritional values and used in subsequent analysis: vegetables; fried potatoes; fruit; manufactured fruit juices; soft drinks; breakfast cereals; milk; dairy products (yoghurt, fermented milk beverages . . .); cold cuts (ham, sausages, salami, mortadella . . .); cheese; jam and honey; chocolate and nut-based spreads (Nutella, peanut butter . . .); butter and margarine; low-fat spreads (light butter, light margarine . . .); ketchup; refined cereals (white bread, pasta and rice); pizza; nuts and seeds (almonds, walnuts, sunflower seeds, raisins . . .); snacks (popcorn, crisps, chips, pancakes . . .); and sweets (candies, chocolate, cakes, desserts, ice creams . . .). Food groups included in the analysis were selected based on their relation to health-related practices and obesity prevalence (13–15). Responses included eight frequency categories of consumption: 'never/less than once a week', '1–3 times a week', '4–6 times a week', '1 time per day', '2 times per day', '3 times per day', '4 or more times per day' and 'I have no idea'. Frequencies were converted into times per week ranging from 0 to 30. Previous findings suggested acceptable reproducibility of the CEHQ-FFQ (16) and found a positive correlation between milk consumption frequencies and potassium and calcium urinary excretion ratios (17).

The number of meals per week consumed at home or at other people's homes was also reported. For

every meal, parents had to choose one of the following frequencies: 'daily', 'only at weekdays', 'only at weekends', 'several times per week' and 'on fewer occasions'.

Physical examinations

Weight was measured in light underwear with an electronic scale (TANITA BC 420 SMA, Tanita Europe GmbH, Sindelfingen, Germany) and height was measured without shoes using a stadiometer (Seca 225, Birmingham, UK). BMI was calculated as weight (kg) divided by squared height (m^2). Skinfolds thicknesses were measured twice with a calliper (Holtain Ltd, Croswell, UK) at the triceps and subscapular sites and the mean of the two measurements was taken. Blood pressure was measured with an electronic sphygmomanometer (Welch Allyn 4200B-E2, Welch Allyn Inc., Skaneateles Falls, NY, USA) in the right arm with the child in a sitting position. Two measurements were taken at 2 min intervals and differences of 5% magnitude lead to take a third measurement. Means of replicate measurements were used in all analyses. All physical examinations were taken by trained fieldworkers.

Physical activity and sedentary behaviours

Parents were asked about the sum of hours that their children spent playing outdoors (weekday and weekend days) in addition to weekly participation in sport club activities. This was done via an outdoor playtime questionnaire (18), which had previously been significantly correlated with objective measures of physical activity (PA) (Spearman $r = 0.20$, $P = 0.003$). Children's weekly participation in sport club activities significantly correlated (Spearman $r = 0.23$, $P < 0.001$) with children's daily time spent in moderate to vigorous activity as measured by accelerometry within the IDEFICS study (unpublished results).

The average hours per week of TV/video/DVD viewing was obtained by asking the parents 'How long does your child usually watch TV/video/DVD per day?' Responses were split into weekdays and weekend days and included five categories: 'not at all', '<30 minutes per day', '<1 hour per day', '1–2 hours per day', '2–3 hours per day' and '>3 hours per day'. The total number of hours per week of TV/video/DVD viewing was calculated.

Physical fitness

Cardiorespiratory fitness (CRF; mL kg^{-1} min^{-1}) was predicted by means of the Léger *et al*. (19) formula using the maximum speed that the child reached during the 20-m shuttle run test. Participants were required to run between two lines 20 m apart following beep signals in gradual increase of speed. When the participant stopped due to fatigue or did not reach the line with the audio signal on two consecutive occasions, the test finished. The final score was computed as the number of stages completed and was used to calculate the maximum oxygen uptake (VO_{2max}) (19).

Biological samples

A detailed description of the blood sampling procedures is published elsewhere (20). Blood samples were obtained after an overnight fast. Blood glucose, TC, HDL-c and TG were assessed at each study centre and serum insulin concentrations were determined in a central laboratory. Insulin resistance as defined by the homeostasis model assessment (HOMA-IR) (21) was calculated via a standard formula from fasting glucose and plasma insulin: HOMA-IR = [insulin (μUI mL^{-1}) × glucose (mg dL^{-1})]/405.

Cardiovascular disease risk score

A continuous score of clustered CVD risk factors was computed using the following variables according to Andersen *et al*. (7): SBP, TG, ratio TC/HDL-c, HOMA and sum of two skinfolds (tricipital and subscapular). Since the 20-m shuttle run test was only performed in children older than 6 years, a second CVD risk score was only obtained for older children containing the CRF variable. Gender- and age group-specific (2–<6 years/6–9 years) *z*-scores were calculated for each risk factor variable. All individual *z*-scores were summed to create two clustered CVD scores: CVD risk score A (without CRF) and CVD risk score B (with CRF), computed only in older children. CRF was multiplied by –1 to indicate higher CVD risk with increasing value. The lower the score the better the overall CVD risk factor profile.

Statistical analysis

The Predictive Analytics SoftWare (version 18; SPSS Inc., Chicago, IL, USA) was used to perform the analyses. Statistical significance was set at $P < 0.05$. Frequencies of food group consumption (times per week) were converted into age-, gender- and study centre-specific tertiles. Food groups with high proportion of non-consumers (>33% non-consumers) were treated differently, i.e. participants with zero consumption were considered as one category. The

Table 1 Values for CVD risk-related factors and anthropometric measures of study participants by age group and gender

	2–<6 years old		6–9 years old	
	Boys (n = 1103)	Girls (n = 982)	Boys (n = 1760)	Girls (n = 1703)
Age (years)	4.4 (0.9)	4.4 (0.9)	7.5 (0.8)	7.5 (0.8)
Height (cm)	107.8 (7.6)	106.5 (7.7)	127.9 (7.4)	126.6 (7.3)
Weight (kg)	18.6 (3.7)	18.0 (3.6)	27.7 (6.5)	27.2 (6.3)
BMI (kg m^{-2})	15.9 (1.8)	15.8 (1.8)	16.8 (2.8)	16.8 (2.8)
Underweight (%)*	165 (15)	130 (13.2)	164 (9.3)	152 (8.9)
Normal weight (%)	804 (72.9)	695 (70.8)	1217 (69.1)	1132 (66.5)
Overweight (%)	92 (8.3)	109 (11.1)	244 (13.9)	292 (17.1)
Obese (%)	42 (3.8)	48 (4.9)	135 (7.7)	127 (7.5)
Sum of two skinfolds (mm)	15.6 (4.7)	17.7 (5.4)	17.9 (8.6)	21.2 (9.2)
DBP (mm Hg)	61.0 (6.2)	62.0 (6.1)	63.3 (6.6)	64.1 (6.3)
SBP (mm Hg)	96.7 (8.3)	96.7 (8.1)	102.8 (8.5)	102.1 (8.6)
TG (mg/dL)	40.9 (22.6)	42.1 (23.7)	40.5 (21.7)	43.1 (25.2)
Total cholesterol (mg dL^{-1})	155.7 (29.3)	157.9 (30.4)	159.5 (29.8)	164.7 (32.2)
HDL-c (mg dL^{-1})	49.1 (13.5)	46.6 (13.6)	54.9 (14.8)	53.8 (14.4)
Glucose (mg dL^{-1})	82.8 (9.2)	81.1 (8.9)	87.8 (9.0)	85.5 (8.7)
Insulin (μU mL^{-1})	3.1 (2.4)	3.5 (2.8)	4.7 (3.1)	5.4 (3.6)
HOMA score	0.7 (0.6)	0.7 (0.6)	1.0 (0.7)	1.2 (0.8)
CRF (mL kg^{-1} min^{-1})	–	–	48.0 (3.0)	47.0 (2.3)
CVD risk score A	–1.24 (2.53)	–0.44 (2.60)	0.12 (2.89)	0.93 (3.06)
CVD risk score B	–	–	–0.001 (2.77)	0.63 (2.80)

*BMI categories according to Cole *et al*. (34).
BMI, body mass index; CRF, cardiorespiratory fitness; CVD, cardiovascular disease; DBP, diastolic blood pressure; HDL-c, high-density lipoprotein cholesterol; HOMA, homeostasis model assessment; SBP, systolic blood pressure; TG, triglycerides. All data expressed as mean (SD).

remaining participants were split into two halves of consumption according to their medians. This resulted in unbalanced tertiles in terms of number of participants. That approach was applied for fried potatoes (only for analysis with CVD risk score A), soft drinks (only for analysis with CVD risk score A), jam and honey, low-fat spreads, and chocolate and nut-based spreads (only for analysis with CVD risk score B). The number of non-consumers for pizza, nuts and fried potatoes (only for analysis with CVD risk score B) food groups were >66%. For these food groups, comparisons were based on consumers and non-consumers. The association between clustered CVD risk and food group consumption was assessed by binary logistic regression. Children above 1 standard deviation (SD) for clustered risk scores were identified as being at risk of CVD (7). Model 1 was adjusted for SES, study centre and the number of meals per week consumed at home or at other people's homes. Model 2 included the covariates of model 1 plus physical activity.

Since only older children had valid data on CRF (n = 1332), additional gender- and study centre-specific tertiles were computed for food groups in those analysis conducted with CVD risk score B. The same approach described above was applied for low consumed food groups.

Results

Descriptive characteristics of participants by age group and gender are shown in Table 1. The risk of having clustered risk factors (dichotomous z-score above 1 SD) according to tertiles of food group consumption is presented separately by age and gender group for CVD risk score A and B.

Data regarding CVD risk score A are presented in Table 2 for boys and Table 3 for girls. Younger boys consuming nuts and seeds were 0.62 (95% CI = 0.40–0.95) times less likely of being at CVD risk than those with no consumption. Considering soft drinks, the odds ratio (OR) of having clustered CVD risk increased for younger boys in tertile 3 (OR = 1.57; 95% CI = 1.00–2.46) compared with those in tertile 1. In addition, the odds of having CVD risk decreased for children in the highest tertile of chocolate and nut-based spreads consumption for both older boys (OR = 0.46; 95% CI = 0.32–0.69)

Table 2 Age-specific odd ratios (OR) for CVD risk score A for tertiles of food groups in boys

	2–<6 years old				6–9 years old			
	Model 1†		Model 2‡		Model 1		Model 2	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Reference for all food groups: tertile 1								
Vegetables (raw and cooked)								
Tertile 2	1.08	0.70,1.67	1.14	0.72,1.79	0.81	0.58,1.14	0.81	0.57,1.15
Tertile 3	1.04	0.66,1.65	1.11	0.69,1.78	0.84	0.60,1.20	0.88	0.61,1.26
Fried potatoes								
Tertile 2	0.75	0.50,1.11	0.74	0.49,1.13	0.90	0.67,1.21	0.85	0.63,1.16
Tertile 3	0.88	0.33,2.37	1.04	0.38,2.85	0.55	0.21,1.45	0.54	0.20,1.44
Fruit								
Tertile 2	0.72	0.45,1.16	0.72	0.44,1.18	0.89	0.61,1.29	0.91	0.62,1.33
Tertile 3	0.67	0.41,1.08	0.69	0.42,1.14	1.34	0.95,1.87	0.94	0.94,1.93
Manufactured juices								
Tertile 2	0.91	0.58,1.42	0.88	0.56,1.41	0.90	0.62,0.29	0.93	0.64,1.35
Tertile 3	1.09	0.68,1.74	1.10	0.67,1.78	1.04	0.62,1.29	1.03	0.71,1.49
Soft drinks								
Tertile 2	0.68	0.38,1.21	0.70	0.38,1.28	0.91	0.62,1.32	0.87	0.59,1.29
Tertile 3	1.41	0.92,2.17	1.57	1.00,2.46	1.05	0.74,1.47	0.98	0.69,1.40
Breakfast cereals								
Tertile 2	1.32	0.83,2.08	1.48	0.92,2.37	1.10	0.78,1.57	1.09	0.76,1.56
Tertile 3	0.88	0.55,1.40	0.93	0.57,1.52	0.77	0.53,1.12	0.73	0.50,1.07
Milk								
Tertile 2	0.85	0.53,1.35	0.85	0.53,1.38	0.89	0.62,1.28	0.83	0.57,1.20
Tertile 3	1.02	0.66,1.58	1.01	0.64,1.59	0.83	0.59,1.17	0.79	0.56,1.13
Dairy products								
Tertile 2	1.25	0.79,1.96	1.24	0.77,1.98	0.96	0.67,1.38	0.99	0.68,1.44
Tertile 3	0.89	0.57,1.38	0.96	0.61,1.52	1.01	0.73,1.41	1.05	0.75,1.47
Cold cuts								
Tertile 2	0.74	0.47,1.16	0.68	0.42,1.10	0.97	0.68,1.39	0.96	0.67,1.38
Tertile 3	0.84	0.53,1.33	0.88	0.55,1.40	1.06	0.74,1.52	1.05	0.73,1.52
Cheese								
Tertile 2	1.23	0.78,1.93	1.33	0.83,2.13	1.15	0.81,1.62	1.12	0.78,1.59
Tertile 3	1.01	0.62,1.62	1.12	0.68,1.83	1.32	0.92,1.89	1.32	0.91,1.91
Jam and honey								
Tertile 2	1.08	0.68,1.70	1.07	0.67,1.71	0.69	0.48,0.99	0.69	0.48,0.99
Tertile 3	0.88	0.50,1.53	0.92	0.51,1.65	1.12	0.74,1.70	1.09	0.71,1.68
Chocolate and nut-based spreads								
Tertile 2	0.95	0.56,1.62	0.95	0.55,1.65	0.84	0.57,1.24	0.76	0.51,1.12
Tertile 3	0.99	0.65,1.51	0.98	0.63,1.52	0.53	0.36,0.76	0.46	0.32,0.69
Butter and margarine								
Tertile 2	0.92	0.53,1.60	0.86	0.48,1.52	1.55	0.99,2.42	1.56	0.99,2.46
Tertile 3	0.70	0.41,1.22	0.74	0.42,1.29	1.10	0.71,1.71	1.10	0.70,1.72
Low-fat spreads								
Tertile 2	1.10	0.55,2.20	1.14	0.55,2.33	0.79	0.46,1.34	0.73	0.42,1.26
Tertile 3	1.44	0.86,2.40	1.47	0.87,2.51	0.91	0.60,1.39	0.90	0.59,1.39

Table 2 Continued

	2–<6 years old				6–9 years old			
	Model 1†		Model 2‡		Model 1		Model 2	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Ketchup								
Tertile 2	0.89	0.54,1.46	0.98	0.59,1.64	1.21	0.81,1.81	1.24	0.82,1.88
Tertile 3	1.26	0.75,2.11	1.34	0.78,2.31	1.31	0.87,1.98	1.30	0.86,1.98
Refined cereals (white bread, pasta, rice)								
Tertile 2	1.28	0.82,2.00	1.30	0.82,2.07	1.56	1.10,2.22	1.48	1.04,2.12
Tertile 3	1.02	0.64,1.62	1.14	0.71,1.82	1.02	0.71,1.48	0.92	0.63,1.35
Pizza*								
Tertile 2	–	–	–	–	–	–	–	–
Tertile 3	1.49	0.99,2.25	1.38	0.89,2.12	1.18	0.86,1.61	1.16	0.85,1.60
Nuts and seeds*								
Tertile 2	–	–	–	–	–	–	–	–
Tertile 3	0.67	0.44,1.01	0.62	0.40,0.95	0.88	0.65,1.20	0.88	0.64,1.21
Snacks								
Tertile 2	0.88	0.47,1.66	0.80	0.42,1.53	0.99	0.70,1.41	1.01	0.71,1.44
Tertile 3	0.87	0.58,1.29	0.88	0.58,1.33	1.05	0.73,1.50	1.02	0.71,1.48
Sweets (candies, chocolate, cakes, desserts, ice creams)								
Tertile 2	0.96	0.60,1.52	0.93	0.58,1.49	0.76	0.54,1.07	0.72	0.50,1.03
Tertile 3	1.44	0.92,2.25	1.42	0.90,2.24	0.74	0.52,1.05	0.69	0.48,0.98

*These food groups have been split into consumers and non-consumers (reference group) due to the high proportion of non-consumers (>66%) in these groups.
†Model 1: adjusted for SES, study centre and number of meals per week consumed at home or at other people's homes.
‡Model 2: adjusted for socioeconomic status (SES), study centre, number of meals per week consumed at home or at other people's homes and total physical activity performed during the week.
CI, confidence interval; CVD, cardiovascular disease.

and girls (OR = 0.60; 95% CI = 0.42–0.86) compared with those with the lowest consumption. Older boys with the highest consumption of sweets showed lower likelihood of having clustered risk (OR = 0.69; 95% CI = 0.48–0.98) than boys in tertile 1. Older girls in tertile 3 of jam and honey were 0.45 (95% CI = 0.26–0.78) times less likely to be at CVD risk than those in tertile 1. The OR for being at risk of clustered CVD increased for older girls with the highest consumption of manufactured juices (OR = 1.45; 95% CI = 1.01–2.10) compared with those with the lowest consumption.

Table 4 presents data on CVD risk score B. Both older boys and girls in the highest tertile of consumption of breakfast cereals were 0.41 (95% CI = 0.21–0.79) and 0.45 (95% CI = 0.22–0.93) times, respectively, less likely to be at CVD risk than their peers in tertile 1. Additionally, the odds of having clustered CVD risk decreased for those boys with the highest consumption of chocolate and nut spreads (OR = 0.20; 95% CI = 0.07–0.56) and sweets (OR = 0.52; 95% CI = 0.27–0.99) when compared with boys in tertile 1.

Discussion

This study investigated the association between food consumption and clustering of CVD risk factors. Examination of clusters of CVD risk factors has been shown to be a more informative approach than single-risk factors in children (22). Additionally, a composite score could compensate in part for fluctuations in the single-risk factors (7). On the other hand, single cut-off points cannot be used to define metabolic abnormalities as they vary with age, gender and pubertal age (23). It is noteworthy, however, that CVD risk factors have also been observed at early ages, mainly among obese children (21,22). For that reason, specific CVD risk scores were computed by age group and gender.

Observed significant associations between CVD risk score A and tertiles of food group consumption persisted in CVD risk score B for boys > 6 years but not in girls. Furthermore, older children with the highest consumption of breakfast cereals were less likely of having clustered CVD risk B compared with

Table 3 Age-specific odd ratios (OR) for CVD risk score A for tertiles of food groups in girls

	2–<6 years old				6–9 years old			
	Model 1†		Model 2‡		Model 1		Model 2	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Reference for all food groups: tertile 1								
Vegetables (raw and cooked)								
Tertile 2	1.13	0.70,1.81	1.15	0.70,1.90	1.02	0.71,1.46	1.04	0.71,1.51
Tertile 3	0.79	0.49,1.30	0.87	0.52,1.44	0.80	0.57,1.13	0.84	0.58,1.20
Fried potatoes								
Tertile 2	1.23	0.81,1.86	1.01	0.65,1.58	1.09	0.81,1.49	1.00	0.73,1.38
Tertile 3	0.21	0.03,1.62	0.25	0.03,2.07	0.87	0.35,2.17	0.80	0.29,2.20
Fruit								
Tertile 2	0.72	0.45,1.16	0.72	0.44,1.18	0.89	0.61,1.29	0.91	0.62,1.33
Tertile 3	0.67	0.41,1.08	0.69	0.42,1.14	1.34	0.95,1.87	0.94	0.94,1.93
Manufactured juices								
Tertile 2	1.18	0.72,1.94	1.16	0.70,1.94	0.91	0.61,1.37	0.90	0.59,1.37
Tertile 3	1.00	0.62,1.60	0.84	0.51,1.39	1.48	1.04,2.11	1.45	1.01,2.10
Soft drinks								
Tertile 2	1.76	1.06,2.93	1.71	1.01,2.90	0.89	0.60,1.31	0.91	0.61,1.37
Tertile 3	1.26	0.76,2.10	1.10	0.64,1.91	1.05	0.73,1.50	1.06	0.72,1.53
Breakfast cereals								
Tertile 2	1.49	0.91,2.45	1.56	0.93,2.60	1.15	0.80,1.64	1.09	0.75,1.58
Tertile 3	1.06	0.65,1.75	1.07	0.63,1.80	0.86	0.60,1.24	0.83	0.56,1.21
Milk								
Tertile 2	0.95	0.59,1.54	1.01	0.61,1.67	0.86	0.61,1.21	0.87	0.61,1.24
Tertile 3	0.97	0.59,1.59	1.00	0.60,1.68	0.85	0.58,1.24	0.84	0.56,1.24
Dairy products								
Tertile 2	0.65	0.39,1.10	0.60	0.35,1.02	1.13	0.79,1.63	1.11	0.76,1.61
Tertile 3	1.05	0.67,1.64	0.89	0.56,1.43	1.19	0.84,1.68	1.20	0.84,1.73
Cold cuts								
Tertile 2	0.55	0.34,0.91	0.54	0.32,0.90	1.18	0.82,1.70	1.18	0.81,1.72
Tertile 3	0.86	0.54,1.38	0.78	0.47,1.28	1.13	0.78,1.63	1.06	0.73,1.56
Cheese								
Tertile 2	0.73	0.46,1.16	0.75	0.46,1.22	0.96	0.67,1.39	0.97	0.66,1.41
Tertile 3	0.72	0.44,1.19	0.81	0.48,1.37	1.00	0.70,1.43	1.03	0.71,1.49
Jam and honey								
Tertile 2	0.78	0.48,1.26	0.78	0.47,1.30	1.11	0.78,1.56	1.11	0.78,1.58
Tertile 3	0.77	0.43,1.37	0.88	0.49,1.59	0.43	0.25,0.74	0.45	0.26,0.78
Chocolate and nut-based spreads								
Tertile 2	0.75	0.44,1.25	0.67	0.39,1.15	0.77	0.50,1.18	0.79	0.50,1.24
Tertile 3	0.94	0.58,1.52	0.91	0.55,1.50	0.65	0.46,0.91	0.60	0.42,0.86
Butter and margarine								
Tertile 2	0.63	0.35,1.14	0.60	0.33,1.11	0.79	0.51,1.21	0.83	0.53,1.30
Tertile 3	0.76	0.42,1.38	0.72	0.39,1.33	0.65	0.42,1.01	0.67	0.43,1.05
Low-fat spreads								
Tertile 2	0.43	0.17,1.07	0.39	0.14,1.04	1.65	1.00,2.72	1.95	1.16,3.29
Tertile 3	1.26	0.76,2.11	1.24	0.73,2.09	1.31	0.86,2.00	1.38	0.89,2.15

Table 3 Continued

	2–<6 years old				6–9 years old			
	Model 1†		Model 2‡		Model 1		Model 2	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Ketchup								
Tertile 2	1.19	0.72,1.97	1.06	0.63,1.78	0.90	0.62,1.32	0.81	0.54,1.21
Tertile 3	1.63	0.93,2.86	1.61	0.90,2.89	1.25	0.82,1.91	1.02	0.65,1.59
Refined cereals (white bread, pasta, rice)								
Tertile 2	1.04	0.64,1.69	1.10	0.66,1.83	0.83	0.59,1.18	0.82	0.57,1.19
Tertile 3	1.34	0.82,2.20	1.36	0.81,2.29	0.79	0.55,1.12	0.79	0.55,1.14
Pizza*								
Tertile 2	–	–	–	–	–	–	–	–
Tertile 3	0.78	0.47,1.29	0.72	0.42,1.24	1.07	0.77,1.47	0.94	0.66,1.32
Nuts and seeds*								
Tertile 2	–	–	–	–	–	–	–	–
Tertile 3	0.90	0.59,1.35	0.94	0.61,1.44	0.95	0.69,1.29	0.95	0.68,1.32
Snacks								
Tertile 2	0.95	0.57,1.55	1.03	0.61,1.71	1.01	0.68,1.49	0.90	0.60,1.36
Tertile 3	0.92	0.56,1.52	0.93	0.55,1.58	0.97	0.69,1.38	0.84	0.58,1.21
Sweets (candies, chocolate, cakes, desserts, ice creams)								
Tertile 2	0.94	0.59,1.49	0.93	0.57,1.50	0.98	0.69,1.41	0.91	0.63,1.32
Tertile 3	0.68	0.42,1.10	0.66	0.40,1.10	1.13	0.79,1.60	1.04	0.72,1.50

*These food groups have been split into consumers and non-consumers (reference group) due to the high proportion of non-consumers (>66%) in these groups.
†Model 1: adjusted for socioeconomic status (SES), study centre and number of meals per week consumed at home or at other people's homes.
‡Model 2: adjusted for SES, study centre, number of meals per week consumed at home or at other people's homes and total physical activity performed during the week.
CI, confidence interval; CVD, cardiovascular disease.

those in tertile 1, whereas no significant differences were observed for CVD risk score A. These differences could be attributed to the effect of the CRF variable. Indeed, it was found that CRF was positively associated with bread and cereals consumption in European adolescent boys (24).

Our findings indicated that higher consumption of soft drinks in younger boys and manufactured juices in older girls were associated with higher risk of clustered CVD risk. In a study (6) performed in Mexican schoolchildren, foods rich in fat and sugar were identified to be related more to CVD risk factors. Ventura *et al*. (25) suggested that at ages 5, 7 and 9 years, those with higher metabolic risk had the highest daily sweetened beverage consumption compared with others being at lower risk. Increased sugar-sweetened beverages consumption have been associated with increased HOMA-IR, SBP, waist circumference and decreased HDL-c concentrations in US adolescents (26) and with increased glucose concentrations in Mexican children (6).

Higher consumption of breakfast cereals decreased the OR of having clustered CVD risk B in both older boys and girls compared with those children with the lowest consumption. These beneficial effects may be explained partly by the presence of wholegrain breakfast cereals, which have been inversely associated to CVD-specific mortality in adults (27). Unexpectedly, older boys and girls with higher consumption of several sugar-rich products, i.e. chocolate and nut-based spreads, jam and honey (girls only), and sweets (boys only) were at lower risk of having clustered CVD compared with those with the lowest consumption. Although these products have more frequently been related to increased CVD or metabolic risk in children (6), one study performed in the United States (28) observed that candy consumption did not negatively affect CVD risk factors in both children and adolescents. Additionally, these products are typically consumed at breakfast. A recent study carried out among Italian adults suggested that frequent consumption of breakfast foods such as breakfast cereals, honey, sugar and jam positively affected their CVD risk profile (29). On the other hand, results observed among younger children are in agreement with a recent study, which associated nut consumption with a decreased prevalence of CVD risk factors, type 2 diabetes and metabolic syndrome in adults (30).

Table 4 Gender-specific odd ratios for CVD risk score B for tertiles of food groups

	Boys				Girls			
	Model 1†		Model 2‡		Model 1		Model 2	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Reference for all food groups: tertile 1								
Vegetables (raw and cooked)								
Tertile 2	0.57	0.32,1.03	0.64	0.35,1.16	1.03	0.57,1.84	1.04	0.56,1.95
Tertile 3	0.50	0.26,0.94	0.54	0.28,1.02	0.80	0.44,1.44	0.89	0.47,1.68
Fried potatoes*								
Tertile 2	–	–	–	–	–	–	–	–
Tertile 3	1.34	0.80,2.24	1.22	0.72,2.08	1.32	0.81,2.15	1.15	0.67,1.97
Fruit								
Tertile 2	0.65	0.35,1.18	0.59	0.32,1.11	1.04	0.55,1.94	1.10	0.57,2.13
Tertile 3	0.63	0.34,1.17	0.59	0.31,1.11	1.23	0.69,2.19	1.29	0.69,2.40
Manufactured juices								
Tertile 2	0.90	0.46,1.78	0.94	0.47,1.88	0.74	0.40,1.37	0.73	0.38,1.41
Tertile 3	0.94	0.53,1.68	0.90	0.50,1.61	1.31	0.72,2.36	1.18	0.62,2.24
Soft drinks								
Tertile 2	1.08	0.52,2.26	1.06	0.49,2.27	0.75	0.37,1.52	0.72	0.33,1.58
Tertile 3	1.29	0.73,2.28	1.16	0.64,2.09	1.05	0.54,2.03	1.02	0.49,2.13
Breakfast cereals								
Tertile 2	0.57	0.32,1.03	0.55	0.29,1.01	0.98	0.56,1.69	1.10	0.61,1.96
Tertile 3	0.41	0.22,0.79	0.41	0.21,0.79	0.44	0.23,0.85	0.45	0.22,0.93
Milk								
Tertile 2	0.63	0.30,1.31	0.53	0.24,1.13	0.85	0.49,1.46	0.97	0.54,1.73
Tertile 3	0.99	0.57,1.71	1.05	0.60,1.85	0.83	0.43,1.61	0.79	0.38,1.64
Dairy products								
Tertile 2	1.42	0.77,2.61	1.45	0.77,2.74	0.53	0.29,0.99	0.54	0.28,1.04
Tertile 3	1.01	0.55,1.84	1.05	0.57,1.95	0.98	0.56,1.73	1.00	0.54,1.86
Cold cuts								
Tertile 2	1.11	0.60,2.10	1.19	0.63,2.22	0.97	0.55,1.74	1.10	0.59,2.04
Tertile 3	1.20	0.64,2.25	1.21	0.63,2.29	0.95	0.53,1.72	1.10	0.58,2.08
Cheese								
Tertile 2	0.76	0.41,1.39	0.73	0.39,1.36	0.88	0.49,1.59	0.87	0.47,1.64
Tertile 3	1.04	0.57,1.90	1.13	0.61,2.10	0.81	0.46,1.43	0.81	0.44,1.50
Jam and honey								
Tertile 2	0.44	0.22,0.90	0.48	0.23,0.98	1.49	0.81,2.76	1.55	0.81,2.97
Tertile 3	0.89	0.43,1.86	1.01	0.47,2.18	0.97	0.44,2.13	1.15	0.49,2.67
Chocolate and nut-based spreads								
Tertile 2	0.73	0.40,1.30	0.63	0.34,1.15	0.96	0.53,1.73	1.17	0.62,2.20
Tertile 3	0.26	0.10,0.69	0.20	0.07,0.56	0.59	0.28,1.26	0.53	0.23,1.20
Butter and margarine								
Tertile 3	1.00	0.48,2.09	1.00	0.47,2.15	0.65	0.32,1.30	0.82	0.39,1.76
Tertile 3	0.70	0.33,1.50	0.70	0.32,1.54	0.41	0.20,0.85	0.57	0.26,1.24
Low-fat spreads								
Tertile 2	1.21	0.59,2.50	1.17	0.55,2.47	2.63	1.29,5.36	3.23	1.51,6.92
Tertile 3	0.79	0.35,1.77	0.87	0.38,1.98	0.33	0.63,2.80	1.30	0.57,2.99

Table 4 Continued

	Boys				Girls			
	Model 1†		Model 2‡		Model 1		Model 2	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Ketchup								
Tertile 3	0.26	0.10,0.69	0.20	0.07,0.56	0.59	0.28,1.26	0.53	0.23,1.20
Tertile 3	0.26	0.10,0.69	0.20	0.07,0.56	0.59	0.28,1.26	0.53	0.23,1.20
Refined cereals (white bread, pasta, rice)								
Tertile 2	1.70	0.94,3.06	1.70	0.94,3.06	0.64	0.35,1.17	0.64	0.35,1.17
Tertile 3	0.81	0.41,1.62	0.81	0.41,1.62	0.67	0.34,1.30	0.67	0.34,1.30
Pizza*								
Tertile 2	–	–	–	–	–	–	–	–
Tertile 3	1.13	0.62,2.07	1.13	0.62,2.08	1.10	0.61,1.97	0.88	0.46,1.71
Nuts and seeds*								
Tertile 2	–	–	–	–	–	–	–	–
Tertile 3	0.80	0.47,1.37	0.79	0.45,1.37	0.84	0.51,1.40	0.80	0.45,1.40
Snacks								
Tertile 2	1.08	0.58,2.01	1.18	0.63,2.24	1.40	0.76,2.58	1.26	0.65,2.45
Tertile 3	0.94	0.51,1.75	0.95	0.51,1.80	1.87	1.05,3.34	1.62	0.87,3.02
Sweets (candies, chocolate, cakes, desserts, ice creams)								
Tertile 2	0.62	0.35,1.12	0.61	0.33,1.11	1.67	0.92,3.00	1.52	0.81,2.85
Tertile 3	0.60	0.32,1.12	0.52	0.27,0.99	1.23	0.66,2.29	0.98	0.49,1.93

*These food groups have been split into consumers and non-consumers (reference group) due to the high proportion of non-consumers (>66%) in these groups.
†Model 1: adjusted for socioeconomic status (SES), study centre and number of meals per week consumed at home or at other people's homes.
‡Model 2: adjusted for SES, study centre, number of meals per week consumed at home or at other people's homes and total physical activity performed during the week.
CI, confidence interval; CVD, cardiovascular disease; NA, not available.

The present study has several limitations. The cross-sectional design does not allow us to conclude that dietary intakes of certain foods causally contributed to a greater CVD risk. Another limiting factor is dietary information has been obtained by proxy-reported food frequency questionnaire, which is subject to error in the total number of foods usually consumed (31). However, the CEHQ-FFQ has previously been shown to give reproducible estimates of the frequency of food group consumption in European children (16,17). Obtained data from FFQs has been considered to be appropriate to explore patterns of intake and relationships with CVD risk factors (32). The large number of non-consumers in each food group might also have influenced observed relationships. Additionally, the attenuation of effect estimates due to misreporting cannot be precluded; however, the prevalence of misreporting in this sample of European children is rather low compared with others (33).

Children with CVD risk score A or B above 1 SD were considered to be at risk. As a result, individuals not being at risk could have been classified as being at risk. Nevertheless, that misclassification would have resulted in an underestimation of the true association between food groups consumption and clustered CVD risk (7). It is important to remark that the sample included in this study differs from the whole study population in terms of mean age, height and weight, so our conclusions can neither be generalized nor extrapolated to the whole IDEFICS sample.

The sample size was large enough to detect associations between food intake and the clustering of CVD risk factors. To our knowledge, this is the largest sample of children recruited from several European countries in which the relationship between CVD risk factors and food intake has been assessed. Furthermore, all procedures were standardized across countries.

In conclusion, our findings suggest that children being more likely to be at greater CVD risk had higher consumption of sugar-sweetened beverages (younger boys and older girls) and lower consumption of nuts and seeds (younger boys), sweets (older boys), jam and honey (older girls), chocolate and nut-based spreads and breakfast cereals (older boys and girls). These results can help to develop strategies

aimed at promoting healthy lifestyles from very early in life by means of encouraging children to have healthier eating patterns and to engage in physical activity regularly. More studies, preferably with a longitudinal design, are needed to explore the relationship between CVD risk factors and food consumption during childhood.

Conflicts of Interest Statement

The authors declare no conflict of interest.

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Artículo VI [Paper VI]:

Is dairy consumption associated with low cardiovascular diseases risk in European adolescents? Results from the HELENA Study

Bel-Serrat S, Mouratidou T, Jiménez-Pavón D, Huybrechts I, Cuenca-García M, Mistura L, Gottrand F, González-Gross M, Dallongeville J, Kafatos A, Manios Y, Stehle P, Kersting M, De Henauw S,Castillo MJ, Hallstrom L, Molnár D, Widhalm K, Marcos A, Moreno LA.

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Is dairy consumption associated with low cardiovascular disease risk in European adolescents? Results from the HELENA Study

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What is already known about this subject

- Although there is no clear evidence of the role of dairy consumption on cardiovascular disease (CVD) risk development in adults, several studies have suggested dairy consumption to have a protective effect.
- Limited evidence on the relationship between milk and dairy products consumption and CVD risk factors among adolescents.

What this study adds

- Dairy consumption was inversely associated with CVD risk in European adolescent girls.
- Higher dairy consumption was associated with lower adiposity and higher cardiorespiratory fitness in both genders.

Summary

Objective: To identify those food groups best discriminating individuals at high/low cardiovascular disease (CVD) risk and to investigate the relationship between dairy consumption and CVD risk factors (individual and scores) in adolescents (12.5–17.5 years) from eight European cities participating in the cross-sectional (2006–2007) HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) project.

Methods: Diet, waist circumference, skin-folds thickness, systolic blood pressure, insulin resistance, triglycerides, total cholesterol/high-density lipoprotein ratio and cardiorespiratory fitness (CRF) were assessed in 511 (49.9% boys) adolescents. Individual z-scores of CVD risk factors were summed to compute sex-specific clustered CVD risk scores.

Results: Dairy emerged as the food group best discriminating adolescents at low/high CVD risk. In both genders, waist circumference and sum of skin-folds were inversely associated with consumption of milk and yogurt, and milk- and yogurt-based beverages, whereas a positive association was observed with CRF. Moreover, CVD risk score ($\beta = -0.230$, $P = 0.001$) was also inversely associated with overall dairy consumption only in girls.

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Discussion: Dairy consumption is associated with lower adiposity and higher CRF in these adolescents. An inverse association between CVD risk score and dairy consumption is also depicted in girls. The study adds further evidence to the scarce literature on the influence of milk and dairy products on adolescents' cardiovascular health.

Keywords: Adolescents, body fat, cardiovascular disease risk, dairy.

Introduction

Clustering of cardiovascular disease (CVD) risk factors, including abdominal obesity, atherogenic dyslipemia, hypertension, insulin resistance and glucose intolerance, has been observed among paediatric populations (1). Early identification of clustering of risk factors, i.e. during childhood and adolescence, is needed because of the increased risk for CVD in adulthood (2). Clustering of CVD risk factors has been suggested as an adequate measure of cardiovascular health (3) given the unavailability of an appropriate criterion for the definition of cardio-metabolic risk in children and adolescents (2).

Dietary behaviours and physical activity (PA) have been associated with clustering of CVD or metabolic risk factors (4,5). Focusing on dietary behaviours, few studies have addressed the relationship between foods and CVD risk factors in adolescents (2). High consumption of fruits and vegetables and low intake of fat- and sugar-rich products are some of the components related to a decreased cardio-metabolic risk among adolescents (4–6). No distinction, however, has been made on which food groups may be associated to a higher extent to cardio-metabolic risk.

Regarding dairy consumption, there is no clear evidence of its role on CVD risk development (7). Some studies have suggested dairy consumption to have a protective effect on the development of CVD risk factors (7); among adolescents, however, there is a lack of studies addressing this association. Therefore, this study aimed (1) to identify which food groups best discriminate adolescents at higher and lower risk of CVD and (2) to investigate in depth the relationship between dairy consumption and individual risk factors and specifically computed CVD risk scores among European adolescents.

Methods

Data were obtained from the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) study ($n = 3528$), a cross-sectional multi-centre study performed in 10 European cities (Athens in Greece, Dortmund in Germany, Ghent in Belgium, Heraklion in Greece, Lille in France, Pécs in Hungary, Rome in Italy, Stockholm in Sweden, Vienna in Austria and Zaragoza in Spain) between 2006 and 2007. General procedures, characteristics and inclusion criteria of HELENA have been described in detail elsewhere (8). Adolescents and their parents gave written informed consent. The study was performed following the ethical guidelines of the Declaration of Helsinki 1964 and ethical approval was obtained by the local Ethical Committee at each study centre.

Adolescents aged 12.5–17.5 years with complete measurements on waist circumference (WC), skinfolds thickness, systolic blood pressure (SBP), triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), glucose, insulin, cardiorespiratory fitness (CRF) and two 24-hour dietary recalls (24-HDRs) were included ($n = 511$, 49.9% boys). Blood samples were drawn after an overnight fast only in one-third of the HELENA participants randomly selected, explaining partly the observed decrease in sample size of the current analysis. Eight out of the 10 study centres were included in the 24-HDR analyses because of lack of available information in Heraklion and Pécs due to logistical reasons. No significant differences ($P < 0.05$), however, were found between included and excluded participants in mean age, weight, height and body mass index (BMI).

Socioeconomic status (SES)

SES was estimated by means of the Family Affluence Scale based on the concept of material conditions in the family. A set of items was chosen to reflect family expenditure and consumption (affluence) (9).

Sedentary behaviours

The average time engaged in two sedentary behaviours (TV viewing and playing with video games) was estimated by means of a self-administered questionnaire previously found to demonstrate good reliability (10).

Physical activity

Uniaxial accelerometers (Actigraph MTI, model GT1M, Manufacturing Technology Inc., Fort Walton

Beach, FL, USA) were used to objectively measure PA (11). At least 3 d of recording with a minimum of 8 h of registration per day was set as an inclusion criterion. Time spent at moderate-to-vigorous PA (MVPA) (>3 metabolic equivalents) was calculated through the following cut-off point: ≥ 2000 counts per minute for MVPA (11).

Food consumption

Diet was assessed by the HELENA-DIAT (Dietary Assessment Tool), a self-administered computer-based tool based on the software YANA-C shown to be appropriate in assessing dietary information of European adolescents (12,13). The software consists of a single 24-HDR structured according to six meal occasions. Adolescents were asked to recall all food and drinks consumed the previous day. Two non-consecutive 24-HDRs within a time span of 2 weeks were obtained from each participant. Questionnaires were completed during school time and assisted by fieldworkers; therefore, no information on Fridays and Saturdays is available.

The German Food Code and Nutrition Data Base was used to calculate energy and nutrient intakes as it is identified the most complete food composition database across Europe in terms of nutrients and food items (14). The usual food consumption of 31 food groups (grams/day) was estimated by the multiple source method which considers the between- and within-person variability of the dietary data (15). The food group 'milk' included both milk and buttermilk and the 'yogurt, milk- & yogurt-based beverages' group included yogurt, yogurt and milk beverages like chocolate milk and probiotic beverages, and 'fromage blanc'. Cheese and milk-based desserts were considered as two separate food groups due to their different nutrient composition. No distinction on the fat content in any of the two food groups, 'milk' and 'yogurt, milk- & yogurt-based beverages' was made.

Physical examinations

Weight and height were measured in underwear and barefoot with an electronic scale (Type SECA 861) and a stadiometer (Type SECA 225). WC, taken at the midpoint between the lowest rib and the iliac crest, was measured with an anthropometric tape (SECA 200). Skin-folds thickness was measured with a Holtain Calliper (Crymych, UK) in triplicate on the left side at biceps, triceps, subscapular and supra-iliac sites. All anthropometric measures were taken following a standardized protocol (16). BMI was calculated as body weight (kg) divided by the height squared (m^2) and was categorized according to Cole *et al*. (17,18). Blood pressure was measured twice, 10 min apart, with an automatic oscillometric device (M6, HEM-7001-E, Omron Healthcare Europe B.V., Hoofddorp, The Netherlands). The lowest value was retained. Pubertal stage was assessed by a physician according to Tanner and Whitehouse (19).

Blood sampling

Blood sampling procedures have previously been described in detail (20). TG, TC, HDL-c and glucose were measured using enzymatic methods (Dade Behring, Schwalbach, Germany). Insulin levels were measured using an Immulite 200 analyzer (DPC Bierman GmbH, Bad Nauheim, Germany). The homeostasis model assessment (HOMA) index calculation was used as a measurement of insulin resistance (21) using the following formula: HOMA index = [insulin (μUI mL^{-1}) × glucose (mg dL^{-1})]/405. The ratio TC/HDL-c was computed.

Cardiorespiratory fitness

CRF was predicted using the maximum speed that an individual reached during the 20-m shuttle run test. During the test, participants ran between two lines 20 m apart following audio signals in gradual increase of speed (0.5 km h^{-1} min^{-1}). When the participant stopped due to fatigue or did not reach the line with the audio signal on two consecutive occasions, the test finished. The last completed stage or half-stage was recorded and used to calculate the maximum oxygen uptake (VO_{2max}) (mL kg^{-1} min^{-1}) by the Léger *et al*. (22) formula (boys and girls: $VO_{2max} = 31.025 + (3.238 \times S \times 3.248 \times A) + (0.1536 \times S \times A)$ (A the age, S the final speed: S = 8 + 0.5 last stage completed).

CVD risk score

A continuous score of clustered CVD risk factors was computed according to Andersen *et al*. (3) using: SBP, sum of four skin-folds (bicipital, tricipital, subscapular and supra-iliac), TG, ratio TC/HDL-c, HOMA index and CRF. Sex-specific z-scores were calculated for each risk factor variable. All individual z-scores were summed to create the clustered CVD risk score. CRF was multiplied by –1 to indicate higher CVD risk with increasing value. The lower the score the better the overall CVD risk factor profile.

Statistical analysis

Distribution of all variables was checked before the analysis. Sum of four skin-folds, WC, HOMA index,

TG, ratio HDL-c and CRF were skewed and therefore were log transformed to improve normality. Significant differences in general characteristics between genders were examined using *t*-test or Mann–Whitney *U*-test for continuous variables and chi-squared test for categorical variables.

Discriminant analysis was used to statistically distinguish participants at high and low CVD risk distribution of food consumption. Specifically, the stepwise method was used to identify the food groups that significantly distinguished participants into the two groups, separately for each CVD risk factor, with all food groups (31 food groups) entered simultaneously. In comparison to the rest of food groups, milk and yogurt, milk- & yogurt-based beverages (cheese and milk-based desserts were entered as separate food groups) showed higher standardized canonical discriminant coefficients (≥0.30) and accounted for greater between-group variability for most CVD risk factors in both genders. Further analysis was focused on these two food groups. One-way analysis of covariance was applied to assess differences in CVD risk factors across sex- and study centre-specific tertiles of overall dairy consumption. Bonferroni correction was used for *post hoc* multiple comparison test. Multiple linear regression analyses were conducted to examine the association of individual CVD risk factors and CVD risk score (dependent variables) with dairy consumption (independent variables). Pubertal maturity, SES, MVPA, sedentary behaviours and daily energy intake were used as covariates. Study centre was also entered as a covariate and the variance explained by the cluster 'centre' was less than 5%. SBP, TG, ratio TC/HDL-c and HOMA index were additionally adjusted by BMI. Predictive Analytics SoftWare (PASW, version 18; SPSS Inc., Chicago, IL, USA) was used to perform the analyses. Statistical significance was set at $P < 0.05$.

Results

Descriptive data are provided in Table 1. Boys had significantly higher weight, height, SBP, glucose, WC, CRF and milk consumption, and lower sum of four skin-folds, insulin and HOMA index than their female peers.

Results from discriminant analysis are displayed in Supporting Information Table S1. Standardized beta coefficients obtained from multiple linear regression are showed in Table 2. An inverse association was observed between milk consumption and sum of skin-folds in boys, and TG and CVD risk score in girls. In both genders, WC was inversely associated with yogurt, and milk- and yogurt-based beverages consumption, whereas a positive association was shown for CRF. Furthermore, an inverse association was found between such variable and z-BMI, sum of skin-folds and CVD risk score in girls. Milk consumption and yogurt, milk- and yogurt-based beverages consumption were summed to examine the additive effect of both variables. In boys, WC ($\beta = -0.176$, $P = 0.020$) and sum of skin-folds ($\beta = -0.154$, $P = 0.023$) were inversely associated with overall dairy consumption whereas a positive association was observed with CRF ($\beta = 0.173$, $P = 0.015$). The same associations were seen among their female peers (WC: $\beta = -0.221$, $P = 0.003$; sum of skin-folds: $\beta = -0.142$, $P = 0.046$; CRF: $\beta = 0.206$, $P = 0.004$). Moreover, z-BMI, TG, ratio TC/HDL-c and CVD risk score ($\beta = -0.230$, $P = 0.001$) were inversely associated with overall dairy consumption in girls. After controlling for multiple testing (Bonferroni method, $P \leq 0.005$), the inverse association between yogurt, milk- and yogurt-based beverages consumption, and WC and sum of skin-folds remained significant in girls. The same was true for WC, CRF and CVD risk score and overall dairy consumption among girls.

Figure 1 shows adjusted means of WC, sum of skin-folds and CVD risk score across sex- and study centre-specific tertiles of overall dairy consumption. *Post hoc* analyses revealed that adolescent girls in the lowest tertile had significantly ($P < 0.05$) higher values for WC (72.0 to 67.8 cm), sum of skin-folds (61.6 to 51.2 mm) and CVD risk score (0.63 to –0.94) than those in the high-consumption tertile. No significant results were observed in boys.

Discussion

In this sample of adolescents, dairy emerged as those food groups best discriminating adolescents at high/low CVD risk. Thereafter, the study explored the relationship between milk and yogurt, milk- and yogurt-based beverages, and CVD risk factors in European adolescents participating in the HELENA study, suggesting that higher consumption of dairy was associated with lower body fat and higher CRF in both genders. Additionally, an inverse association between clustered CVD risk score and overall dairy consumption was observed in girls. To our knowledge, this is the first study addressing these associations in adolescents.

Dairy consumption has been associated with reduced risk of CVD and metabolic syndrome in 6- to 18-year-old children and adolescents (6) and in adults (23,24). Pereira *et al*. (24) also found that dairy

Table 1 Descriptive characteristics of the study participants stratified by gender

	Boys (n = 255)		Girls (n = 256)		P-value
	Mean	SD	Mean	SD	
Age (years)	14.8	1.3	14.6	1.2	0.197
Weight (kg)	60.9	13.8	55.7	11.3	<0.001*
Height (cm)	170.0	9.6	161.8	7.3	<0.001*
BMI (kg m^{-2})	20.9	3.6	21.2	3.6	0.400
Underweight (%)§	5.9		7.8		–
Normal weight (%)	75.3		73.8		–
Overweight (%)	11.8		13.7		–
Obese (%)	7.1		4.7		–
SES (%)					<0.001‡
Low (0–2)	4.7		16.0		–
Medium (3–5)	60.2		52.3		–
High (6–8)	35.0		31.6		–
Pubertal maturity (%)					0.134
Stage 1	1.6		0.0		–
Stage 2	9.8		6.7		–
Stage 3	20.4		27.3		–
Stage 4	44.3		42.3		–
Stage 5	23.9		23.7		–
SBP (mm Hg)	119.9	13.8	112.7	11.7	<0.001*
Glucose (mg dL^{-1})	92.2	6.9	88.5	6.3	<0.001*
CVD risk score	–0.4	3.6	–0.3	3.6	0.733
	Median	25th–75th percentile	Median	25th–75th percentile	
Milk consumption (g d^{-1})	141.0	36.0–275.0	88.3	24.0–178.6	<0.001†
Yogurt, milk- and yogurt-based beverages consumption¶ (g d^{-1})	11.0	6.9–122.2	14.0	6.7–122.7	0.687
Energy consumption (kcal d^{-1})	2501	2031.1–3104.6	1891	1585.0–2325.9	<0.001†
MVPA (min d^{-1})	66.3	51.4–83.3	48.0	26.9–58.8	<0.001†
Sedentary behaviours (min d^{-1})	138.2	75.0–210.0	107.1	75.0–184.3	0.012†
WC (cm)	72.0	67.6–77.6	69.3	64.8–74.7	<0.001†
Sum of four skin-folds (mm)	35.0	26.5–55.1	54.8	42.6–75.4	<0.001†
TG (mg dL^{-1})	58.0	43.0–79.0	61.0	47.2–80.7	0.068
TC (mg dL^{-1})	152.0	134.0–168.0	166.0	146.0–183.5	<0.001†
HDL-c (mg dL^{-1})	53.0	47.0–58.0	56.0	49.0–65.0	<0.001†
Ratio TC/HDL-c	2.8	2.5–3.2	2.9	2.5–3.3	0.239
Insulin (μIU mL^{-1})	8.0	5.9–14.7	9.1	6.5–12.8	<0.001†
HOMA index	1.8	1.3–2.5	2.0	1.4–2.8	0.020†
CRF (mL kg^{-1} min^{-1})	47.1	41.4–51.4	36.8	33.3–41.2	<0.001†

*P < 0.05 by means of independent samples t-test.
†P < 0.05 by means of Mann–Whitney U-test.
‡P < 0.05 by means of chi-squared test.
§BMI categories according to Cole *et al*. (17,18).
¶Yogurt, milk- and yogurt-based beverages group includes yogurt, yogurt and milk beverages, probiotic beverages, 'fromage blanc'.
BMI, body mass index; CRF, cardiorespiratory fitness; CVD, cardiovascular disease; HDL-c, high-density lipoprotein cholesterol; HOMA, homeostasis model assessment; MVPA, moderate-to-vigorous physical activity; SBP, systolic blood pressure; SES, socioeconomic status; TC, total cholesterol; TG, triglycerides; WC, waist circumference.
SES: Socioeconomic status based on FAS. Pubertal maturity based on Tanner stage.

consumption was inversely associated with the incidence of all CVD risk factors, i.e. obesity, abnormal glucose homeostasis, hypertension and dyslipemia in overweight black and white men and women. We also observed inverse associations with individual CVD risk factors, i.e. z-BMI, WC and sum of skin-folds in both genders, and TG and ratio TC/HDL-c in girls.

Table 2 Standardized regression coefficients examining the association of milk and yogurt, milk- and yogurt-based beverages consumption with the individual CVD risk factors, and the clustered CVD risk score

	Milk (g d^{-1})		Yogurt, milk- and yogurt-based beverages† (g d^{-1})		Overall dairy consumption‡ (g d^{-1})	
	β§	*P*-value	β§	*P*-value	β§	*P*-value
Boys						
z-BMI	–0.096	0.192	–0.065	0.368	–0.107	0.148
WC	–0.132	0.084	–0.158*	0.035	–0.176*	0.020
Sum of four skin-folds	–0.144*	0.034	–0.086	0.199	–0.154*	0.023
SBP	–0.009	0.895	–0.001	0.983	–0.008	0.908
TG	0.049	0.505	0.034	0.465	0.055	0.458
Ratio TC/HDL-c	0.017	0.823	0.090	0.226	0.054	0.478
HOMA index	0.056	0.400	0.078	0.231	0.080	0.228
CRF	0.133	0.062	0.148*	0.034	0.173*	0.015
CVD risk score	–0.059	0.385	–0.008	0.903	–0.052	0.450
Girls						
z-BMI	–0.060	0.407	–0.156*	0.030	–0.156*	0.034
WC	–0.093	0.211	–0.212*	0.004	–0.221*	0.003
Sum of four skin-folds	0.017	0.805	–0.235*	0.001	–0.142*	0.046
SBP	0.012	0.865	0.025	0.726	0.027	0.709
TG	–0.165*	0.024	–0.024	0.743	–0.162*	0.032
Ratio TC/HDL-c	–0.138	0.052	–0.054	0.455	–0.157*	0.031
HOMA index	–0.103	0.141	–0.005	0.939	–0.095	0.190
CRF	0.099	0.166	0.181*	0.010	0.206*	0.004
CVD risk score	–0.140*	0.041	–0.165*	0.015	–0.230*	0.001

*$P < 0.05$.
†Yogurt, milk- and yogurt-based beverages group includes yogurt, yogurt and milk beverages, probiotic beverages, 'fromage blanc'.
‡Overall dairy consumption includes milk consumption and yogurt, milk- and yogurt-based beverages consumption (yogurt, yogurt and milk beverages, probiotic beverages, 'fromage blanc').
§Adjusted by Tanner status, centre, SES, moderate-to-vigorous physical activity, sedentary behaviours and total energy intake. SBP, TG, ratio TC/HDL-c and HOMA index were additionally adjusted by BMI.
BMI, body mass index; CRF, cardiorespiratory fitness; CVD, cardiovascular disease; HDL-c, high-density lipoprotein cholesterol; HOMA, homeostasis model assessment; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; WC, waist circumference.

Observational studies have inversely associated dairy consumption with body weight and body fat in children and adolescents (25–28). In accordance with our findings, US adolescents (28) consuming more servings of dairy per day had about 5 mm (girls) and 3.4 mm (boys) less subcutaneous body fat, calculated via their sum of two skin-folds, and lower z-BMI compared with those consuming less. Abreu *et al*. (25) also observed that milk consumption was inversely related to body fat percentage ($\beta = -0.143$, $P = 0.030$) and BMI ($\beta = -0.167$, $P = 0.013$) in girls. Nevertheless, we found an inverse association between sum of skin-folds and milk consumption only in boys. WC was not included in the clustered CVD risk score, but we also examined its association with dairy consumption. Our results were in agreement with other studies which reported an inverse association between dairy consumption and WC in adolescents (26,27), which may be translated into a lower risk of metabolic syndrome among these subjects. Although available data on diet-CRF associations are limited, in a previous report of HELENA adolescents ($n = 1492$), CRF was consistently associated with high milk and dairy products consumption in both boys and girls (29).

CVD risk score and dairy consumption were significantly associated in girls but not in boys. Although differences were not always significant, girls showed worse values for most of the individual CVD risk factors, i.e. higher sum of skin-folds, TG, ratio TC/HDL-c, HOMA and lower CRF than boys. This leads authors to suggest that the association between milk and dairy products and CVD risk score may depend on the initial status of individual CVD risk factors. Other authors (25) have hypothesized that the interactions between dietary calcium (and milk and dairy products) and body fat may differ across different thresholds of percentage of body fat. It might be applicable to the remaining CVD risk factors, which would partially explain the inverse

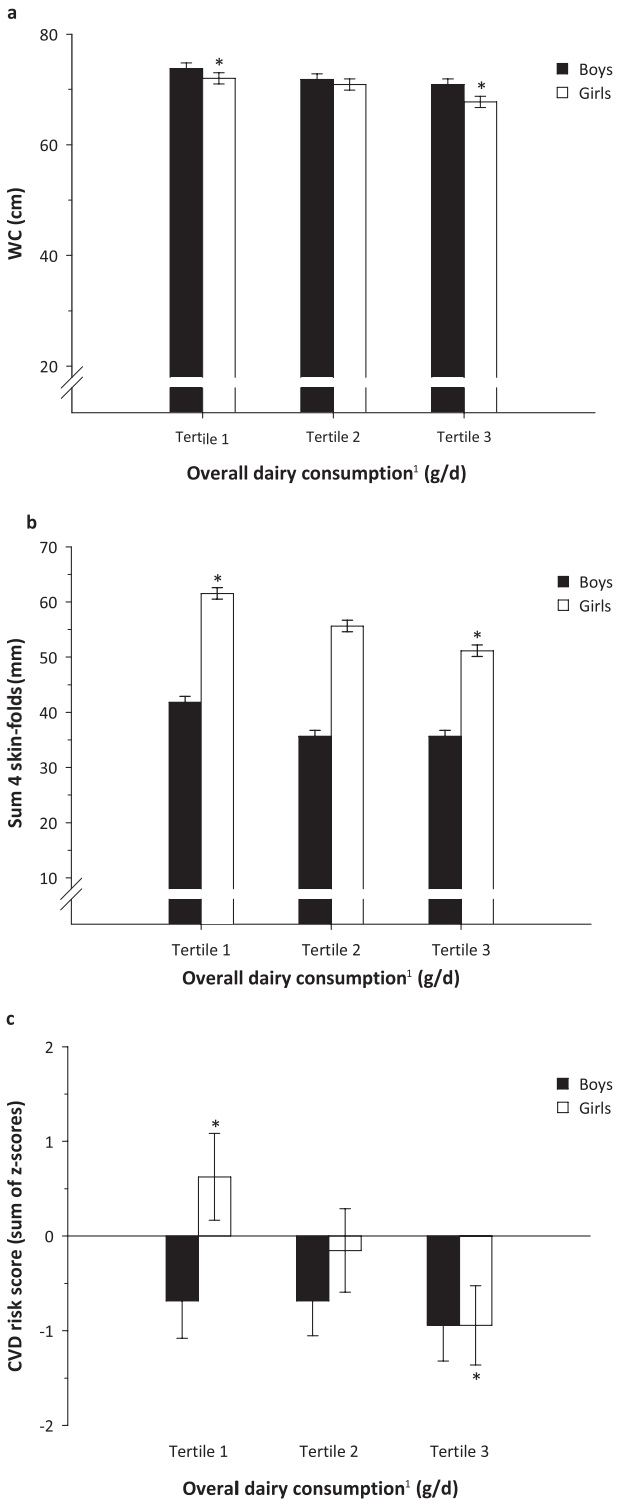

Figure 1 Mean (SE) WC (a), sum of four skin-folds (b), and CVD risk score (c) by tertiles of overall dairy consumption.
Black bars = boys; white bars = girls.
*$P < 0.05$ across tertiles of combined milk and yogurt, milk- and yogurt-based beverages consumption after Bonferroni correction for *post hoc* multiple comparisons. Covariates: pubertal maturity, study centre, SES, sedentary behaviours, moderate-to-vigorous physical activity and daily energy intake.
[1]Overall dairy consumption includes milk consumption and yogurt, milk- and yogurt-based beverages consumption (includes yogurt, yogurt and milk beverages, probiotic beverages, 'fromage blanc').
Values have been back-transformed by raising 10 to the power (unlog) to make easier interpretability.
Median overall dairy consumption:
Tertile 1: 51.4 g d^{-1} (boys), 40.9 g d^{-1} (girls)
Tertile 2: 225.9 g d^{-1} (boys), 166.1 g d^{-1} (girls)
Tertile 3: 432.8 g d^{-1} (boys), 343.5 g d^{-1} (girls)
CVD, cardiovascular disease; WC, waist circumference.

association found between TG and ratio TC/HDL-c and dairy consumption in girls but not in boys.

The potential benefits of dairy products could be due to multiple dairy components. Bioactive peptides derived from milk-based proteins seem to be involved in several mechanisms such as satiety response, regulation of insulinemia and blood pressure, uptake of free radicals, and lipid profile abnormalities (30). Findings on the effect of dairy fat on CVD risk factors are not consistent given the diverse composition of dairy foods. Milk fat has been shown to raise serum HDL-c (31), and fermented dairy foods may lower plasma cholesterol (7). Additionally, calcium, magnesium and potassium content of dairy foods seems to have positive effects on CVD risk factors (23,24). Dairy calcium has been inversely related to hypertension and a lower 9-year increase in plasma TG levels (23) as well as to body fat through its effect on lipogenesis (32). These results are of great interest because girls can engage in unhealthy weight-control behaviours, resulting in a decreased consumption of calcium and other micronutrients (33).

The cross-sectional design of this study does not allow us to draw causal associations. Although dietary assessment is subject to measurement errors, the method has been shown to be appropriate in collecting detailed dietary information in adolescents (12,13). More than two measurements would have been desirable to compensate for day-to-day variation and to estimate usual dietary intake more accurately; however, dietary intake was corrected for between- and within-person variability to mitigate in part such limitation (34). No dietary measurements were collected on Fridays and Saturdays. Energy intake tends to increase on weekends (35); for that reason, collection of these data would have been desirable to capture the apparent modification on dietary habits.

A continuous CVD risk score was computed using risk factors related to the metabolic syndrome. The clustered CVD risk score has been shown as a better measure of cardiovascular health in children and adolescents rather than single risk factors (3). Additionally, the score partially compensates for day-to-day fluctuations quite common when considering only single risk factors (3).

In conclusion, these findings showed a positive role of milk and dairy products consumption on adiposity and CRF, and as a result, on clustered CVD risk score in adolescents. Intervention strategies aiming at preventing obesity and CVD risk in adolescents should promote regular dairy consumption as part of a healthy diet. This study adds evidence to the limited literature about the influence of dairy products consumption on adolescents' CVD health; a dietary component often understudied but related to a number of chronic diseases.

Conflict of interest statement

No conflicts of interest were declared.

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FG, MGG, JD, AK, YM, PS, MK, SDH, MJC, DM, KW, AM and LAM designed and directed the study; SBS, DJP, IH, MCG, LM and LH conducted research; SBS wrote the manuscript, analyzed the data and generated figures; TM, DJP and LAM participated in data interpretation. SBS, TM, DJP, IH, MCG, LM, FG, MGG, JD, AK, YM, PS, MK, SDH, MJC, LH, DM, KW, AM and LAM critically reviewed the manuscript. All authors read and approved the final manuscript.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Summary of interpretative measures for stepwise two-group discriminant analysis of study participants at high and low risk of CVD risk factors and clustered CVD risk score.

Appendix

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Table S1. Summary of interpretative measures for stepwise two-group discriminant analysis of study participants at high and low risk of CVD risk factors and clustered CVD risk score.

	Males		Females	
	Univariate F ratio*	**Standardized canonical coefficients†**	**Univariate F ratio**	**Standardized canonical coefficients**
WC				
Sweet bakery products (g/day)	5	0.655	-	-
Soups & bouillon (g/day)	3	0.514	-	-
Yogurt, milk- & yogurt-based beverages[1] (g/day)	9	0.748	11	1.000
Sum 4 skinfolds				
Sweet bakery products (g/day)	16	0.564		
Snacks (g/day)	13	0.427	9	0.478
Sugar, honey & jam (g/day)	5	0.329	-	-
Chocolate (g/day)	13	0.337	-	-
Alcoholic beverages (g/day)	3	0.353	-	-
Milk (g/day)	6	0.425	-	-
Pasta, rice & other cereals (g/day)	-	-	4	0.610
Confectionery non-chocolate products (g/day)	-	-	9	0.469
Vegetable oils (g/day)	-	-	1	-0.412
Fruit & vegetable juices (g/day)	-	-	4	0.378
Eggs (g/day)	-	-	4	0.329
Yogurt, milk- & yogurt-based beverages (g/day)	-	-	11	0.501
SBP				
Sweet bakery products (g/day)	9	0.716	8	0.559
Chocolate (g/day)	4	-0.453	-	-
Milk (g/day)	5	0.470	-	-
Cheese (g/day)	4	-0.462	-	-
Margarine (g/day)	-	-	10	-0.625
Soups & bouillon (g/day)	-	-	8	0.587
TG				
Potatoes & starch roots (g/day)	7	0.787	-	-
Fruit (g/day)	4	0.618	-	-
Bread and rolls (g/day)	-	-	7	0.824
Milk (g/day)	-	-	4	-0.666
Ratio TC/HDL-c				
Sauces (g/day)	5	0.745	-	-
Meat (g/day)	4	-0.725	-	-
Sweet bakery products (g/day)	-	-	9	0.845
Butter & animal fats (g/day)	-	-	1	0.555
Fruit (g/day)	-	-	3	-0.671
HOMA index				
Snacks (g/day)	3	0.464	-	-
Butter & animal fats (g/day)	12	0.809	-	-
Coffee & tea (g/day)	6	0.549	-	-
Vegetable oils (g/day)	-	-	4	0.609
Fruit & vegetable juices (g/day)	-	-	7	0.799
CRF				
Sweet bakery products (g/day)	5	0.586	-	-
Vegetable oils (g/day)	14	-0.717	-	-
Milk (g/day)	11	0.512	-	-
Sugar, honey & jam (g/day)	-	-	2	-0.381
Confectionery non-chocolate products (g/day)	-	-	10	0.531
Pulses (g/day)	-	-	1	-0.337
Soup (g/day)	-	-	7	0.407
Eggs (g/day)	-	-	6	0.604

Yogurt, milk- & yogurt-based beverages (g/day)	-	-	6	0.472
Milk based desserts (g/day)	-	-	1	-0.430
Meat substitutes & vegetarian products (g/day)	-	-	3	-0.342
CVD risk score				
Chocolate (g/day)	8	0.793	-	-
Milk (g/day)	6	0.699	-	-
Sweet bakery products (g/day)	-	-	10	0.567
Eggs (g/day)	-	-	9	0.576
Yogurt, milk- & yogurt-based beverages (g/day)	-	-	7	0.569

SBP, systolic blood pressure; WC, waist circumference; HOMA, homeostasis model assessment; TG, triglycerides; TC, total cholesterol; HDL-c, high density lipoprotein cholesterol; CRF, cardiorespiratory fitness; CVD, cardiovascular diseases.

*Univariate F ratio: ratio of between-groups variability to the within-groups variability

†Standardized canonical coefficients: partial contribution (discriminating power) of each variable to the discriminate function controlling for all other variables in the equation.

[1]Yogurt, milk- & yogurt-based beverages group includes yogurt, yogurt and milk beverages, probiotic beverages, “fromage blanc”.

Artículo VII [Paper VII]:

Clustering of multiple lifestyle behaviours and its association to cardiovascular risk factors in children: the IDEFICS study

Bel-Serrat S, Mouratidou T, Santaliestra-Pasías AM, Iacoviello L, Kourides YA, Marild S, Molnár D, Reisch L, Siani A, Stomfai S, Vanaelst B, Veidebaum T, Pigeot I, Ahrens W, Krogh V, Moreno LA.

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ORIGINAL ARTICLE

Clustering of multiple lifestyle behaviours and its association to cardiovascular risk factors in children: the IDEFICS study

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BACKGROUND/OBJECTIVES: Individual lifestyle behaviours have independently been associated with cardiovascular diseases (CVD) risk factors in children. This study aimed to identify clustered lifestyle behaviours (dietary, physical activity (PA) and sedentary indicators) and to examine their association with CVD risk factors in children aged 2–9 years.

SUBJECTS/METHODS: Participants included 4619 children (51.6% boys) from eight European countries participating in the IDEFICS cross-sectional baseline survey (2007–2008). Insulin resistance, total cholesterol/high-density lipoprotein cholesterol ratio, triglycerides, sum of two skinfolds and systolic blood pressure (SBP) *z*-scores were summed to compute a CVD risk score. Cluster analyses stratified by sex and age groups (2 to <6 years; 6–9 years) were performed using parental-reported data on fruit, vegetables and sugar-sweetened beverages (SSB) consumption, PA performance and television video/DVD viewing.

RESULTS: Five clusters were identified. Associations between CVD risk factors and score, and clusters were obtained by multiple linear regression using cluster 5 ('low beverages consumption and low sedentary') as the reference cluster. SBP was positively associated with clusters 1 ('physically active'; $\beta = 1.34$; 95% confidence interval (CI): 0.02, 2.67), 2 ('sedentary'; $\beta = 1.84$; 95% CI: 0.57, 3.11), 3 ('physically active and sedentary'; $\beta = 1.45$; 95% CI: 0.15, 2.75) and 4 ('healthy diet'; $\beta = 1.83$; 95% CI: 0.50, 3.17) in older boys. A positive association was observed between CVD risk score and clusters 2 ($\beta = 0.60$; 95% CI: 0.20, 1.01), 3 ($\beta = 0.55$; 95% CI: 0.14, 0.97) and 4 ($\beta = 0.60$, 95% CI: 0.18, 1.02) in older boys.

CONCLUSIONS: Low television/video/DVD viewing levels and low SSB consumption may result in a healthier CVD profile rather than having a diet rich in fruits and vegetables or being physically active in (pre-)school children.

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Keywords: cardiovascular diseases; lifestyle; diet; exercise; sedentary lifestyle; child

INTRODUCTION

Increased risk of CVD is characterized by a cluster of metabolic abnormalities, such as abdominal obesity, atherogenic dyslipemia, hypertension, insulin resistance and glucose intolerance.[1] CVD remains the leading cause of mortality in both middle- and high-income countries[2] in parallel to the onset of atherosclerosis and related risk factors occurring early in childhood.[3,4] This makes an early diagnosis and implementation of guidelines for primary prevention essential in young populations.[3] In view of no universally accepted paediatric definition of metabolic syndrome in children, clustering of CVD risk factors has been shown as an appropriate measure of cardiovascular health in young populations.[5]

Individual observations of several lifestyle behaviours, that is, dietary habits, physical activity (PA) or sedentary behaviours, have separately been associated with clustering of CVD or metabolic risk factors. For instance, high fruits and vegetables (F&V) and low sugar-sweetened beverages (SSB) consumption has inversely been associated with metabolic risk and/or individual risk factors in children.[6,7] Similarly, PA levels have also been inversely associated with metabolic risk factors during childhood.[8,9] This inverse relationship was stronger for moderate and vigorous PA levels than for light or sedentary activity levels.[10] Moreover, sedentary behaviours such as excessive television (TV) viewing or computer use have been linked to increased risk of CVD in children,[11,12] regardless of PA levels.[13,14] In addition, and given the contradictory findings on PA and eating habits,[15] cluster analysis, referring to the co-occurrence of several lifestyle behaviours, might offer an alternative to explore their relationship to several chronic diseases, including CVD in children.

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Author Contributions: LAM, VK, WA, IP, SM, DM, AS, LI, LR and TV designed and directed the study; SBS, AMSP, YAK, SS and BV conducted research; SBS wrote the manuscript; SBS, TM, AMSP and LAM analysed the data; TM and LAM participated in data interpretation; SBS had primary responsibility for final content. SBS, TM, AMSP, LAM, VK, WA, IP, SM, DM, AS, LI, LR, TV, YAK, SS and BV critically reviewed the manuscript. All authors read and approved the final manuscript.

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To improve the understanding of the association between co-occurrence of lifestyle behaviours and the onset of CVD risk factors in children, the present study aimed (1) to identify clustered lifestyle behaviours based on dietary, PA and sedentary-selected indicators and (2) to examine the relationship between individual risk factors and defined clusters, and a specifically computed CVD risk score in a large sample of European children.

SUBJECTS AND METHODS

Study population

The baseline survey (2007–2008) of the IDEFICS (Identification and prevention of Dietary and lifestyle-induced health Effects in children and InfantS) project was carried out in eight European countries: Italy, Estonia, Cyprus, Belgium, Sweden, Germany, Hungary and Spain, in children aged 2–9 years. A total of 16 224 children (51% of those invited) met the IDEFICS inclusion criteria: complete data on weight, height, age and sex. More details about the study procedures have been published elsewhere.[16]

Those subjects with complete measurements on triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), glucose, insulin, systolic blood pressure (SBP), and complete data on F&V, SSB consumption, PA performance and self-reported TV/video/DVD viewing were included ($n = 4619$; 51.6% boys). The study sample differed with respect to mean age, height and weight from the total IDEFICS sample, as participants were older, taller and heavier ($P < 0.05$); however, no significant differences were observed for mean *z*-body mass index.

All procedures involving human subjects/patients were approved by the Ethics Committee at each study centre. Written informed consent was obtained by the parents of all participating children.

Measurements

Socioeconomic status. Socioeconomic status was estimated by means of the International Standard Classification of Education.[17] The maximum International Standard Classification of Education level of both parents was considered.

F&V and SSB consumption. F&V and SSB consumption was obtained by means of the validated food frequency section of the Children's Eating Habits Questionnaire.[18–20] The questionnaire was completed by parents, querying on the number of times the child had eaten or drunk food items included in the questionnaire during a typical week in the previous month. Responses included eight frequency categories of consumption: 'never/less than once a week', '1–3 times per week', '4–6 times per week', 'once a day', 'twice a day', '3 times per day', '$\geqslant$4 times per day' and 'I have no idea'. Frequencies were converted into times per week ranging from 0 to 30. From the 43 food groups initially included in the questionnaire, we only used: cooked vegetables, potatoes and beans; raw vegetables; fresh fruits and sugar-sweetened drinks, on the basis of the fact that only these food groups were included within the IDEFICS intervention key messages. According to the purposes of the present analysis, vegetables and fruit responses were summed into one group.

Physical activity. Information on PA was assessed by means of a self-administered parentally reported questionnaire querying, 'Is your child member in a sports club?', with response category (i) yes and (ii) no. If 'yes', they were additionally asked, 'How much time (in hours) does he/she spend doing sport in a sports club per week?'. A 'no' response was coded as 0 h spent doing sports. This question significantly correlated with children's daily time spent in moderate-to-vigorous activity, as measured by accelerometer within this sample of children (Spearman $r = 0.18$, $P < 0.01$). All children participating in the IDEFICS study, regardless of sex, age or country, were asked to wear accelerometers for at least 3 consecutive days (2 weekdays and 1 weekend day), unless they had any physical handicap or constrained movement. The device was worn at the right hip underneath the clothes and during waking hours. From the initial IDEFICS study sample ($n = 16\,224$), a total of 7451 (45.9%) children had complete and valid accelerometer data (minimum of 3 recording days). In the present study sample, accelerometer data was available only in 2621 (56.7%) children.

Sedentary behaviours. Parents were asked via questionnaire, 'How long does your child usually watch TV/video/DVD per day?'. Responses were split into weekdays and weekends, and included five categories: 'not at all', '<30 min per day', '<1 h per day', '1–2 h per day', '2–3 h per day' and '>3 h per day'. The average hours per week of TV/video/DVD viewing was calculated as follows: ((weekdays $\times$ 5) + (weekends $\times$ 2))/7.

Physical examinations. Weight and height were measured in light underwear and in bare feet, with an electronic scale (Tanita BC 420 SMA, Tokyo, Japan) and a stadiometer (Seca 225, Seca Ltd., Birmingham, UK), respectively. Body mass index was calculated as body weight (kg) divided by the height squared (m^2). Skinfolds thicknesses were measured twice with a Holtain calliper (Holtain Ltd., Croswell, UK) at the triceps and subscapular sites, and the mean was used for the analysis. Blood pressure was measured with an electronic sphygmomanometer (Welch Allyn 4200B-E2, Welch Allyn Inc., Skaneateles Falls, NY, USA)[21] in the right arm, with the child in sitting position. Two measurements were taken at 2 min intervals. Differences higher than 5% of magnitude lead to a third measurement. Means of replicate measurements were used in all analyses.

Biological samples. Blood sampling procedures have been described previously elsewhere.[22] Blood sampling was performed after an overnight-fast. Blood glucose, TC, HDL-c and TG were assessed at each study centre by point-of-care analysis (Cholestech LDX analyzer, Cholestech Corp., Hayward, CA, USA).[23] Serum insulin concentrations were determined by luminescence immunoassay Immulite 2000 (Siemens, Eschborn, Germany) in a central laboratory. Insulin resistance was defined by the homoeostasis model assessment (HOMA)[24] and calculated from fasting glucose and plasma insulin via a standard formula: HOMA = (insulin (μIU/ml) $\times$ glucose (mg/dl))/405. The TC/HDL-c ratio was computed.

CVD risk score. A continuous score of clustered CVD risk factors was computed according to Andersen *et al.*[8]: SBP, TG, ratio TC/HDL-c, HOMA and sum of two skinfolds. Age- and sex-specific *z*-scores were calculated for each risk factor, considering children younger than 6 years old (2 to <6 years) as one group and older than 6 years (6–9 years) as the second group. All individual *z*-scores were added up to create the clustered CVD risk score. The lower the risk score the more the overall cardiovascular profile improved.

Statistical analysis

All analyses were performed using the Predictive Analytics SoftWare (PASW, version 18; SPSS Inc., Chicago, IL, USA) stratified by age and sex. Sum of two skinfolds, HOMA, ratio TC/HDL-c and TG concentrations were transformed by natural logarithm because of their skewed distribution.

Cluster analysis was performed to create age group- and sex-specific lifestyle behaviours. F&V and SSB consumption, PA and TV/video/DVD viewing were included as indicators and were standardized because of the variation in means and variances among them.[25] The analysis was split into two steps in which a combination of hierarchical and non-hierarchical clustering methods was applied.[26] In the first step, Ward's method was used based on squared Euclidean distances[27] as hierarchical cluster analysis. To reduce the high sensitivity of Ward's method to outliers, univariate outliers (values >3 s.d. above or below the mean) and multivariate outliers (those with high values of the Mahalanobis distance) for any of the four variables tested were removed ($n = 444$). In the second step, an iterative non-hierarchical *K*-means clustering procedure was applied in which initial cluster centres based on Ward's hierarchical method were used as nonrandom starting points.

As instability of the results could be a limitation of cluster analysis, the third step was to test the stability of cluster solution. The sample was randomly split into two subsamples in which the clustering procedure was repeated. The degree of agreement was calculated by comparing the new clusters obtained in both subsamples with those of the total sample by means of Cohen's Kappa (κ). Agreement was excellent, ranging from 0.904 to 0.963.[28]

Analysis of variance and analysis of covariance were performed to describe clusters characteristics. Bonferroni correction was used for *post hoc* multiple comparisons test. Multiple linear regression models were carried out to examine the association of clusters (independent variables) with the individual risk factors and CVD risk score (dependent variables). Clusters were converted into dummy variables. 'Low beverages and low sedentary' cluster was chosen as the reference, as it showed the lowest values for most individual risk factors and CVD risk score within the age- and sex-specific groups. All analyses were adjusted for parental socio-economic status and study centre.

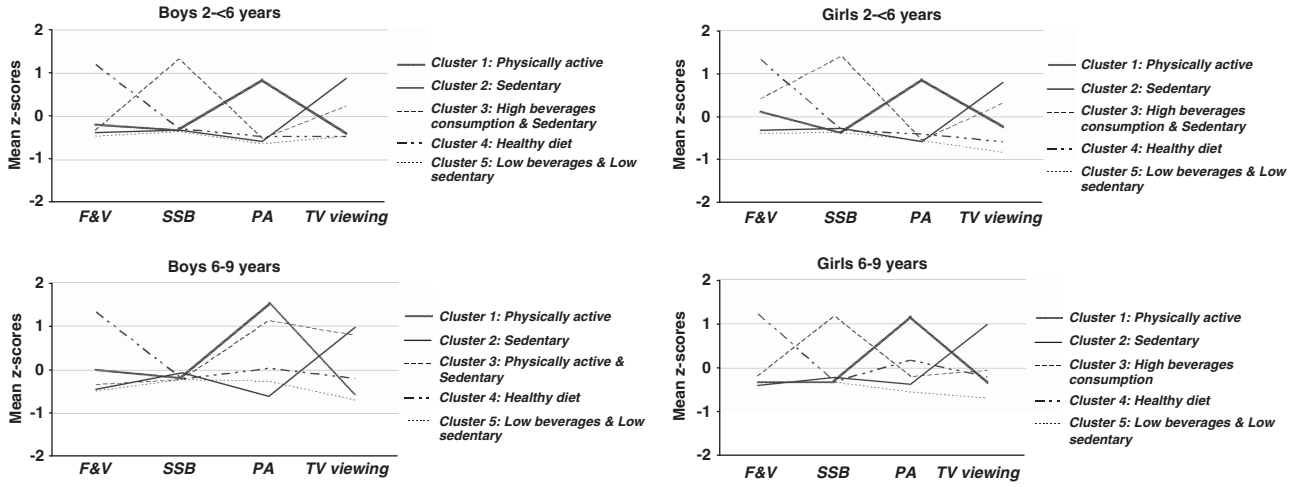

Figure 1. Age- and sex-specific cluster solutions according to mean *z*-scores on lifestyle indicators

Table 1. Differences in lifestyle indicators, individual risk factors and CVD risk score by identified clusters in 2 to <6-year-old children

	2 to <6 years old									
	Physically active (C1) n = *97*		*Sedentary (C2)* n = *257*		*High beverage consumption and sedentary (C3)* n = *56*		*Healthy diet (C4)* n = *201*		*Low beverage consumption and low sedentary (C5)* n = *316*	
Boys	*Mean*	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*
Age (years)	5.0	0.7[a,b,c,d]	4.6	0.8[a,e]	4.3	0.7[b]	4.4	0.8[c,f]	4.2	0.9[d,e,f]
z-BMI	0.1	1.2	0.1	1.2	−0.2	0.9	−0.1	0.8	−0.0	0.9
F&V (times per week)	27.4	10.9[a,b,c,d]	23.5	12.3[a,g]	24.4	11.3[h]	54.6	11.7[c,f,g,h]	21.9	9.2[d,f]
SSB (times per week)	1.0	1.8[b]	1.0	1.6[i]	10.9	3.5[b,h,i,j]	1.7	2.1[h]	0.7	1.4[j]
PA (hours per week)	2.4	0.9[a,b,c,d]	0.2	0.5[a,g]	0.3	0.6[b]	0.4	0.7[c,f,g]	0.1	0.3[d,f]
TV viewing (hours per week)	7.0	3.0[a,b,d]	13.3	3.1[a,e,g,i]	10.2	3.3[b,h,i,j]	6.7	3.5[f,g,h]	5.3	2.3[d,e,f,j]
Sum two skinfolds	15.4	1.3	15.4	1.3	14.9	1.3	14.7	1.3	15.0	1.2
SBP (mm Hg)	96.5	8.0	97.1	8.6	96.7	8.0	96.4	8.0	96.4	8.4
HOMA index	0.57	2.30[d]	0.49	2.31	0.52	2.32	0.45	2.22	0.45	2.26[d]
Ratio TC/HDL-C	3.2	1.3	3.2	1.4	3.3	1.3	3.2	1.3	3.3	1.4
TG	40.8	1.7	34.5	1.6	35.6	1.7	35.8	1.6	36.1	1.6
CVD risk score	0.8	2.7	1.2	2.6	1.2	2.8	1.5	2.4	1.4	2.5
	Physically active (C1) n = *97*		*Sedentary (C2)* n = *236*		*High beverage consumption and sedentary (C3)* n = *45*		*Healthy diet (C4)* n = *141*		*Low beverage consumption and low sedentary (C5)* n = *327*	
Girls	*Mean*	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*
Age (years)	4.8	0.7[a,c,d]	4.4	0.8[a]	4.5	0.8	4.2	0.9[c]	4.3	0.8[d]
z-BMI	−0.1	1.0	0.1	1.0	−0.1	1.1	0.0	0.9	−0.1	1.0
F&V (times per week)	33.1	13.0[a,b,c,d]	24.7	11.8[a,g,i]	38.8	16.4[b,h,i,j]	56.8	10.8[c,f,g,h]	23.1	9.3[d,f,j]
SSB (times per week)	0.5	1.3[a,b]	1.2	1.8[a,e,i]	11.3	3.5[b,h,i,j]	1.0	1.7[h]	0.7	1.5[e,j]
PA (hours per week)	2.4	0.8[a,b,c,d]	0.2	0.4[a,g]	0.2	0.6[b]	0.5	0.8[c,f,g]	0.2	0.5[d,f]
TV viewing (hours per week)	7.7	3.6[a,b,c,d]	12.9	3.0[a,e,g,i]	10.5	3.9[b,h,i,j]	9.1	3.2[c,f,g,h]	4.8	2.2[d,f,g,j]
Sum two skinfolds	17.9	1.3	17.2	1.3	16.6	1.4	16.9	1.3	16.4	1.3
SBP (mm Hg)	97.4	7.9	96.8	7.8	95.3	8.8	97.8	8.3	96.3	8.4
HOMA index	0.54	2.04	0.58	2.19	0.65	2.32	0.48	2.36	0.53	2.13
Ratio TC/HDL-C	3.3	1.4	3.5	1.4	3.6	1.4	3.4	1.3	3.5	1.4
TG	42.1	1.5	33.9	1.6	37.0	1.6	36.3	1.6	36.8	1.7
CVD risk score	−0.1	2.4	−0.5	−2.6	−0.6	2.7	−0.6	2.7	−0.6	2.7

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; HDL-c, high-density lipoprotein cholesterol; HOMA, homoeostatic assessment model; PA, physical activity; SBP, systolic blood pressure; SSB, sugar-sweetened beverages; TC, total cholesterol; TG, triglycerides. Values in the same row with common superscript letters are significantly different ($P<0.05$) after Bonferroni correction for *post hoc* multiple comparisons. [a]$P<0.05$ between C1 and C2. [b]$P<0.05$ between C1 and C3. [c]$P<0.05$ between C1 and C4. [d]$P<0.05$ between C1 and C5. [e]$P<0.05$ between C2 and C5. [f]$P<0.05$ between C4 and C5. [g]$P<0.05$ between C2 and C4. [h]$P<0.05$ between C3 and C4. [i]$P<0.05$ between C2 and C3. [j]$P<0.05$ between C3 and C5.

RESULTS

Cluster analysis resulted in a five cluster solution as the most suitable to define CVD risk-related behaviours in the four age- and sex-specific groups. Distinguishing characteristics of each cluster were indicated by high or low *z*-scores, and are shown in Figure 1. Each cluster was named according to its predominant characteristics.

Results from analysis of variance are reported by sex and age group in Tables 1 and 2. Cluster 1 (C1, 'physically active') comprised subjects with high levels of PA whereas cluster 2 (C2, 'sedentary') was characterized by high levels of TV/video/DVD viewing. The characteristics of cluster 3 (C3) varied by age group. The 'high beverage consumption and sedentary' cluster among children 2 to <6 years was described by high levels of SSB consumption and TV/video/DVD viewing. C3 ('physically active and sedentary') in older boys included subjects with high levels of PA and TV video/DVD viewing. Older girls in C3 ('high beverage consumption') presented the highest mean consumption of SSB. High F&V and low SSB consumption characterized cluster 4 (C4, 'healthy diet') in all children. Children within cluster 5 (C5, 'low beverages consumption and low sedentary') were characterized by low levels of SSB consumption and TV/video/DVD viewing, as well as low PA levels and F&V consumption. No significant differences in body mass index were observed among clusters for any age- and sex-specific group.

Means and s.d. for individual risk factors and CVD risk score across clusters are presented in Tables 1 and 2, separately by sex and age group. Results of multiple linear regression analysis are shown in Tables 3 and 4 after adjustment for covariates. In younger boys, a positive relationship between HOMA ($\beta = 0.28$, 95% confidence interval (CI): 0.09–0.46) and C1 was observed. The same was true for C2 in older girls ($\beta = 0.14$, 95% CI: 0.05–0.24) and C3 ($\beta = 0.19$, 95% CI: 0.08–0.29) in older boys. Sum of two skinfolds and SBP were positively associated with C2 ($\beta = 0.06$, 95% CI: 0.01–0.10) and C4 ($\beta = 1.70$, 95% CI: 0.10–3.30), respectively, in younger girls. A positive association was seen between sum of two skinfolds and the 'sedentary' cluster ($\beta = 0.08$, 95% CI: 0.02–0.13); and ratio TC/HDL-c and 'healthy

Table 2. Differences in lifestyle indicators, individual risk factors and CVD risk score by identified clusters in 6–9-year-old children

	6–9 years old									
	Physically active (C1) n = *257*		*Sedentary (C2)* n = *301*		*Physically active and sedentary (C3)* n = *271*		*Healthy diet (C4)* n = *257*		*Low beverage consumption and low sedentary (C5)* n = *370*	
Boys	*Mean*	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*
Age (years)	7.6	0.8	7.4	0.9[a]	7.7	0.8[a,b,c]	7.4	0.8[c]	7.4	0.8[b]
z-BMI	−0.1	0.8[d]	0.1	1.2[d]	0.0	0.9	−0.1	1.0	−0.0	1.0
F&V (times per week)	31.0	12.5[d,e,f,g]	22.1	12.3[d,h]	23.9	12.3[e,c,b]	56.8	11.8[f,h,c,i]	21.1	9.3[g,b,i]
SSB (times per week)	1.7	2.9[d]	2.5	3.4[d,a,h,j]	1.5	2.3[a]	1.6	2.6[h]	1.5	2.7[j]
PA (hours per week)	3.4	0.9[d,e,f,g]	0.2	0.4[d,a,h,j]	2.8	0.9[e,a,c,b]	1.1	1.1[f,h,c,i]	0.7	0.8[g,j,b,i]
TV viewing (hours per week)	6.3	2.1[d,e,f,g]	13.8	3.0[d,a,h,j]	12.9	2.9[e,a,c,b]	8.0	3.4[f,h,c,i]	5.5	2.2[g,j,b,i]
Sum two skinfolds	16.0	1.4	17.6	1.5	16.9	1.4	15.8	1.5	15.9	1.5
SBP (mm Hg)	103.0	9.0	103.8	8.5[h]	103.1	8.0	102.9	8.8	101.7	8.8[h]
HOMA index	0.75	1.99[e]	0.89	2.01	0.92	1.96[e,b]	0.79	2.08	0.79	2.02[b]
Ratio TC/HDL-C	2.9	1.4	3.0	1.3	2.9	1.3	3.0	1.3	2.9	1.3
TG	36.9	1.6	35.0	1.6	35.4	1.6	36.6	1.6	34.1	1.6
CVD risk score	48.9	3.3[d]	47.2	2.6[d]	48.2	3.3	48.0	2.7	47.8	2.9
	Physically active (C1) n = *301*		*Sedentary (C2)* n = *353*		*High beverage consumption (C3)* n = *94*		*Healthy diet (C4)* n = *243*		*Low beverage consumption and low sedentary (C5)* n = *399*	
Girls	*Mean*	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*
Age (years)	7.6	0.8	7.5	0.8	7.6	0.7	7.5	0.8	7.5	0.8
z-BMI	0.0	1.0	0.0	1.0	−0.1	0.9	−0.1	1.0	−0.0	1.0
F&V (times per week)	24.8	11.7[f]	23.2	11.6[a,h]	27.3	13.5[a,c]	54.7	10.6[c,f,h,i]	24.3	10.6[i]
SSB (times per week)	1.0	1.8[d,e]	1.6	2.0[a,d,h,j]	9.9	3.6[a,b,c,e]	1.0	1.5[c,h]	0.9	1.5[b,j]
PA (hours per week)	2.9	0.9[d,e,f,g]	0.5	0.8[a,d,h,j]	0.8	0.9[b,c,d,e]	1.4	1.0[c,f,h,i]	0.3	0.5[b,g,i,j]
TV viewing (hours per week)	7.3	3.3[d,e,g]	13.9	2.9[a,d,h,j]	8.7	3.3[a,b,e]	8.0	3.5[h,i]	5.6	2.2[b,g,i,j]
Sum two skinfolds	19.6	1.5	20.4	1.5	18.5	1.4	19.1	1.5	19.3	1.5
SBP (mm Hg)	101.8	8.5	102.4	8.8	102.3	8.1	101.7	8.2	102.1	8.6
HOMA index	0.92	2.04	1.00	2.03[j]	0.96	1.83	0.89	1.99	0.87	1.92[j]
Ratio TC/HDL-C	3.1	1.3	3.1	1.3	3.1	1.3	3.1	1.4	3.1	1.4
TG	39.3	1.6	37.6	1.6	37.8	1.7	35.9	1.7	34.9	1.6
CVD risk score	47.5	2.6	46.8	2.4	47.1	1.2	47.1	2.2	47.1	2.2

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; F&V, fruits and vegetables; HDL-c, high-density lipoprotein cholesterol; HOMA, homoeostatic assessment model; PA, physical activity; SBP, systolic blood pressure; SSB, sugar-sweetened beverages; TC, total cholesterol; TG, triglycerides. Values in the same row with common superscripts letters are significantly different ($P < 0.05$) after Bonferroni correction for *post-hoc* multiple comparisons. [a]$P < 0.05$ between C2 and C3. [b]$P < 0.05$ between C3 and C54. [c]$P < 0.05$ between C3 and C5. [d]$P < 0.05$ between C1 and C2. [e]$P < 0.05$ between C1 and C3. [f]$P < 0.05$ between C1 and C4. [g]$P < 0.05$ between C1 and C5. [h]$P < 0.05$ between C2 and C4. [i]$P < 0.05$ between C4 and C5. [j]$P < 0.05$ between C2 and C5.

Table 3. Unstandardized regression β-coefficients (95% CI) examining the relationship between individual risk factors and the CVD risk score and identified clusters among 2 to <6-year-old children

Cluster solutions	*Sum two skinfolds*	*SBP*	*HOMA*	*Ratio TC/HDL-c*	*TG*	*CVD risk score*
Boys						
Physically active	0.03 (−0.03, 0.08)	0.43 (−1.44, 2.31)	0.28 (0.09, 0.46)*	−0.03 (−0.10, 0.04)	0.03 (−0.03, 0.09)	0.44 (−0.10, 0.09)
Sedentary	0.03 (−0.01, 0.07)	0.48 (−0.87, 1.84)	0.07 (−0.06, 0.20)	−0.01 (−0.03, 0.04)	0.01 (−0.03, 0.06)	0.22 (−0.59, 0.61)
High beverage consumption and sedentary	−0.01 (−0.08, 0.06)	0.37 (−1.97, 2.70)	0.08 (−0.15, 0.31)	0.004 (−0.08, 0.09)	0.02 (−0.06, 0.10)	0.17 (−0.50, 0.84)
Healthy diet	−0.02 (−0.06, 0.03)	0.17 (−1.20, 1.63)	0.004 (−0.14, 0.15)	−0.01 (−0.06, 0.04)	0.01 (−0.04, 0.06)	−0.03 (−0.45, 0.40)
Low beverage consumption and low sedentary	Reference	Reference	Reference	Reference	Reference	Reference
Girls						
Physically active	0.05 (−0.005, 0.11)	1.46 (−0.39, 3.30)	−0.02 (−0.20, 0.16)	−0.06 (−0.13, 0.01)	−0.04 (−0.11, 0.02)	0.005 (−0.54, 0.55)
Sedentary	0.06 (0.01, 0.10)*	0.36 (−1.01, 1.73)	0.05 (−0.08, 0.18)	0.01 (−0.04, 0.06)	0.02 (−0.03, 0.07)	0.34 (−0.07, 0.74)
High beverage consumption and sedentary	0.005 (−0.08, 0.09)	−1.43 (−4.00, 1.14)	0.09 (−0.15, 0.34)	0.01 (−0.08, 0.11)	0.000 (−0.10, 0.10)	0.02 (−0.74, 0.78)
Healthy diet	0.03 (−0.02, 0.08)	1.70 (0.10, 3.30)*	−0.10 (−0.25, 0.05)	−0.02 (−0.09, 0.04)	−0.01 (−0.07, 0.05)	0.05 (−0.42, 0.52)
Low beverage consumption and low sedentary	Reference	Reference	Reference	Reference	Reference	Reference

Abbreviations: CI, confidence intervals; CVD, cardiovascular disease; HDL-c, high-density lipoprotein cholesterol; HOMA, homoeostatic assessment model; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides. The model is adjusted for socioeconomic status and study centre. *$P<0.05$.

Table 4. Unstandardized regression β-coefficients (95% CI) examining the relationship between individual risk factors and the CVD risk score and identified clusters among 6–9-year-old children

Cluster solutions	*Sum two skinfolds*	*SBP*	*HOMA*	*Ratio TC/HDL-c*	*TG*	*CVD risk score*
Boys						
Physically active	0.02 (−0.04, 0.08)	1.34 (0.02, 2.67)*	−0.02 (−0.13, 0.09)	0.03 (−0.02, 0.07)	0.01 (−0.03, 0.05)	0.29 (−0.13, 0.71)
Sedentary	0.08 (0.02, 0.13)*	1.84 (0.57, 3.11)*	0.10 (−0.01, 0.20)	0.01 (−0.03, 0.05)	0.01 (−0.03, 0.05)	0.60 (0.20, 1.01)*
Physically active and sedentary	0.05 (−0.004, 0.11)	1.45 (0.15, 2.75)*	0.19 (0.08, 0.29)*	−0.002 (−0.05, 0.04)	0.004 (−0.04, 0.05)	0.55 (0.14, 0.97)*
Healthy diet	0.02 (−0.04, 0.08)	1.83 (0.50, 3.17)*	0.06 (−0.05, 0.17)	0.05 (0.01, 0.10)*	0.04 (0.000, 0.08)	0.60 (0.18, 1.02)*
Low beverage consumption and low sedentary	Reference	Reference	Reference	Reference	Reference	Reference
Girls						
Physically active	−0.01 (−0.06, 0.05)	−0.04 (−1.33, 1.25)	0.07 (−0.03, 0.18)	0.003 (−0.04, 0.05)	−0.005 (−0.05, 0.04)	0.07 (−0.35, 0.49)
Sedentary	0.03 (−0.02, 0.08)	0.18 (−1.06, 1.41)	0.14 (0.05, 0.24)*	−0.02 (−0.06, 0.03)	−0.003 (−0.05, 0.04)	0.24 (−0.16, 0.64)
High beverage consumption	−0.04 (−0.12, 0.05)	0.24 (−1.71, 2.18)	0.05 (−0.10, 0.20)	−0.03 (−0.10, 0.04)	−0.01 (−0.08, 0.06)	−0.13 (−0.76, 0.50)
Healthy diet	0.01 (−0.05, 0.07)	0.48 (−0.92, 1.89)	0.10 (−0.01, 0.21)	0.01 (−0.04, 0.06)	0.002 (−0.05, 0.05)	0.23 (−0.22, 0.69)
Low beverage consumption and low sedentary	Reference	Reference	Reference	Reference	Reference	Reference

Abbreviations: CI, confidence intervals; CVD, cardiovascular disease; HDL-c, high-density lipoprotein cholesterol; HOMA, homoeostatic assessment model; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides. The model is adjusted for socioeconomic status and study centre. *$P<0.05$.

diet' cluster (β = 0.05, 95% CI: 0.01–0.10) in older boys. SBP was also positively associated with C1 to C4 within this group. CVD risk score turned out to be positively associated with C2 (β = 0.60, 95% CI: 0.20–1.01), C3 (β = 0.55, 95% CI: 0.14–0.97) and C4 (β = 0.60, 95% CI: 0.18–1.02) in 6–9-year-old boys.

DISCUSSION

The study aimed to examine the co-occurrence of four lifestyle behaviours, that is, F&V and SSB consumption, PA and TV/video/DVD viewing, and its association to individual CVD risk factors and age- and sex-specific CVD risk score. Other studies performing cluster analysis in children[15,29] and adolescents[30] have dealt with similar variables as health- or obesity-related behaviours. A total of five age- and sex-specific clusters were identified and characterized by one or even two predominant lifestyle behaviours with the exception of C5, defined by low levels for all indices. This is in agreement with Ottevaere *et al.*[30] showing that behaviours do not have to occur simultaneously. We have also observed a sex influence in the composition of clusters being stronger in older children. That could be explained by sex differences in dietary habits as hypothesized by Vereecken *et al.*[31] as well as unbalanced patterns of PA.[32]

Unexpectedly, high F&V consumption and high PA levels did not concur within one cluster representing the 'healthy' group, as occurred in other studies carried out in 10-year-old children.[15] Sabbe *et al.*,[15] however, pointed out that there is no predisposition for children who are engaged into a specific lifestyle behaviour to be also involved in another one. This has been observed in studies of adults,[33] suggesting conscious compensation of the lack of a specific healthy behaviour by adhering to another healthy one. Although this behaviour has not been investigated in children yet, it may be plausible in our study as data was completely parent-reported.

Although CVD risk factors have more frequently been observed among older children, several related risk factors have also been detected in young obese children.[34,35] This possibly indicates the potential implications of different lifestyle behaviours in school and preschool children in addition to sex influences also observed in our study. For that reason, CVD risk score as well as clustering of lifestyle behaviours have been specifically calculated by sex and age group, that is, 2 to <6 years (preschool children) and 6–9 years (school children).

As expected, clusters characterized by high levels of TV/video/DVD viewing (C2 and C3, only in older boys) were adversely associated with individual risk factors and clustered CVD risk score, predominantly in older boys. A recent systematic review concluded that an increase in sedentary behaviours expressed as hours of total screen time was associated with increased risk for markers of CVD in children and adolescents.[36] In addition, it was suggested that TV viewing >1.2 h per day was associated with an increase in SBP.[36] Sedentary behaviours seem to have an important role in the development of CVD risk-related factors, even when values are slightly below the maximum values established for total screen time.

Although PA has previously been inversely associated with clustered metabolic risk in children,[9,12] our results suggested low levels of sedentary behaviours and low consumption of SSB to have a major role on the CVD risk profile than PA *per se*. PA was negatively associated with many CVD risk factors as blood pressure, TC, HDL-c, TG, insulin resistance and SBP among others.[10] Our findings showed C1 to be positively associated with HOMA and SBP values in younger boys and older boys, respectively. This might suggest that decreasing sedentary behaviours and SSB consumption may be more favourable, at least in terms of insulin resistance and SBP, rather than being physically active above a specific threshold. In addition, higher consumption of SSB has been also related to increased insulin resistance in adolescents.[37] The same hypothesis could be plausible for C4, characterized by having a healthy diet. A decrease in TV/video/DVD viewing as well as in SSB consumption may be more beneficial for some individual risk factors that is, sum of two skinfolds in younger girls and older boys, and the ratio TC/HDL-c and CVD risk score among older boys, than higher consumption of F&V.

This study has limitations that should be taken into account when interpreting the results. The cross-sectional nature of the study does not allow us drawing causal relationships. Data on diet, PA and TV/video/DVD viewing were based on parent reports, which are subject to measurement error like social desirability or misreporting, despite showing acceptable reliability in our study.[18,19] Despite the methodological limitations of questionnaires, their low cost and ease of administration in large samples have led them to be the most common tool used to subjectively assess PA and sedentary behaviours.[38] It is possible that sedentary behaviours and PA levels have been underestimated. Indeed, children PA performance was estimated according to their membership in a sport club, and children who responded 'no' were assumed to have spent 0 min doing PA. This represents a major study limitation, as PA performed outside a sport club such as playing sports in the neighbourhood or at school was not taken into account. In addition, young children would be less likely to enroll into a sports club but playing outdoors than older children. Sports club participation, however, has been shown to be positively associated with PA performance in children.[39]

The main strengths of this study are the large sample size, comprising diverse geographical areas of Europe, and the use of standardized procedures. To our knowledge, this is the first study investigating dietary, PA and sedentary behaviours as clusters of lifestyle behaviours, and their relationship to CVD risk factors in European (pre)school children.

In conclusion, our data suggest that low levels of TV/video/DVD viewing and low SSB consumption are associated with a healthier cardiovascular profile than having a diet rich in F&V or being physically active. These findings emphasize the importance of early life-prevention strategies aimed at preventing the onset of CVD risk-related factors. Further longitudinal studies should focus on examining the stability and/or evolution of the clusters together with the tracking of CVD risk factors later in life.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Artículo VIII [Paper VIII]:

Sedentary behaviour and clustered metabolic risk in adolescents: The HELENA study

Rey-López JP, Bel-Serrat S, Santaliestra-Pasías A, de Moraes AC, Vicente-Rodríguez G, Ruiz JR, Artero EG, Martínez-Gómez D, Gottrand F, De Henauw S, Huybrechts I, Polito A, Molnar D, Manios Y, Moreno LA

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Sedentary behaviour and clustered metabolic risk in adolescents: The HELENA study☆

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Sedentary behaviour;
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Abstract *Background and aims:* Although sedentary behaviours are linked with mortality for cardiovascular reasons, it is not clear whether they are negatively related with cardio-metabolic risk factors. The aim was to examine the association between time engaged in television (TV) viewing or playing with videogames and a clustered cardio-metabolic risk in adolescents.

Methods and results: Sedentary behaviours and physical activity were assessed in 769 adolescents (376 boys, aged 12.5–17.5 years) from the HELENA-CSS study. We measured systolic blood pressure, HOMA index, triglycerides, TC/HDL-c, VO_2max and the sum of four skinfolds, and a clustered metabolic risk index was computed. A multilevel regression model (by Poisson) was performed to calculate the prevalence ratio of having a clustered metabolic risk. In boys,

☆ A list with all the HELENA members is shown as Supplementary data.

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playing >4 h/day with videogames (weekend) and moderate to vigorous PA (MVPA) was associated with cardio-metabolic risk after adjustment for age, maternal education and MVPA. In contrast, TV viewing was not associated with the presence of cardio-metabolic risk.
Conclusion: In boys, playing with videogames may impair cardio-metabolic health during the adolescence. Adolescents should be encouraged to increase their participation in physical activity of at least moderate intensity to obtain a more favourable risk factor profile.

Introduction

Nowadays, the presence of cardio-metabolic risk factors (dyslipidemia, glucose intolerance, hypertension and obesity) is highly prevalent in young people from developed countries. For example, in a recent study carried out with American youth half of them presented at least one cardio-metabolic risk factor [1]. This is of concern for the future population health, as cardio-metabolic risk factors in children and adolescents predict coronary heart disease [2] and mortality in adulthood [3]. Therefore, actions designed to improve cardio-metabolic health during the first decades of life are urgently needed to reverse this situation.

There is increasing evidence that physically active subjects have a better cardio-metabolic profile than less active ones [4]. However, less is known about the impact of behaviours that elicit low energy expenditures, namely sedentary behaviours, on different cardio-metabolic risk factors. Martinez-Gómez et al. [5] found that adolescents with a high level of sedentary behaviour (using accelerometry) had less favourable systolic blood pressure, triglycerides and glucose levels and a higher cardiovascular risk score. In contrast, in a representative sample of US adolescents [6], neither the volume nor pattern of sedentary behaviour (with accelerometry) or computer use were found to be predictors of high cardiovascular risk score. Furthermore, controversial data exist on the association between TV viewing and cardio-metabolic risk [6,7]. On the other hand, no study has examined the association between videogames playing and indexes of cardio-metabolic risk.

From a methodological perspective, the use of a clustered cardio-metabolic risk score is recommended because it can compensate for day-to-day fluctuations observed when using the single risk factors [4]. The Healthy Lifestyle in Europe by Nutrition in Adolescence cross-sectional study (HELENA-CSS) brings with it the opportunity to gain a better insight into the relationship of sedentary behaviours with cardio-metabolic health in adolescents.

The main objective of this study was to examine the relationships between sedentary behaviours (TV viewing and videogames) and clustered cardio-metabolic risk in adolescents.

Methods

Study population

The HELENA-CSS aimed to describe the lifestyle and nutritional status of European adolescents. Data collection took place between October 2006 and December 2007 in the following cities: Athens and Heraklion in Greece, Dortmund in Germany, Ghent in Belgium, Lille in France, Pecs in Hungary, Rome in Italy, Stockholm in Sweden, Vienna in Austria, and Zaragoza in Spain. Further information about the study design has been published elsewhere [8]. Participants were recruited at schools. To ensure that the heterogeneity of social background of the population would be represented, schools were randomly selected after stratification by school zone or district. In cases where the selected schools refused to participate, a second list of substitute schools had already been drawn up. Up to three classes from two grades were selected per school. A class was considered eligible if the participation rate was at least 70%. The general inclusion criteria for HELENA were age range of 12.5—17.5 years, not participating simultaneously in another clinical trial, and free of any acute infection during the last week [8].

From a sample of 3528 adolescents who met the HELENA general inclusion criteria, one third of the school classes were randomly selected in each centre for blood collection, resulting in a total of 1089 adolescents. For the purposes of the present study, adolescents with valid data for sedentary behaviour, food habits, accelerometry, cardiorespiratory fitness, total cholesterol, HDL-c, insulin, glucose, systolic blood pressure and triceps, biceps, subscapular and supra-iliac skinfolds were finally included in the analysis ($n = 769$). The study sample did not differ in sex distribution, median age, median body mass index (BMI) and mean values of cardiorespiratory fitness from the full HELENA sample (all $p > 0.05$).

The study was performed following the ethical guidelines of the Declaration of Helsinki 1975 (as revised in 1983). The study was approved by the Research Ethics Committee of each city involved. Written informed consents were obtained from both the adolescents and their parents.

Maternal education

The level of education attained by mothers was considered as the indicator of socioeconomic status. Adolescents ticked one category: primary education; lower secondary education; higher secondary education; or higher education/university degree. Compared with other socioeconomic indicators, maternal education is the one showing the strongest association with unhealthy outcomes (such as adiposity) in children and adolescents [9].

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Physical examination

Waist circumference, height, weight and four skinfolds thicknesses (on the left side from biceps, triceps, subscapular, supra-iliac) were measured following a standardized protocol. The definition of obesity (including overweight) was based on international BMI cut-offs proposed by Cole et al. [10] from several different countries. Systolic and diastolic blood pressure measurements were taken twice (10 min apart) and the lowest value was retained (OMROM M6).

Screen-time

Time engaged in two sedentary behaviours (TV viewing and playing with videogames) was estimated by asking adolescents: 'During weekdays, how many hours do you usually spend watching TV or playing with videogames' and 'During weekend days, how many hours do you usually spend watching TV or playing with videogames?' They selected one category: 1) 0 min, 2) >0–30 min, 3) >30–60 min, 4) >60–120 min, 5) >120–180 min, 6) >180–240 min, and 7) >240 min. In addition, adolescents reported whether they had a TV or game console devices in their bedroom.

The reliability (1-week test–retest) of the HELENA sedentary behaviour questionnaire was studied in 183 adolescents (79 boys, 104 girls; age range 12.5–17.5 years). Cohen's kappa values using quadratic weights showed a substantial agreement (>0.7) [11].

Objectively measured physical activity

Uni-axial accelerometers (Actigraph MTI, model GT1M, Manufacturing Technology Inc., Fort Walton Beach, FL, USA) were used to measure physical activity (PA) [12]. Adolescents were instructed to place the monitor underneath clothing, on their lower back, using an elastic waistband, and wear it for seven consecutive days. They had to wear the accelerometer during all waking hours and were allowed to remove it only during water-based activities and while sleeping. We excluded from the analysis bouts of 20 continuous minutes of 0 activity intensity counts, considering these periods as non-wearing time. Monitor wearing time was calculated by subtracting non-wear time from the total registered time for the day. At least three days of recording [12], with a minimum of 8 h registered per day was set as an inclusion criterion. The time sampling interval was set at 15 s. The time spent at moderate to vigorous physical activity (MVPA) (>3 metabolic equivalents) was calculated on the basis of the following cut-off points: ≥2000 counts per minute for moderate PA [4] and ≥4000 counts per minute for vigorous PA [12,13].

Cardiorespiratory fitness

Participants ran between two lines spaced 20 m apart, keeping the pace with audio signals. The initial speed was 8.5 km/h, and each minute speed was increased by 0.5 km/h. Participants had to run in a straight line and to pivot on the lines. The test finished when subjects stopped due to fatigue or when they failed to reach the end line concurrent with the signals on two consecutive occasions. The last completed stage or half-stage was recorded. Finally, the maximal oxygen consumption (VO_2max) in ml/kg/min was estimated by the Leger equation (boys and girls: $VO_2\text{max} = 31.025 + (3.238 \times S \times 3.248 \times A) + (0.1536 \times S \times A)$ (A the age; S the final speed) ($S = 8 + 0.5$ last stage completed) [14].

Metabolic risk factors

Blood samples were obtained for a third of the HELENA-CSS participants. Blood samples (24.3 ml) were collected by venipuncture at school between 8 and 10 o'clock in the morning after a 10-h overnight fast. Centrifugation was performed at room temperature. Blood was collected in heparinized tubes, immediately placed on ice and centrifuged within 30 min (3500 r.p.m. for 15 min) to avoid haemolysis. Immediately after centrifugation, the samples were stored and transported at 4–7 °C (for a maximum of 14 h) to the central laboratory in Bonn (Germany) and stored there at −80 °C until assayed. Triglycerides, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c) and glucose were measured using enzymatic methods (Dade Behring, Schwalbach, Germany). Insulin levels were measured using an Immulite 200 analyser (DPC Bierman GmbH, Bad Nauheim, Germany). The homeostasis model assessment (HOMA) calculation was used as a measurement of insulin resistance [15].

A clustered metabolic risk index was created from the following variables: systolic blood pressure, HOMA index, triglycerides, TC/HDL-c, VO_2max and the sum of four skinfolds. The standardized value of each variable was calculated as follows: (value − mean)/SD, separately for boys and girls and by 1-yr age groups. For variables characterized by a lower metabolic risk with increasing values (VO_2max), Z scores were multiplied by −1. To create the metabolic risk score, all the Z scores were summed, where the lowest values are indicative of a better cardiometabolic risk profile. Finally, all those subjects at or above age and gender specific 1SD were identified at metabolic risk, similar to previous studies [4,16].

Statistical analysis

The main objective was to examine associations between a clustered metabolic risk index and sedentary behaviours. Because observations were dependent of centre and school, we performed a multilevel analysis. Data are shown as medians and interquartile ranges, unless otherwise stated. Poisson regression with robust variance adjustment, which is recommended for high prevalence outcomes [17] (by multilevel regression models using random effects intercept [18]), was performed to calculate the prevalence ratio (PR) with a confidence interval of 95% (95% CI). The random intercept model was chosen because the group showed significant differences in physical activity and sedentary behaviour levels among countries.

Some potential confounders were taken into account. Maternal education (compared with other socioeconomic indicators) is strongly associated with adiposity in young

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people [9]. Because some sedentary behaviours could be (a priori) associated with low MVPA levels, the adjusted analysis was performed according to a hierarchical framework previously developed into two levels: Model 1: age and maternal education level; 2) model 1 and MVPA. For a variable to be retained in the model, the significance level was set at $p < 0.20$. The *Wald* test for heterogeneity was used to check the significance level (5% alpha) of dichotomous variables and the linear trend was used for ordinal categorical variables. Analyses were conducted separately by gender since sex interactions were found between gender and sedentary behaviour. A significance level was considered when p values were <0.05. Statistical analyses were performed using STATA 12.0.

Results

Descriptive characteristics for girls and boys are shown in Table 1. Boys were taller, heavier and had a larger waist circumference than girls ($p < 0.001$), but non-significant differences were observed for BMI values or for the prevalence of obesity. In addition, boys accumulated more daily minutes of MVPA than girls ($p < 0.001$). The prevalence of time watching TV was similar between sexes, although a higher proportion of boys reported having a TV in their bedroom compared to girls (62% vs. 57%, respectively; $p < 0.05$). For playing with videogames, boys reported a higher prevalence and availability of game console devices in their bedrooms than girls (all $p < 0.001$). A similar prevalence of metabolic risk was observed for both genders (15% for boys and 17% for girls; $p = 0.46$). The multilevel regression model showed that (in boys) playing >4 h/day with videogames during weekend days was associated with metabolic risk after adjustment for age, maternal education and MVPA (Model 2, in Table 3). Watching TV was not associated with metabolic risk in both sexes. MVPA was inversely associated with metabolic risk in boys: PR = 0.99 (0.98–0.99), but not in girls PR = 0.99 (0.98–1.00) (Table 2).

Discussion

The main findings of this study were: 1) In boys, playing videogames >4 h/day (on weekend days) was associated with the presence of a clustered metabolic risk; 2) TV viewing was not associated with metabolic risk in either sex; 3) In boys, time spent in MVPA was inversely associated with clustered metabolic risk.

In adults, high levels of TV time are associated with an increased risk of death from all causes and cardiovascular reasons [19]. Potential pathways linking sedentary behaviours to all-cause and cardiovascular mortalities are diverse and need exploration [20]. High exposure to food advertisements increases the consumption of unhealthy snacks and the total amount of food consumed [8]. Nonetheless, our data and previous studies [7] do not support the hypothesis of increased metabolic risk associated with TV viewing during adolescence. For example, in a cross-sectional study of European children and adolescents, Ekelund et al. [7] did not find an association between TV viewing and clustered metabolic risk. On the other hand, a recent study in children aged 4–18 years found that total sedentary time (which reflects the total of sedentary behaviours, screen based or not) was unrelated to established cardio-metabolic risk factors after additional adjustment for MVPA [20]. Taken together, these results suggest that the pathogenic effects of TV viewing or

Table 1 Characteristics of the study population.

	Boys ($n = 376$)	Girls ($n = 393$)	P
Age (years)	14.8 (13.8–15.7)	14.7 (13.8–15.7)	0.47
Height (cm)	170 ± 9.7	161 ± 7.3	***
Weight (kg)	60 (51.8–69.1)	54.7 (48.4–61.3)	***
BMI (kg/m^2)	20.5 (18.6–23.1)	20.7 (18.8–23.1)	0.41
Waist (cm)	72.6 (67.9–78.2)	69 (64.5–74.6)	***
Obesity (including overweight; *n*, %) by Cole	85 (23)	79 (20)	0.43
Maternal education			
Primary (*n*, %)	24 (7)	49 (13)	*
Lower secondary (n, %)	92 (26)	93 (25)	
Higher secondary (n, %)	109 (30)	123 (32)	
University degree (n, %)	131 (37)	114 (30)	
MVPA (min/day) ($n = 254/281$)	67 (52–84)	50 (36–61)	***
TV weekdays			
0–2 h/day (*n*, %)	241 (64)	267 (67)	0.48
2–4 h/day (*n*, %)	113 (30)	103 (26)	
>4 h/day (*n*, %)	24 (6)	26 (7)	
TV weekend days			
0–2 h/day (*n*, %)	143 (39)	151 (38)	0.95
2–4 h/day (*n*, %)	156 (42)	168 (43)	
>4 h/day (*n*, %)	72 (19)	73 (19)	
TV at bedroom (*n*, %)	238 (62)	222 (57)	*
VG weekdays			
0–2 h/day (*n*, %)	177 (47)	321 (82)	***
2–4 h/day (*n*, %)	116 (31)	57 (14)	
>4 h/day (*n*, %)	83 (22)	15 (4)	
VG weekend days			
0–2 h/day (*n*, %)	140 (38)	272 (69)	***
2–4 h/day (*n*, %)	84 (23)	74 (19)	
>4 h/day (*n*, %)	149 (40)	46 (12)	
VG at bedroom (*n*, %)	180 (50)	90 (23)	***
Metabolic risk (*n*, %)	55 (15)	65 (17)	0.46

BMI: body mass index; MVPA: moderate to vigorous physical activity; VPA: vigorous physical activity; VG: videogames. Quantitative variables are shown as median (interquartile range) or mean and standard deviation according the type of distribution of each variable. Number of subjects and percentage are noted for categorical variables. *P* values show sex differences using *T*-student, *U* Mann–Whitney or Pearson chi-square: *$P \leq 0.05$; **$P < 0.01$; ***$P < 0.001$.

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Table 2 Prevalence ratio (PR) and 95% confidence interval (CI) for having a clustered metabolic risk according to sedentary behaviours in females.

		VG weekdays (n = 393) PR (95% CI)			VG weekend (n = 392) PR (95% CI)			VG at bedroom (n = 388) PR (95% CI)	
		<2 h/day	2–4 h/day	>4 h/day	<2 h/day	2–4 h/day	>4 h/day	No	Yes
Model Null	−1.85 (−2.21–−1.50)								
Alpha	0.16								
AIC	368.6								
Model 1		Ref.	1.59 (0.85–2.95)	1.51 (0.51–4.44)	Ref.	0.92 (0.46–1.80)	1.60 (0.81–3.19)	Ref.	1.25 (0.71–2.21)
Cons				0.28 (0.005–13.71)			0.28 (0.01–14.2)		0.44 (0.01–14.1)
Alpha				0.13			0.15		0.11
AIC				370.8			370.7		358.9
Model 2		Ref.	1.59 (0.86–2.95)	1.52 (0.52–4.47)	Ref.	0.92 (0.46–1.80)	1.60 (0.80–3.18)	Ref.	1.27 (0.72–2.23)
Cons				0.15 (0.09–0.23)			0.15 (0.09–0.25)		0.16 (0.10–0.25)
Alpha				0.13			0.15		0.11
AIC				372.7			372.6		360.5
		TV weekdays (n = 393) PR (95% CI)			TV weekend (n = 392) PR (95% CI)			TV at bedroom (n = 392) PR (95% CI)	
		<2 h/day	2–4 h/day	>4 h/day	<2 h/day	2–4 h/day	>4 h/day	No	Yes
Model 1		Ref.	1.0 (0.56–1.77)	1.38 (0.57–3.38)	Ref.	1.24 (0.69–2.24)	1.77 (0.89–3.51)	Ref.	1.60 (0.90–2.85)
Cons				0.18 (0.001–7.92)			0.13 (0.001–6.03)		0.55 (0.02–15.05)
Alpha				0.16			0.10		0.06
AIC				309.5			304.2		301.8
Model 2		Ref.	0.99 (0.55–1.96)	1.37 (0.56–3.36)	Ref.	1.24 (0.68–2.24)	1.77 (0.89–2.52)	Ref.	1.59 (0.90–2.83)
Cons				0.17 (0.09–0.31)			0.16 (0.03–8.53)		0.22 (0.10–0.73)
Alpha				0.16			0.10		0.06
AIC				303.1			297.3		295.7

Model 1: adjusted PR for age and maternal education. Model 2: adjusted PR for age, maternal education and moderate to vigorous PA.

sedentary time on cardiovascular health may take several decades to be clinically important. In fact, a recent systematic review that described the prospective relationship between childhood sedentary behaviour and health indicators concluded that there is insufficient evidence for an independent effect of sedentary behaviours on various cardiovascular risk factors [21]. However, we found that playing with videogames during weekend days (>4 h/day) was associated with metabolic risk in boys. In a similar way, playing videogames was the only form of sedentary behaviour that was independently associated with increased BP and lipids in Canadian adolescents [22]. Further research is needed to replicate our findings (using longitudinal designs) and to elucidate the possible mechanisms linking videogames playing with cardio-metabolic risk. A recent laboratory study carried out with adolescents, video game playing increased food intake in the absence of hunger [23]. Video game playing could impair satiety signals or alternatively, promote eating through the mental stress-induced reward system [23]. In contrast to another study [22], we only found association between videogames and cardio-metabolic risk in boys. However, in the former study authors commented that it is possible that sex differences had emerged with more balanced sample on sex (males comprised about 30%).

Similarly, an inverse association of MVPA with cardio-metabolic risk was only found in boys. This could be partially due to the fact that girls are less physically active than boys. In our study a higher proportion of boys (56.8% of boys vs. 27.5% of girls) met the physical activity recommendations of at least 60 min/day of MVPA [12]. In addition, there is mounting evidence that physical activity mitigates several risk factors more [24] and produces higher adaptations in men than women. For example, women may

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Table 3 Prevalence ratio (PR) and 95% confidence interval (CI) for having a clustered metabolic risk according to sedentary behaviours in males.

		VG weekdays (n = 376) PR (95% CI)			VG weekend (n = 373) PR (95% CI)			VG at bedroom (n = 360) PR (95% CI)	
		<2 h/day	2–4 h/day	>4 h/day	<2 h/day	2–4 h/day	>4 h/day	No	Yes
Model Null	0.14 (0.08–0.26)								
Alpha	0.78								
AIC	314								
Model 1		Ref.	1.11 (0.54–2.27)	1.59 (0.80–3.18)	Ref.	1.55 (0.64–3.71)	**2.18 (1.02–4.63)**	Ref.	1.18 (0.66–2.11)
Cons				0.15 (0.003–7.08)			0.06 (0.001–3.36)		0.23 (0.01–10.53)
Alpha				0.68			0.66		0.81
AIC				308.2			304.7		297.3
Model 2		Ref.	1.12 (0.55–2.30)	1.55 (0.78–3.10)	Ref.	1.52 (0.63–3.63)	**2.14 (1.02–4.50)**	Ref.	1.15 (0.64–2.08)
Cons				0.15 (0.08–0.30)			0.11 (0.05–0.25)		0.18 (0.09–0.35)
Alpha				0.50			0.49		0.59
AIC				302.4			298.7		290.5
		TV weekdays (n = 376) PR (95% CI)			TV weekend (n = 371) PR (95% CI)			TV at bedroom (n = 369) PR (95% CI)	
		<2 h/day	2–4 h/day	>4 h/day	<2 h/day	2–4 h/day	>4 h/day	No	Yes
Model 1		Ref.	1.17 (0.64–2.13)	1.52 (0.59–3.36)	Ref.	1.12 (0.58–2.17)	1.47 (0.70–3.09)	Ref.	1.01 (0.54–1.88)
Cons				0.18 (0.001–7.92)			0.13 (0.001–6.03)		0.14 (0.4–46)
Alpha				0.76			0.75		0.77
AIC				309.5			304.2		301.8
Model 2		Ref.	1.19 (0.65–2.19)	1.75 (0.67–4.58)	Ref.	1.16 (0.60–2.24)	1.70 (0.80–3.60)	Ref.	0.92 (0.49–1.73)
Cons				0.17 (0.09–0.31)			0.16 (0.08–0.32)		0.22 (0.10–0.73)
Alpha				0.58			0.55		0.57
AIC				303.1			297.3		295.7

Model 1: adjusted PR for age and maternal education. Model 2: adjusted PR for age, maternal education and moderate to vigorous PA. Statistically significant values are showed in bold.

necessitate bouts of higher intensity to evoke chronic myocardial benefits than men [24]. Nonetheless, physical activity unquestionably plays an essential role in the prevention and treatment of chronic diseases.

The present study has several strengths. To the best of our knowledge, this is the first study that has examined the association between the use of videogames and the presence of clustered cardio-metabolic risk. Second, we included the clustered cardio-metabolic score because it counteracts daily fluctuations that are typically observed when single risk factors are used. Third, we used a multilevel analysis to control the influence of contextual variables (i.e. cities and school), because several studies have shown its influence on physical activity and sedentary behaviour patterns [25]. Furthermore, because an inverse relation between time spent playing with videogames and daily physical activity has been reported [26], data were additionally adjusted for MVPA (Model 2). Finally, the sample used in this study was obtained from a diverse geographical area in Europe (North, West, East and South).

Study limitations

Although the present study does not provide evidence of an independent effect of certain screen-time related behaviours (TV viewing) on cardio-metabolic health, some limitations must be considered. First, it is important to bear in mind that we examined a reduced period of life (adolescence) in a cross-sectional manner, and that associations between TV viewing and clustered metabolic risk may show up in other age groups (adults) or during follow-up studies

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[27]. Second, although excessive video game playing could have adverse cardio-metabolic consequences, this type of activity can be less prevalent later in life. Third, although the clustered risk marker is a good index of cardio-metabolic health, the inclusion of novel, emerging cardiovascular risk factors [28] may help to provide insight into the pathogenesis attributed to sedentary behaviours.

Our main public health message is that to achieve optimal cardio-metabolic health, adolescents should be encouraged to increase their participation in physical activity of at least moderate intensity rather than just reducing sedentary behaviours. An excessive amount of time playing videogames may impair cardio-metabolic health during adolescence, but more studies are needed before any conclusion can be made.

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Conflict of interest

None declared.

The content of this article reflects only the authors' views, and the European community is not liable for any use that may be made of the information contained therein.

Appendix A. Supplementary materials

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.numecd.2012.06.006.

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5. Discusión [Discussion]

5.1 Validez de los métodos de valoración de la dieta

La evaluación de los métodos de medida utilizados en un estudio de investigación es un paso previo y necesario para poder determinar el grado de precisión de las medidas realizadas. Por lo que se refiere a la dieta, este proceso es esencial puesto que una información incorrecta puede llevar a asociaciones erróneas entre factores dietéticos y enfermedades o marcadores de enfermedad (108).

En la presente Tesis Doctoral se ha examinado la validez de dos métodos de valoración de la dieta, un CFCA y un recuerdo dietético de 24 horas, para ser utilizados en niños europeos de 2 a 10 años. En nuestro caso, esta evaluación cobra especial relevancia ya que, debido a la edad de los participantes, la información relativa a la dieta fue obtenida a través de sus padres.

En primer lugar, se valoró la precisión de dos recuerdos de 24 horas para estimar la IE usando medidas objectivas de GET obtenidas con la técnica del agua doblemente marcada considerada como el método de referencia (109). Los resultados mostraron un buen grado de acuerdo entre la EI y el GET tanto para la muestra en general como para subgrupos de edad, género, país de procedencia y estado ponderal. Este buen grado de acuerdo entre la EI y el GET podría explicarse por la medida adicional de las comidas en la escuela. Esto reduciría el error causado por aquellas tomas de alimentos que no tienen lugar bajo la supervisión de un adulto, así como por el uso de fotos de tamaños de porciones para valorar de forma más precisa el tamaño de la porción consumida. Además, el hecho de que nuestra muestra fuera de conveniencia, también debería tenerse en consideración ya que es probable que estos individuos estuvieran mucho más motivados.

Sin embargo, a nivel individual, las diferencias entre la IE y el GET fueron bastante amplias; hecho que podría ser explicado por la presencia de error aleatorio, incluyendo la

variación día a día, la cual desaparecería en el análisis del grupo. Nuestros resultados concuerdan con otros estudios en los que el grado de acuerdo fue mejor a nivel grupal que a nivel del individuo (110-113). Cuando la ingesta dietética se obtiene a través de los padres, tanto la infra- como la sobre-declaración de la ingesta pueden estar causadas mayoritariamente por ingestas de alimentos que no tienen lugar bajo el control parental o por dificultades para estimar el tamaño de las porciones, pero también podría ser intencionadas debido al efecto de la deseabilidad social.

También se estudió la validez relativa del CEHQ-FFQ en comparación con el recuerdo dietético de 24 horas. Nuestro estudio de validación de un CFCA es el más amplio, en cuanto a tamaño de muestra se refiere, llevado a cabo en niños de estas edades (2-9 años) y realizado a través de ingestas de alimentos y no de nutrientes. Además, este estudio de validación ofrece un nuevo enfoque para validar CFCAs ya que hemos usado porciones/día en lugar de gramos/día, la unidad de medida más utilizada en la actualidad en este tipo de estudios. Los resultados obtenidos mostraron amplias diferencias en cuanto a la validez relativa entre los diferentes grupos de alimentos, lo cual pone de manifiesto la importancia de validar métodos de valoración de la dieta a través de grupos de alimentos en lugar de nutrientes. Se observó que las ingestas medias del CEHQ-FFQ eran mayores que las del recuerdo 24 horas, una tendencia que también se ha observado previamente en otros estudios llevados a cabo en niños (114, 115). Además, los resultados obtenidos sugieren que la sobre-declaración era más frecuente en los grupos de alimentos consumidos de forma episódica, lo cual podría explicarse por la dificultad del recuerdo de 24 horas para capturar alimentos consumidos de forma no frecuente, sobre todo si tenemos en cuenta que las dietas de los niños presentan una alta variabilidad y los hábitos dietéticos cambian muy rápidamente (4). Las correlaciones también tendían a ser más fuertes para aquellos grupos de alimentos con mayor frecuencia de consumo. Aún así, las correlaciones observadas fueron entre moderadas y bajas, lo cual no sorprende ya que las correlaciones obtenidas a través de los instrumentos de valoración de la

frecuencia de consumo son generalmente más bajas en poblaciones de niños y adolescentes que en adultos (4). Esto podría ser atribuido al hecho de que las ingestas fueron obtenidas a través de los padres, lo que hace que la información sobre la dieta esté siempre sujeta a su habilidad para recordar de forma precisa la ingesta de alimentos de sus hijos (9). Además, la capacidad del cuestionario para clasificar a los individuos según cuartiles de ingesta fue bastane limitada; sin embargo, los porcentajes de acuerdo y de clasificación errónea estaban dentro del rango obtenido por otros estudios (116-118). El grado de acuerdo entre los dos métodos, evaluado a través de la Kappa ponderada, fue también bajo.

No se observaron grandes diferencias en los coeficientes de correlación y el grado de acuerdo en cuanto a los dos grupos de edad examinados, pre-escolar (2-<6 años) y escolar (6-9 años), puesto que los valores fueron bastante similares para la mayoría de los grupos de alimentos. No obstante, cabe destacar que los coeficientes de correlación para alimentos consumidos con mayor frecuencia como la fruta, los cereales de desayuno o la leche, entre otros, fueron considerablemente mayores en el grupo de pre-escolares en comparación con los niños en edad escolar. Así mismo, el grado de acuerdo entre cuestionarios fue también mayor en este tipo de alimentos para los niños en edad pre-escolar. El hecho de que la ingesta de alimentos de los más pequeños tenga lugar con mayor frecuencia bajo la supervisión de los padres (3, 9) podría explicar estos resultados, ya que los padres tendrían mayor capacidad para registrar la dieta de sus hijos de forma más precisa.

La falta de acuerdo entre estos dos métodos de valoración de la dieta puede ser debida a varias razones. Por un lado, y tal y como se ha mencionado anteriormente, el hecho de que la dieta haya sido obtenida a través de los padres constituye un factor importante. Además, la naturaleza de la dieta de los niños y la falta de un método de referencia ideal para evaluar la validez de los CFCA también contribuiría a explicar los resultados obtenidos (119). Adicionalmente, el que los tamaños de las porciones no fueran valorados podría ser una explicación del bajo grado de acuerdo encontrado puesto que los alimentos consumidos en

pequeñas cantidades se habrían sobre-estimado mientras que aquellos consumidos en mayores cantidades habrían sido infra-estimados.

Por otro lado, la información del recuerdo de 24 horas en Hungría fue recogida siguiendo una metodología distinta a la del resto de países, lo que representa una de las mayores limitaciones del estudio. Por ello, todos los análisis se llevaron a cabo con y sin los datos húngaros y observamos que, cuando estos eran excluidos, la fuerza de las asociaciones entre el CEHQ-FFQ y el recuerdo 24 horas aumentaba, así como los valores de la kappa ponderada y los porcentajes de individuos clasificados correctamente. Consecuentemente, las proporciones de individuos clasificados erróneamente disminuyeron. Esto indica que la diferencia en la forma de administrar un mismo método, en este caso el recuerdo de 24 horas, influye de manera notable en la precisión de la información obtenida, más aún cuando dicha información es acerca de la dieta del individuo.

Para poder valora la dieta de los adultos en estudios epidemiológicos se ha aconsejado combinar métodos de valoración de la dieta, concretamente dos recuerdos de 24 horas no consecutivos en combinación con un CFCA, (120). Sin embargo, existe una falta de recomendaciones para medir la dieta en niños, pero el uso combinado del recuerdo de 24 horas y el CFCA también podría ayudar a mejorar la estimación de la ingesta en poblaciones pediátricas.

5.2 Dieta y factores de riesgo cardiovascular

La presente Tesis Doctoral incluye un tercer artículo metodológico en el que se investigó la asociación entre los aminoácidos (AA) dietéticos y los lípidos plasmáticos, importantes factores de riesgo cardiovascular, así como el papel que podía ejercer sobre esta relación la ingesta de grasa. Los análisis iniciales revelaron una asociación inversa entre la ingesta de AA y los niveles plasmáticos de TG, colesterol total , LDL-c y apolipoproteína B, el índice colesterol total/HDL-c y el índice apolipoproteína B/apolipoproteína A1 en las chicas

adolescentes y con los niveles plasmáticos de TG y el índice colesterol total/HDL-c en los chicos. Sin embargo, estás asociaciones dejaron de ser significativas tras ajustar los análisis por la ingesta de grasa.

A pesar de que previamente se han llevado a cabo estudios sobre la asociación entre AA y diversos factores de riesgo cardiovascular como la obesidad, la resistencia a la insulina y la tensión arterial (121-124), entre otros, la evidencia científica disponible acerca de la asociación entre los AA dietéticos y el perfil lipídico sérico es muy limitada y no ofrece una idea clara. Los mecanismos sobre los que subyace el efecto de la suplementación de AA en los lípidos séricos todavía no se conocen (125). Por ejemplo, se ha observado que la suplementación de la dieta con AA esenciales disminuye los niveles séricos de TG, colesterol total y lipoproteínas de muy baja densidad (VLDL-c) en personas mayores (125). En general, en los estudios llevados a cabo sobre este tema, los análisis no se han ajustado por variables potencialmente confusoras como la ingesta total de grasa y, además, se han llevado a cabo en poblaciones de personas mayores y no en adolescentes, lo cual limita su comparabilidad con nuestros resultados.

En cuanto a la ingesta de grasa, especialmente la grasa saturada, se ha venido observando que existe una asociación positiva con los niveles plasmáticos de LDL-c y colesterol total (126); sin embargo, un reciente meta-análisis de estudios epidemiológicos prospectivos ha puesto de manifiesto que no hay evidencia científica suficiente para concluir que la grasa saturada dietética esté asociada con un incremento del riesgo cardiovascular (126). Por otro lado, se ha observado que el contenido de grasa saturada de la carne roja, una de las mayores fuentes de proteína y, consecuentemente, de AA, podría ser en parte responsable de la asociación positiva entre este alimento y el riesgo cardiovascular (127). El hecho de que en nuestro caso la asociación inicial entre la ingesta de AA y los lípidos plasmáticos desapareciera al ajustar los análisis por la ingesta de grasa total, nos lleva a sugerir que el papel positivo que los AA puedan tener sobre el perfil de lípidos sérico quedaría

neutralizado cuando los AA, o las proteínas, son ingeridas junto con la grasa, principalmente la grasa saturada. Sin embargo, la ingesta elevada de proteínas no implica únicamente la ingesta elevada de carnes rojas, sino también de otros alimentos ricos en proteína como el pescado, la carne de ave, la leche y los productos lácteos, los frutos secos, los huevos o los productos de origen vegetal, entre otros. De hecho, se ha mostrado que las ingestas de carnes de ave y de lácteos tienen un efecto neutro sobre el riesgo cardiovascular (128). Además, un estudio llevado a cabo por Mangravite et al. (129) puso de manifiesto que la fuente de proteína dietética podría modificar los efectos de la grasa saturada en las lipoproteínas aterogénicas; de hecho, los autores sugirieron que la asociación entre la grasa saturada y el riesgo cardiovascular podría variar según el contexto dietético en el que la grasa saturada fuera consumida.

Aparte de los AA, y siguiendo con los lípidos plasmáticos, también se examinó su asociación con la ingesta de macronutrientes. Además, puesto que previamente se había observado en el estudio HELENA que la grasa corporal contribuía a la variación de los lípidos plasmáticos (130), también se valoró el papel que el grado de grasa corporal ejercía sobre esta asociación. En general, los resultados obtenidos mostraron que el perfil de lípidos séricos estaba inversamente asociado a la grasa dietética y positivamente asociado a los hidratos de carbono. Además, dichas asociaciones se vieron afectadas según el grado de grasa corporal de los adolescentes, es decir, se observaron asociaciones entre la ingesta de grasa y los lípidos plasmáticos principalmente en aquellos con un nivel elevado de grasa corporal.

El papel que ejerce la ingesta de hidratos de carbono sobre el perfil lipídico plasmático es muy complejo y parece ser adverso (131). Se ha observado que la ingesta de hidratos de carbono disminuye los niveles séricos de HDL-c y aumenta los de TG en adultos (131) y en niños (132). Por otro lado, se ha sugerido que los lípidos plasmáticos también estarían controlados por las proteínas procedentes de la dieta (133); sin embargo, la información disponible acerca de la asociación entre ingesta de proteínas y lípidos plasmáticos todavía es

muy limitada. Por otro lado, el papel que la ingesta de grasa puede ejercer sobre el perfil lipídico depende del tipo de grasa estudiado. De hecho, el perfil de ácidos grasos de la dieta es el mayor determinante de la concentraciones séricas de colesterol (134).

La variabilidad en la respuesta de los lípidos a la dieta depende de varios factores, siendo el exceso de adiposidad uno de los más importantes (135). Se ha observado que los individuos obesos presentan una respuesta menor a las intervenciones dietéticas destinadas a mejorar el perfil de lípidos sérico (135). El hecho de que en nuestros resultados hayamos observado asociaciones entre la ingesta de macronutrientes y los lípidos plasmáticos solamente en aquellos individuos con niveles elevados de masa grasa o solamente con niveles bajos de masa grasa, pero en pocas ocasiones simultáneamente en los dos grupos, sugiere que estas asociaciones dependen del grado inicial de grasa, independientemente de la definición de obesidad utilizada: adiposidad general (IMC-z y suma de pliegues) o adiposidad central (índice cintura-altura). En adultos, algunos autores ya han indicado el papel que puede ejercer el grado de adiposidad en estas asociaciones (136, 137). Por otro lado, modelos predictivos han mostrado que, tanto en niños como en adultos, el peso corporal parece predecir la respuesta de los lípidos séricos a distintas dietas (138).

Tras valorar la asociación entre los macronutrientes y los lípidos plasmáticos, se consideró también interesante evaluar la relación entre la ingesta de alimentos y un indicador de riesgo cardiovascular agrupado en niños europeos de entre 2 y 9 años. Nuestros resultados mostraron que una ingesta elevada de bebidas refrescantes azucaradas en los niños de menor edad (2-<6 años) y de zumos manufacturados en las chicas más mayores (6-9 años) estaba asociada con un mayor riesgo de indicador agrupado de riesgo cardiovascular. Estos resultados coinciden con aquellos obtenidos en un estudio llevado a cabo por Ventura et al. (139) en el cual se observó que a las edades de cinco, siete y nueve años, aquellos niños con mayor riesgo metabólico presentaban mayores ingestas de bebidas refrescantes azucaradas en comparación con aquellos con menor riesgo.

Por otro lado, una ingesta elevada de cereales de desayuno disminuyó la odds ratio (OR) de tener riesgo cardiovascular tanto en chicos como en chicas de mayor edad en comparación con aquellos con un menor consumo. Esto podría ser debido a la presencia de cereales integrales, puesto que previamente se han relacionado inversamente con la mortalidad por enfermedades cardiovasculares en adultos (140). Además, también observamos que los chicos y chicas mayores con un consumo elevado de algunos alimentos ricos en azúcar como crema de cacao o de frutos secos, mermelada y miel (sólo en chicas), y dulces (sólo en chicos) mostraban menor riesgo de tener indicador agrupado de riesgo cardiovascular en comparación con aquellos con una ingesta menor. Cabe destacar que estos alimentos se consumen típicamente en el desayuno. De hecho, un estudio reciente llevado a cabo en adultos italianos ha puesto de manifiesto que un consumo frecuente de alimentos consumidos en el desayuno, como los cereales de desayuno, la miel, el azúcar y la mermelada disminuye el riesgo metabólico y de enfermedad cardiovascular en el futuro (141).

También se examinó la asociación entre el consumo de alimentos y los factores de riesgo cardiovascular en adolescentes. En este caso, primero investigamos qué alimentos diferenciaban mejor a aquellos individuos con alto riesgo cardiovacular de aquellos con bajo riesgo cardiovascular. De entre todos los grupos de alimentos, la leche y los productos lácteos, como el yogur y las bebidas lácteas a base de leche y yogur, fueron los dos grupos de alimentos que mejor desempeñaban dicha función. Por ello, posteriormente exploramos la asociación entre estos dos grupos de alimentos y los factores de riesgo cardiovascular y se observó que un consumo elevado de lácteos, incluyendo tanto la leche como los productos derivados, estaban asociados con una menor cantidad de grasa corporal y con un mayor nivel de condición física tanto en chicos como en chicas adolescentes. Además, los resultados mostraron una asociación inversa entre el indicador agrupado de riesgo cardiovascular y el consumo de lácteos en las chicas. El consumo de lácteos ya ha sido asociado previamente con un menor riesgo cardiovascular y de síndrome metabólico en niños y adolescentes de 6 a 18

años (57) y en adultos (142, 143). Otros estudios observacionales también han relacionado inversamente el consumo de lácteos con el peso corporal, la grasa corporal y la circunferencia de la cintura tanto en niños como en adolescentes (144-147). Estos beneficios potenciales de los lácteos podrían explicarse por la presencia de múltiples componentes como los péptidos bioactivos derivados de las proteínas de la leche, la grasa propia de los lácteos y algunos minerales, concretamente, el calcio, el magnesio y el potasio. Entre todos ellos, parece que el calcio desempeña el papel más importante puesto que se ha relacionado inversamente con la hipertensión arterial y con una disminución de los niveles plasmáticos de TG después de nueve años (142), así como con la grasa corporal, gracias a su efecto sobre la lipogénesis (148).

5.3 Estilos de vida y factores de riesgo cardiovascular

Aparte de la dieta, otros factores como la actividad física y los comportamientos sedentarios también se han relacionado previamente con el riesgo cardiovascular. Por ello, examinamos la presencia concomitante de cuatro comportamientos relacionados con los estilos de vida (consumo de frutas y verduras, consumo de bebidas refrescantes azucaradas, actividad física y ver la televisión/vídeo/DVD), así como su asociación con factores de riesgo cardiovascular en niños europeos. Se identificaron un total de cinco clusters o conjuntos específicos por género y por grupo de edad. Cada cluster se caracterizó por tener uno o dos comportamientos de estilos de vida predominantes, con la excepción de un cluster, que estaba definido por niveles bajos de todos los comportamientos. Un estudio previo ya puso de manifiesto que este tipo de comportamientos no tendrían porqué darse de forma simultánea en un mismo sujeto (149). También observamos una influencia del género en la composición de los clusters, siendo más fuerte en los niños de mayor edad (6-9 años). Esto podría explicarse por diferencias de género en cuanto a hábitos dietéticos (150) y a patrones de actividad física (151).

En cuanto a la asociación entre los clusters obtenidos y los factores de riesgo cardiovascular, observamos que los clusters caracterizados por niveles elevados de ver la televisión/vídeo/DVD estaban asociados con un mayor riesgo cardiovascular, predominantemente en los chicos mayores. Estos resultados estarían de acuerdo con una revisión sistemática en la que se concluyó que un incremento de los comportamientos sedentarios, expresados como horas totales de pantalla, estaba asociado con un aumento del riesgo de marcadores cardiovasculares en niños y adolescentes (65).

En lo que se refiere a la actividad física, se ha asociado inversamente con el riesgo metabólico agrupado en niños (72, 73); sin embargo, teniendo en cuenta nuestros resultados, parece ser que niveles bajos de comportamientos sedentarios y bajas ingestas de bebidas refrescantes azucaradas desempeñarían un papel más importante sobre el riesgo cardiovascular que la actividad física por sí misma. Esta misma hipótesis también podría ser válida para el cluster caracterizado por una dieta sana, definida a través de un consumo elevado de frutas y verduras. Tal y como ocurría con la actividad física, bajos niveles de comportamientos sedentarios y bajas ingestas de bebidas refrescantes azucaradas ejercerían un papel mucho más positivo sobre algunos factores de riesgo cardiovascular que una dieta rica en frutas y verduras.

También investigamos la asociación entre el tiempo que los adolescentes pasaban viendo la televisión y jugando a videojuegos así como la actividad física de intensidad moderada e intensa que realizaban y un indicador de riesgo cardio-metabólico agrupado. En los chicos, jugar durante más de cuatro horas a videojuegos durante el fin de semana se asoció con la presencia de riesgo cardio-metabólico mientras que no se observó ninguna asociación con ver la televisión en ninguno de los dos sexos. Además, el tiempo invertido en realizar actividad física moderada e intensa se asoció inversamente con el indicador de riesgo cardio-metabólico agrupado en los chicos adolescentes. Tanto nuestros resultados como los obtenidos por otros autores (73) no apoyan la hipótesis de que ver la televisión esté asociado

con un mayor riesgo metabólico durante la adolescencia. Por otro lado, también cabe destacar que el efecto que el tiempo de sedentarismo pueda tener sobre la salud cardiovascular pueda necesitar varias décadas para manifestarse clínicamente. Sin embargo, sí que observamos una asociación con jugar a videojuegos durante los fines de semana en los chicos. Cabe destacar que jugar a los videojuegos fue el único comportamiento sedentario que se asoció con un aumento de la tensión arterial y de los lípidos plasmáticos en adolescents canadienses (152). Un estudio reciente llevado a cabo también en adolescentes puso de manifiesto que jugar a videojuegos incrementaba la ingesta de alimentos en ausencia de hambre (153). Este comportamiento podría alterar las señales de saciedad o promover la ingesta a través del sistema de recompensa mental inducido por el estrés (153).

Así mismo, el que observáramos una asociación entre la actividad física moderada-intensa y el riesgo cardio-metabólico en chicos pero no en chicas podría explicarse en parte por el hecho de que las chicas tienden a ser menos activas físicamente que los chicos. En nuestro estudio, una mayor proporción de chicos que de chicas cumplía con las recomendaciones de actividad física, es decir, realizar al menos 60 minutos/día de actividad física moderada-intensa (154). Además, hay cierta evidencia de que la actividad física atenúa algunos factores de riesgo cardiovascular (155), produciendo mayores adaptaciones en los varones que en las mujeres. De este modo, sería necesario que las mujeres realizaran actividad física de mayor intensidad para alcanzar los mismos beneficios crónicos cardiovasculares que los hombres (155).

5.4 Implicaciones para la salud pública

Los resultados obtenidos en la presente Tesis Doctoral pueden tenerse en cuenta para promover estilos de vida más saludables en niños y adolescentes europeos. El hecho de que la ingesta de determinados alimentos junto con niveles bajos de sedentarismo esté asociada desde edades tempranas con un menor riesgo cardiovascular destaca la importancia de tener

en cuenta los estilos de vida a la hora diseñar estrategias de prevención de factores de riesgo cardiovascular, como la obesidad, entre otros, puesto que dichos factores tienden a persistir durante la edad adulta.

En lo que se refiere a los estudios de validación de métodos de valoración de la dieta (**Artículos I** y **II**), se podría pensar que no tienen una implicación directa sobre la salud pública de los individuos. Sin embargo, estos métodos constituyen herramientas para que los investigadores puedan obtener información sobre la dieta en poblaciones pediátricas y, consecuentemente, poder establecer nuevas asociaciones o confirmar las ya existentes entre ingesta de alimentos y/o nutrientes y enfermedades o marcadores de enfermedad, y así aumentar la evidencia existente sobre las mismas.

Como se ha mencionado a lo largo de esta Tesis Doctoral, la presencia de factores de riesgo cardiovascular ya no sólo se da en adultos, sino también en niños y adolescentes, lo cual supone un tema de preocupación para la comunidad científica y un riesgo para la salud pública. Por ello, en diciembre de 2011, el Panel de Expertos sobre Pautas Integradas para la Salud Cardiovascular y la Reducción del Riesgo en Niños y Adolescentes (Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents: Summary Report) (156) publicó un documento con el objetivo de desarrollar pautas basadas en la evidencia científica para tratar los factores de riesgo cardiovascular conocidos y así ayudar a los sanitarios que desarrollen su labor en poblaciones pediátricas a promover la salud cardiovascular y a identificar y tratar factores de riesgo específicos desde la infancia hasta la edad adulta temprana.

En cuanto a la dieta, estas nuevas pautas no sólo se basan en las recomendaciones para alcanzar un aporte adecuado de nutrientes en niños y adolescentes en crecimiento, sino que añaden evidencia acerca de la eficacia de ciertos cambios dietéticos para reducir el riesgo cardiovascular a partir de una revisión de la evidencia existente. Sin embargo, todas las recomendaciones se presentan mayoritariamente en términos de nutrientes y no de

alimentos, lo cual reduce su aplicación a la dieta habitual de los individuos. Por otro lado, el panel de expertos concluyó que hay evidencia suficientemente fuerte y consistente para decir que una buena nutrición desde el nacimiento tiene beneficios profundos sobre la salud y es capaz de disminuir el riesgo cardiovascular futuro, lo cual pone de manifiesto la importancia de seguir una dieta saludable desde los primeros años de vida del individuo, y justifica trabajos de investigación focalizados en la alimentación de las poblaciones más jóvenes, como la presente Tesis Doctoral.

A nivel nacional, no obstante, se dispone de un documento en el que se han desarrollado recomendaciones dirigidas a prevenir la obesidad. Se trata de *"La estrategia para la nutrición, actividad física y prevención de la obesidad (NAOS)"*, elaborada en 2005 por el Ministerio de Sanidad y Consumo, con el objetivo de mejorar los hábitos alimentarios y de promover la realización de actividad física de la población española y reducir así la elevada prevalencia de obesidad. Entre las recomendaciones relacionadas con la ingesta de alimentos, destacan el consumo de al menos cinco raciones de frutas y verduras al día (400 gr/día); el moderar el consumo de productos ricos en azúcares simples, como golosinas, dulces y refrescos y el no prescindir nunca de un desayuno completo, compuesto por lácteos, cereales (pan, galletas, cereales de desayuno…) y frutas. Aunque estas pautas son específicas para la prevención de la obesidad, podrían ser aplicables a la salud cardiovascular puesto que la obesidad es un factor de riesgo cardiovascular muy importante. Estas recomendaciones son similares a las del Panel Expertos, ya que también ponen de manifiesto la necesidad de consumir alimentos a base de plantas como las frutas y las verduras para reducir el aporte calórico y aumentar el de vitaminas, minerales y fibra así como reducir el consumo de bebidas azucaradas.

Nuestros resultados (**Artículos V** y **VII**) estarían de acuerdo con la recomendación de disminuir la ingesta de bebidas azucaradas puesto que hemos observado que así se disminuye el riesgo cardiovascular, al menos en niños europeos. Con respecto al tema del desayuno, los

resultados obtenidos (**Artículos V** y **VI**) apoyarían estas recomendaciones de forma indirecta puesto que la ingesta de lácteos parece mejorar el grado de obesidad y el perfil cardiovascular en adolescentes y la ingesta de cereales de desayuno y otros alimentos dulces como la mermelada o la miel, entre otros, disminuiría el riesgo cardiovascular en niños. La importancia de estos resultados radica en el hecho de que todos ellos son alimentos que se consumen típicamente en el desayuno, por lo que aparte de todas las ventajas relacionadas con tomar el desayuno todos los días, también se sumarían los beneficios potenciales de los alimentos consumidos en él.

En lo que se refiere a la actividad física, tanto la estrategia NAOS como el Panel de Expertos aconsejan la práctica de ejercicio de manera regular. Éste último recomienda al menos una hora de actividad moderada-intensa cada día de la semana en niños mayores de cinco años. Existe una fuerte evidencia de que un incremento de la actividad física de intensidad moderada-intensa está asociada con menor tensión arterial sistólica y diastólica, disminución de la grasa corporal y del IMC, mejoras de la condición física, niveles séricos de colesterol total, LDL-c y TG más bajos, mayores concentraciones plasmáticas de HDL-c y una disminución de la resistencia a la insulina durante la infancia y la adolescencia. Nosotros también hemos observado que la realización de actividad física moderada-intensa disminuye el riesgo cardio-metabólico, pero únicamente en chicos adolescentes, lo cual coincidiría con la evidencia científica disponible acerca de los beneficios de la práctica regular de ejercicio sobre el perfil cardiovascular.

Por otro lado, ambos documentos coinciden con la Asociación Americana de Pediatría en que es necesario reducir el tiempo de pantalla a dos horas al día, puesto que la disminución del tiempo de sedentarismo está asociada de forma convincente con una reducción de la obesidad y con un perfil cardiovascular favorable. El **Artículo VIII** ha mostrado que jugar a videojuegos durante más de cuatro horas los fines de semanas está asociado con un mayor riesgo cardio-metabólico en los chicos adolescentes; por ello, es lógico pensar que una

reducción en el tiempo dedicado a esta actividad mejoraría el perfil cardio-metabólico. Es más, una de las deducciones principales obtenidas en esta Tesis Doctoral (**Artículo VII**) es que niveles bajos de comportamientos sedentarios, como ver la televisión/vídeo/DVD, junto con una baja ingesta de bebidas azucaradas estaría asociado, en mayor grado si cabe, con un perfil cardiovascular más saludable que la actividad física y una dieta rica en frutas y verduras. A pesar de esto, estos resultados deben ser interpretados con cautela puesto que idealmente la disminución del tiempo de pantalla debería ir acompañada de un incremento de la actividad física de intensidad moderada-intensa y de una dieta saludable, variada y con una ingesta mínima de 5 porciones de frutas y vegetales al día para así alcanzar una salud cardiovascular óptima.

Cabe destacar la contribución de nuestros resultados a la evidencia científica, puesto que se ha observado que existe un vacío de conocimiento en lo que se refiere a la asociación entre la dieta y los factores de riesgo cardiovascular, principalmente en lo que se refiere a ingestas de alimentos, en niños y adolescentes. Es por ello por lo que hasta el momento no se dispone de recomendaciones específicas sobre la ingesta de alimentos en esta población dirigidas a prevenir o reducir el riesgo cardiovascular.

En resumen, la sociedad de consumo en la que vivimos conduce a la población a dedicar mucho tiempo al ocio, el cual suele estar asociado a ingestas excesivas de alimentos no saludables. Este hecho cobra importancia durante la adolescencia, cuando es relativamente habitual que los adolescentes tengan hábitos alimentarios poco saludables, como una forma de reafirmar su identidad a través de la comida. Estos hábitos se suelen caracterizar por ingestas elevadas de alimentos ricos en grasa saturadas y azúcares simples. Actualmente, esto representa un riesgo importante para la salud pública del siglo XXI que se hace evidente a través de la elevada prevalencia de obesidad y de sus co-morbilidades que se observan en las poblaciones pediátricas de los países desarrollados desde hace unas décadas. A estos hábitos de alimentación poco saludables se une el hecho de que nos encontremos en una etapa de

revolución tecnológica, caracterizada por una disminución considerable de la práctica de ejercicio y un incremento de las conductas sedentarias debidas al uso excesivo de dispositivos electrónicos. Todo esto contribuye de forma notable a un empeoramiento de la salud cardiovascular en niños y adolescentes. Por todo ello, es necesario destacar la importancia de llevar a cabo estudios de intervención o longitudinales para confirmar estos resultados y poder desarrollar estrategias que sean efectivas para la mejora de la salud cardiovascular durante la infancia y la adolescencia y prevenir futuras enfermedades crónicas durante la edad adulta.

6. Aportaciones principales de la tesis doctoral

Artículo I. A nivel de grupo, se observó un buen grado de acuerdo entre la ingesta de energía estimada por los padres y gato energético total medido con la técnica del agua doblemente marcada, incluso en el grupo de niños con sobrepeso y obesidad. A nivel individual, por el contrario, las diferencias entre estos valores variaron ampliamente.

Artículo II. La fortaleza de la asociación entre las ingestas estimadas por el *Children's Eating Habits Questionnaire-Food Frequency Questionnaire* y el recuerdo dietético de 24-horas era variable según el grupo de alimento y el grupo de edad. Además, el *Children's Eating Habits Questionnaire-Food Frequency Questionnaire* mostró una limitada capacidad para clasificar a los niños en función de su ingesta de grupos de alimentos.

Artículo III. Las ingestas de aminoácidos se asociaron inversamente con el perfil lipídico sérico; sin embargo, dichas asociaciones desaparecieron cuando se tuvo en cuenta la ingesta de grasa como variable confusora.

Artículo IV. La ingesta de grasa se asoció con un mejor perfil lipídico a través de la disminución de los valores séricos de triglicéridos, LDL-c, apolipoproteína B y los índices de colesterol total/HDL-c y apolipoproteína B/apolipoproteína A1 y del aumento de las concentraciones séricas de HDL-c mientras que la ingesta de hidratos de carbono se asoció inversamente a HDL-c y apolipoproteína A1 séricos y positivamente al índice colesterol total/HDL-c. Además, dichas asociaciones difirieron según la masa grasa del individuo, es decir, las asociaciones entre la ingesta de grasa y carbohidratos y los lípidos plasmáticos se observaron principalmente en aquellos adolescentes con más grasa corporal.

Artículo V. Un consumo elevado de bebidas azucaradas y una baja ingesta de frutos secos y semillas, dulces, miel y mermelada, cremas de cacao y frutos secos y de cereales de desayuno se asoció con una mayor probabilidad de riesgo cardiovascular.

Artículo VI. La ingesta de leche y productos lácteos se asoció inversamente con el índice de masa corporal, triglicéridos séricos, el índice colesterol total/HDL-c y el indicador agrupado de riesgo cardiovascular en chicas adolescentes y con la circunferencia de la cintura y la suma de cuatro pliegues cutáneos en ambos géneros. Por otro lado, se observó una relación positiva entre la ingesta de lácteos y la condición física en todos los adolescentes, independientemente de su género.

Artículo VII. Niveles bajos de ver la televisión/video/DVD y un bajo consumo de bebidas azucaradas se asociaron inversamente con el HOMA, suma de dos pliegues cutáneos, tensión arterial sistólica, índice colesterol total/HDL-c y con el indicador agrupado de riesgo cardiovascular en niños europeos.

Artículo VIII. Jugar a los videojuegos durante los fines de semana durante más de cuatro horas se asoció con la presencia de riesgo cardiovascular agrupado, mientras que no se observó ninguna asociación con ver la televisión en ninguno de los dos géneros. El tiempo invertido en realizar actividad física de carácter moderado-intenso se asoció inversamente al indicador agrupado de riesgo cardio-metabólico.

6. Main thesis contributions

Manuscript I. Good agreement between proxy-reported energy intake and measured total energy expenditure was observed at a group level – even in overweight/obese children – whereas individual differences values varied widely.

Manuscript II. The strength of the association between estimates of the *Children's Eating Habits Questionnaire-Food Frequency Questionnaire* and the 24-hour dietary recall varied by food group intakes and by age group. Furthermore, the *Children's Eating Habits Questionnaire-Food Frequency Questionnaire* showed low ability to rank children according to their food groups intakes.

Manuscript III. Inverse associations were observed between amino acids intakes and serum lipid profile, however, such associations did not persist when dietary fat was considered as a confounding factor.

Manuscript IV. Dietary fat had a beneficial role on serum lipids levels by lowering serum levels of triglycerides, LDL-c, apolipoprotein B and total cholesterol/HDL-c and apolipoprotein B/apolipoprotein A1 ratios and rising HLD-c concentrations whereas carbohydrates were adversely associated with lipid profile by decreasing HDL-c and Apo 1 serum concentrations and increasing total cholesterol/HDL-ratio. In addition, the above mentioned associations varied according to adolescents' body fat status, i.e. associations between fat and carbohydrates intake and blood lipids were mainly observed among adolescents within the high body fat group.

Manuscript V. High consumption of sugar sweetened beverages and low consumption of nuts and seeds, sweets, jam and honey, chocolate and nut-based spreads and breakfast cereals were associated with higher likelihood of being at risk of cardiovascular disease.

Manuscript VI. Milk and dairy products consumption were inversely associated with body mass index, serum triglycerides, total cholesterol/HLD-c ratio and clustered cardiovascular disease risk score in girls and with waist circumference and sum of four skinfolds in both genders. Additionally, a positive association between dairy consumption and cardiorespiratory fitness was observed among all adolescents regardless of gender.

Manuscript VII. Low levels of television/video/DVD viewing and low sugar sweetened beverage consumption were inversely associated with HOMA, sum of two skinfolds, systolic blood pressure, total cholesterol/HDL-c ratio and clustered cardiovascular disease risk score in European children.

Manuscript VIII. Playing videogames for more than 4 hours a day during the weekend was associated with the presence of clustered cardiovascular disease risk. No association with television viewing was observed in neither of both genders. Additionally, time engaged in moderate-to-vigorous physical activity was inversely associated with clustered cardi-metabolic risk.

7. Conclusiones

Artículo I. Dos recuerdos dietéticos de 24-horas cumplimentados por los padres, incluyendo ayudas visuales y el registro de los alimentos consumidos en el colegio, son una medida válida de la ingesta de energía a nivel de grupo, pero son insuficientes para evaluar las ingestas individuales.

Artículo II. El nivel de acuerdo entre las ingestas, estimadas por los padres a través del *Children's Eating Habits Questionnaire-Food Frequency Questionnaire* y mediante dos recuerdos dietéticos de 24-horas, es bajo para la mayoría de los grupos de alimentos en niños entre 2 y 9 años.

Artículo III. La ingesta de aminoácidos parece estar asociada con un mejor perfil lipídico en adolescentes europeos; sin embargo, dicha asociación es dependiente de la ingesta grasa.

Artículo IV. La ingesta de grasa está asociada con un mejor perfil lipídico en adolescentes sanos mientras que la ingesta de hidratos de carbono está asociada con un peor perfil lipídico. A pesar de que estas asociaciones varían en función de la masa grasa corporal, parece que el papel que ejerce el grado de obesidad del individuo sobre los lípidos séricos es independiente de la definición usada para definirla, es decir, obesidad general u obesidad central.

Artículo V. Aquellos niños con una mayor probabilidad de tener riesgo cardiovascular elevado consumen mayor cantidad de bebidas azucaradas y tienen una menor ingesta de frutos secos y semillas, dulces, miel y mermelada, cremas de cacao y frutos secos y cereales de desayuno.

Artículo VI. La ingesta de leche y productos lácteos ejerce un papel positivo en la adiposidad y en la condición física de los adolescentes y, como resultado, en el riesgo cardiovascular.

Artículo VII. Niveles bajos de ver la televisión/vídeo/DVD y un bajo consumo de bebidas azucaradas están asociados con un perfil cardiovascular más sano que tener una dieta rica en frutas y vegetales o ser físicamente activo.

Artículo VIII. Jugar demasiado tiempo a los videojuegos, principalmente durante los fines de semana, perjudica la salud cardio-metabólica de los adolescentes. Aparte de reducir su nivel de sedentarismo, un incremento de la actividad física de intensidad por lo menos moderada mejora su salud cardio-metabólica.

7. Conclusions

Manuscript I. Two proxy-reported 24-hour dietary recalls including visual aids and school meal reporting are a valid measure for energy intake at group level but no sufficient to evaluate individual intakes.

Manuscript II. There is low agreement for the majority of food groups examined by the proxy-estimated *Children's Eating Habits Questionnaire-Food Frequency Questionnaire* and two 24-hour dietary recalls in 2-9 years old children.

Manuscript III. It remains unclear whether AA intakes are associated with a healthier lipid profile in adolescents and, therefore, with CVD risk, but it seems that such potential association is dependent of fat intake, at least based on the evidence from this sample of European adolescents.

Manuscript IV. Fat intake is associated with a better serum lipid profile while carbohydrates intake is associated with an adverse lipid profile in healthy adolescents. Although these associations differed according to body fat status, general and central body fatness seemed to play a similar role on serum lipids and on its association with diet.

Manuscript V. Children more likely to be at greater cardiovascular disease risk have higher consumption of sugar sweetened beverages and lower consumption of nuts & seeds, sweets, jam & honey, and chocolate & nut-based spreads and breakfast cereals.

Manuscript VI. Milk and dairy products consumption have been shown to have a positive role on adiposity and cardiorespiratory fitness, and as a result, on clustered cardiovascular disease risk score in adolescents.

Manuscript VII. Low levels of TV/video/DVD viewing and low SSB consumption are associated with a healthier CV profile compared to having a diet rich in F&V or being physically active.

Manuscript VIII. Spending too much time per day playing videogames, mainly during weekends, may impair cardio-metabolic health during adolescence. Additionally, adolescents should increase their participation in physical activity of at least moderate intensity rather than just reducing sedentary behaviours in order to improve their cardio-metabolic health.

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"El único lugar donde el éxito viene antes que el trabajo es en el diccionario"

(Albert Einstein)

"The only place success comes before work is in the dictionary"

(Albert Einstein)

Apéndice [Appendix]

Factor de impacto de las revistas y ranking en 2012 en "ISI *Web o Knowledge – Journal Citation Reports (JCR)*" dentro de sus áreas temáticas correspondientes.

[Impact factor and ranking of each Journal in 2012 in "ISI *Web o Knowledge – Journal Citation Reports (JCR)*" within their subject categories].

Artículos publicados o aceptados [*Published or accepted manuscripts*]:

	Revista *[Journal]*	**Factor de Impacto** *[Impact factor]*
Artículo I.	Clinical Nutrition	3.298
	Ranking in 2012 ISI JCR: 19/76 (Nutrition and Dietetics)	
Artículo II.	Public Health Nutrition	2.250
	Ranking in 2012 ISI JCR: 34/76 (Nutrition and Dietetics) 47/158 (Public, Environmental and Occupational Health)	
Artículo V.	Pediatric Obesity	2.276
	Ranking in 2012 ISI JCR: 29/121 (Pediatrics)	
Artículo VI.	Pediatric Obesity	2.276
	Ranking in 2012 ISI JCR: 29/121 (Pediatrics)	
Artículo VII.	European Journal of Clinical Nutrition	2.756
	Ranking in 2012 ISI JCR: 25/76 (Nutrition and Dietetics)	
Artículo VIII.	Nutrition, Metabolism and Cardiovascular Diseases	3.978
	Ranking in 2012 ISI JCR: 11/76 (Nutrition and Dietetics) 29/122 (Cardiac and Cardiovascular Systems) 37/121 (Endocrinology and Metabolism)	

Agradecimientos [Acknowledgements]

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Anexos [Annexes]

A continuación se adjunta una copia del resumen del artículo descriptivo-metodológico de cada estudio:

- **The IDEFICS cohort: design, characteristics and participation in the baseline survey.**

Ahrens W, Bammann K, Siani A, Buchecker K, De Henauw S, Iacoviello L, Hebestreit A, Krogh V, Lissner L, Marild S, Molnár D, Moreno LA, Pitsiladis YP, Reisch L, Tornaritis M, Veidebaum T, Pigeot I, on behalf of the IDEFICS Consortium. *Int J Obes* 2011; **35 Suppl 1**: S3-S15.

Background: The European IDEFICS (Identification and prevention of dietary- and lifestyle induced health effects in children and infants) study was set up to determine the aetiology of overweight, obesity and related disorders in children, and to develop and evaluate a tailored primary prevention programme.

Objective: This paper focuses on the aetiological element of the multicentre study, the measures and examinations, sociodemographic characteristics of the study sample and proportions of participation.

Design: Prospective cohort study with an embedded intervention study that started with a baseline survey in eight countries in 2007–2008.

Subjects and measurements: Baseline participants of the prospective cohort study were 16 224 children aged 2–9 years. Parents reported sociodemographic, behavioural, medical, nutritional and other lifestyle data for their children and families. Examinations of children included anthropometry, blood pressure, fitness, accelerometry, DNA from saliva and physiological markers in blood and urine. The built environment, sensory taste perception and other mechanisms of children's food choices and consumer behaviour were studied in subgroups.

Results: Between 1507 and 2567, children with a mean age of 6.0 years and an even sex distribution were recruited from each country. Of them, 82% lived in two-parent families. The distribution of standardised income levels differed by study sample, with low-income groups being strongly represented in Cyprus, Italy and Germany. At least one 24-h dietary recall was obtained for two-thirds of the children. Blood pressure and anthropometry were assessed in more than 90%. A 3-day accelerometry was performed in 46%, motor fitness was assessed in 41%, cardiorespiratory fitness in 35% and B11% participated in taste perception tests. The proportion of children donating venous blood, urine and saliva was 57, 86 and 88%, respectively.

Conclusion: The IDEFICS cohort provides valuable data to investigate the interplay of social, environmental, genetic, physiological and behavioural factors in the development of major diet- and lifestyle-related disorders affecting children at present.

- **Design and implementation of the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) Cross-Sectional Study.**

Moreno LA, De Henauw S, González-Gross M, Kersting M, Molnár D, Gottrand F, Barrios L, Sjöström M, Manios Y, Gilbert CC, Leclercq C, Widhalm K, Kafatos A, Marcos A; on behalf of the HELENA Study Group. *Int J Obes* 2008; **32 Suppl 5**: S4-S11.

Objective: To provide an overview of the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study (HELENA-CSS) design, with particular attention to its quality control procedures. Other important methodological aspects are described in detail throughout this supplement.

Design: Description of the HELENA-CSS sampling and recruitment approaches, standardization and harmonization processes, data collection and analysis strategies and quality control activities.

Results: The HELENA-CSS is a multi-centre collaborative study conducted in European adolescents located in urban settings. The data management systems, quality assurance monitoring activities, standardized manuals of operating procedures and training and study management are addressed in this paper. Various quality controls to ensure collection of valid and reliable data will be discussed in this supplement, as well as quantitative estimates of measurement error.

Conclusion: The great advantage of the HELENA-CSS is the strict standardization of the fieldwork and the blood analyses, which precludes to a great extent the kind of immeasurable confounding bias that often interferes when comparing results from isolated studies**.**

Estudio IDEFICS

Cuestionario de frecuencia de consumo de alimentos (CEHQ-FFQ)

En el último mes, ¿con qué frecuencia ha consumido su hijo/a los siguientes alimentos y bebidas?
Por favor, limítese a las cuatro últimas semanas y excluya las comidas del colegio o guardería.

	Nunca/ menos de una vez por semana	**1 - 3 veces por semana**	**4 – 6 veces por semana**	**1 vez al día**	**2 veces al día**	**3 veces al día**	**4 o más veces al día**	**No lo sé**
Vegetales								
Verduras, patatas y legumbres cocinadas (también combinadas en el mismo plato)	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Patatas fritas, croquetas de patata	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Vegetales crudos (mezclados en la ensalada, zanahoria, pepino, lechuga, tomate, etc.)	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Frutas								
Frutas frescas (también licuadas) ***sin*** azúcar añadido	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Frutas frescas (también licuadas) ***con*** azúcar añadido	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Bebidas								
Agua	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Zumos de frutas (zumo de naranja, manzana, melocotón, piña,etc.)	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Bebidas edulcoradas incluyendo bebidas deportivas, té en lata o embotellado, refrescos, etc.	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Coca-cola light o bebidas refrescantes sin azúcar	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8

	Nunca/ menos de una vez por semana	1 - 3 veces por semana	4 – 6 veces por semana	1 vez al día	2 veces al día	3 veces al día	4 o más veces al día	No lo sé
Cereales de desayuno								
Cereales de desayuno azucarados o que se les ha añadido azúcar y muesli azucarado (ej. Corn flakes, crispies, etc.)	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Papillas, copos de avena, cereales no azucarados, muesli natural	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Leche								
Leche no azucarada	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Leche azucarada (ej. con azúcar, chocolate, cola-cao, miel, etc.)	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
	Qué tipo de leche consume su hijo/a habitualmente: Entera ❍ Semi-desnatada /desnatada ❍							
Yogur								
Yogur natural o kéfir sin azúcar	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Yogur azucarado y bebidas lácteas fermentadas (ej. Actimel®, LC1®, etc.)	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
	Qué tipo de yogur consume su hijo/a habitualmente: Entero ❍ Semi-desnatado /desnatado ❍							

	Nunca/ menos de una vez por semana	1 - 3 veces por semana	4 – 6 veces por semana	1 vez al día	2 veces al día	3 veces al día	4 o más veces al día	No lo sé
Pescado								
Pescado fresco o congelado, sin freír	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Pescado frito y varitas de pescado	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Carne y productos cárnicos								
Productos loncheados y conservados, o listos para cocinar (ej. fiambres, embutidos, jamón, hamburguesas etc.)	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Huevos								
Huevos fritos o huevos revueltos	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Huevos duros o escalfados	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Mayonesa y productos derivados de la mayonesa (ej. Ligeresa, salsa rosa, etc.)	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Productos sustitutivos de la carne y productos de soja								
Tofu, tempé, leche de soja, yogures de soja, etc.)	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Queso								
Queso en lonchas o para untar (ej. Philadelphia, tranchetes. etc.)	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Queso rallado	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Productos para untar								
Mermelada, miel	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Nocilla o crema de avellanas para untar	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8

	Nunca/ menos de una vez por semana	1 - 3 veces por semana	4 – 6 veces por semana	1 vez al día	2 veces al día	3 veces al día	4 o más veces al día	No lo sé
Mantequilla, margarina en pan	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Productos bajos en grasa en pan (ej. Mermelada, etc.)	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Ketchup	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Productos hechos a base de cereales								
Pan blanco, panecillos blancos, biscotes blancos	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Pan integral , panecillos integrales, biscotes integrales	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Pasta, fideos, arroz	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Cuscús, bulgur, etc.	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Pizza como plato principal	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Sandwiches (rellenos con queso, carne, vegetales, etc)	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Aperitivos								
Frutos secos y semillas y frutas secas (ej. Pipas, cacahuetes, pasas etc.)	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
patatas fritas, aperitivos de maíz, palomitas de maíz, etc (ej. *Cheetos, Lay's, risketos,* etc.)	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
tortas o bollos, pasteles (ej. Tarta de manzana, crepes, palmeras de hojaldre, etc.)	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
chocolate, barritas de chocolate (Mars, Lions, Kit Kat, etc.)	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8

	Nunca/ menos de una vez por semana	1 - 3 veces por semana	4 – 6 veces por semana	1 vez al día	2 veces al día	3 veces al día	4 o más veces al día	No lo sé
caramelos, chucherías, gominolas, etc.)	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
galletas, pasteles envasados, tartas (ej.Donuts, bollycao, cañas de chocolate, etc.)	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8
Helados, polos, sorbetes de fruta(ej. Mágnum, calippo etc.)	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5	❍ 6	❍ 7	❍ 8

Cuestionario sobre actividad física y comportamientos sedentarios

Actividades de tiempo libre y patrón de consumo

44. ¿Cuánto tiempo pasa al día su hijo/a jugando <u>en el patio o las calles cerca de casa</u>(casas de amigos, vecinos o familiares)?

Por favor, indique para cada intervalo horario:

	0 minutos	***1-15 minutos***	***16-30 minutos***	***31-60 minutos***	***Más de 60 minutos***
Desde que se levanta hasta el mediodía	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5
Mediodía hasta las 18:00	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5
18:00 hasta la hora de dormir	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5

45. ¿Cuánto tiempo pasa normalmente su hijo/a al día en el parque, el patio del recreo o en otras zonas recreativas (Ej. piscina, zoo, parque de diversiones)?

Por favor, indique para cada intervalo horario. Incluya el tiempo que pasa en la guardería o la escuela.

	0 minutos	***1-15 minutos***	***16-30 minutos***	***31-60 minutos***	***Más de 60 minutos***
Desde que se levanta hasta el mediodía	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5
Mediodía hasta las 18:00	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5
18:00 hasta la hora de dormir	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5

46. Piense por un momento en el típico día de entre semana de su hijo/a durante el último mes. ¿Cuánto tiempo diría que pasa su hijo jugando fuera en un día típico de entre semana?

|___|___| horas |___|___| minutos

47. Ahora piense en el típico día de fin de semana de su hijo/a durante el último mes. ¿Cuánto tiempo diría que pasa su hijo jugando fuera en un día típico de fin de semana?

|___|___| horas |___|___| minutos

48. ¿Es miembro su hijo/a de algún club deportivo?

❍ 1 Sí

❍ 2 No ➔➔➔ **Por favor, continúe con la pregunta 49**

¿Cuánto tiempo pasa a la semana haciendo ejercicio en el club deportivo?

|___|___| horas |___|___| minutos

¿Qué tipo de deporte practica su hijo/a en el club deportivo?
Por favor, marque la opción que corresponda.

❍ 1 fútbol
❍ 1 natación
❍ 1 tenis
❍ 1 gimnasia rítmica
❍ 1 Otra. Por favor, especificar:
_____

49. ¿Es su hijo/a capaz de…

	Sí	***No***
…montar en bicicleta?	❍ 1	❍ 2
…nadar?	❍ 1	❍ 2
…ir en patines?	❍ 1	❍ 2

50. ¿Cuánto tiempo suele ver su hijo/a la televisión/vídeo/DVD por día?

	Nada en absoluto	*Menos de 30 min. por día*	*Menos de 1 hora por día*	*Aprox. 1-2 horas por día*	*Aprox. 2-3 horas por día*	*Más de tres horas por día*
Entre semana	❍ 0	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5
Sábado/domingo	❍ 0	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5

51. ¿Cuánto tiempo suele usar su hijo/a el ordenador o la consola al día?

	Nada en absoluto	*Menos de 30 min. por día*	*Menos de 1 hora por día*	*Aprox. 1-2 horas por día*	*Aprox. 2-3 horas por día*	*Más de tres horas por día*
Entre semana	❍ 0	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5
Sábado/domingo	❍ 0	❍ 1	❍ 2	❍ 3	❍ 4	❍ 5

Cuestionario sobre el nivel socioeconómico

Información socio-demográfica

66. ¿Cuál es el nivel más alto de educación escolar que usted y su cónyuge/pareja tienen?
Por favor, marcar solamente uno por persona.

	Yo	*Cónyuge/pareja*
Primaria /EGB	❍ 1	❍ 1
Secundaria /ESO	❍ 2	❍ 2
Formación profesional	❍ 3	❍ 3
Ciclos formativos de grado superior	❍ 4	❍ 4
Bachillerato/ BUP/COU	❍ 5	❍ 5
Sin graduación (todavía)	❍ 8	❍ 8
Otros/desconocido	❍ 9	❍ 9

67. ¿Cuál es el nivel más alto de cualificación profesional que usted y su cónyuge/pareja tienen?
Por favor, marcar solamente uno por persona.

	Yo	*Cónyuge/pareja*
Formación profesional	❍ 1	❍ 1
Ciclos formativos de grado superior	❍ 2	❍ 2
Diplomatura universitaria/ingeniería técnica	❍ 3	❍ 3
Licenciatura/ingeniería superior	❍ 4	❍ 4
Doctorado	❍ 5	❍ 5
No formado (todavía)	❍ 8	❍ 8
Desconocido/otros	❍ 9	❍ 9

68. ¿Cuál de los siguientes enunciados describe mejor su estado ocupacional actual y el de su cónyuge/pareja?
Por favor, marcar solamente uno por persona.

	Yo	***Cónyuge/pareja***
Trabajo a tiempo completo (30 horas o más a la semana)	❍ 1	❍ 1
Trabajo a tiempo parcial (menos de 30 horas a la semana)	❍ 2	❍ 2
Estudio o voy a la universidad	❍ 3	❍ 3
No tengo trabajo remunerado	❍ 4	❍ 4
Retirado (también jubilación anticipada)	❍ 5	❍ 5
Baja temporal de la empresa (ej. baja por maternidad o paternidad)	❍ 6	❍ 6
En el paro, desde hace menos de un año	❍ 7	❍ 7
En el paro, desde hace un año o más	❍ 8	❍ 8
En asistencia pública (asistencia social)	❍ 9	❍ 9
Otro, por favor especifique: _____	❍ 10	❍ 10

69. ¿En que posición laboral están actualmente ocupados usted y su cónyuge/pareja?

Si usted o su cónyuge/pareja ya no están ocupados o actualmente no están ocupados, por favor, indique la última posición laboral.

	Yo	***Cónyuge/pareja***
Obrero		
Obrero no cualificado	❍ 1	❍ 1
Obrero semi-cualificado	❍ 2	❍ 2
Obrero cualificado, artesano	❍ 3	❍ 3
Maestro artesano, capataz	❍ 4	❍ 4
Patrón o autónomo (incluyendo la ayuda de miembros de la familia)		
Agricultor y/o ganadero autónomo	❍ 1	❍ 1
Autónomo, trabajador por cuenta propia	❍ 2	❍ 2
Patrón con hasta 9 empleados	❍ 3	❍ 3
Patrón con 10 o más empleados	❍ 4	❍ 4
Ayudo a algún miembro de la familia	❍ 5	❍ 5
Empleado		
Empleado (ej. dependiente, recepcionista, oficinista)	❍ 1	❍ 1
Empleado cualificado (ej. auxiliar contable, auxiliar dental)	❍ 2	❍ 2
Empleado altamente cualificado o con funciones de gestión (ej. científico, jefe de departamento)	❍ 3	❍ 3
Empleado con extensas funciones ejecutivas (ej. director, director general, junta directiva)	❍ 4	❍ 4
Funcionario público		
Categoría A	❍ 1	❍ 1
Categoría B	❍ 2	❍ 2
Categoría C	❍ 3	❍ 3
Categoría D	❍ 4	❍ 4
Categoría E	❍ 5	❍ 5
No trabajo	❍ 6	❍ 6

70. ¿Cuáles son los ingresos mensuales familiares, es decir, el beneficio neto que usted (en total) percibe a parte de impuestos y de retenciones?

Cuando decimos familiares nos referimos a todos aquellos que están residiendo en el mismo hogar que el niño/a seleccionado y que también participan en los gastos. Por favor, incluya también ingresos procedentes de alquileres o arrendamientos, pensiones, subvenciones para los niños, pensiones alimenticias, etc.

	hasta	*800 €*	❍ 1
800 €	hasta	*1050 €*	❍ 2
1050 €	hasta	*1300 €*	❍ 3
1300 €	hasta	*1550 €*	❍ 4
1550 €	hasta	*1900 €*	❍ 5
1900 €	hasta	*2500 €*	❍ 6
2500 €	hasta	*3000 €*	❍ 7
3500 €	hasta	*4000 €*	❍ 8
	Por encima de	*4000 €*	❍ 9

Estudio HELENA

Cuestionario de sedentarismo

7788399663

Healthy Lifestyle in Europe by Nutrition in Adolescence — HELENA — Sixth Framework Programme 2002 - 2006

1. Cuántas horas al día pasas ...

	ninguna	menos de media hora	de media a una hora	de una a dos horas	de dos a tres horas	de tres a cuatro horas	cuatro o más horas
viendo la televisión							
un día de colegio:	○	○	○	○	○	○	○
un día de fin de semana:	○	○	○	○	○	○	○
jugando con juegos en el ordenador							
un día de colegio:	○	○	○	○	○	○	○
un día de fin de semana:	○	○	○	○	○	○	○
jugando con la videoconsola							
un día de colegio:	○	○	○	○	○	○	○
un día de fin de semana:	○	○	○	○	○	○	○
navegando en internet por razones que no están relacionadas con el estudio							
un día de colegio:	○	○	○	○	○	○	○
un día de fin de semana:	○	○	○	○	○	○	○
navegando en internet por motivos de estudio							
un día de colegio:	○	○	○	○	○	○	○
un día de fin de semana:	○	○	○	○	○	○	○
estudiando sin utilizar internet							
un día de colegio:	○	○	○	○	○	○	○
un día de fin de semana:	○	○	○	○	○	○	○

2. cuántas horas duermes normalmente por la noche ...

durante los días de semana: ☐☐,☐ horas por noche

durante el fin de semana: ☐☐,☐ horas por noche

3. si realizas alguna actividad académica o de ocio complementaria al colegio (idiomas, ajedrez clases de repaso, clases the música) aparte del tiempo de estudio personal ¿cuántas horas supone a la semana?

☐☐,☐ horas por semana

4. Cuando estás comiendo con tu familia ¿coméis delante de la televisión?

○ todos los días en cada comida
○ todos los días en 1 0 2 comidas
○ no todos los dias pero mas de 2 comidas a la semana
○ no más de 1 0 2 comidas a la semana
○ escasas veces
○ nunca

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5. tienes en casa ...

televisión:	○ no	○ si, 1	○ si, 2	○ si, 3 o más
ordenador:	○ no	○ si, 1	○ si, 2	○ si, 3 o más
videoconsola:	○ no	○ si, 1	○ si, 2	○ si, 3 o más

tienes en tu habitación ...

televisión:	○ no	○ si
ordenador:	○ no	○ si
videoconsola:	○ no	○ si

tiene tu hermano / hermana en su habitación ...

televisión:	○ no	○ si	○ no tengo hermanos viviendo en casa
ordenador:	○ no	○ si	○ no tengo hermanos viviendo en casa
videoconsola:	○ no	○ si	○ no tengo hermanos viviendo en casa

6. sin contar las comidas principales, cuántas veces ...

	nunca	menos de una vez a la semana	1-2 días por semana	3-4 días por semana	(casi) todos los días	varias veces al día
bebes algo mientras ves la televisión	○	○	○	○	○	○
comes algo mientras ves la televisión	○	○	○	○	○	○

¿qué comes y bebes mientras ves la televisión? (marca todas las respuestas oportunas de esta lista)

○ normalmente no bebo nada	☐ refrescos light	☐ infusiones
☐ agua	☐ refrescos azucarados	☐ café
☐ leche o productos derivados	☐ cerveza	☐ otras bebidas: .....
☐ zumo de frutas		
○ normalmente no como nada	☐ snack salado (patatas)	☐ bocadillo
☐ fruta	☐ bollería	☐ productos lácteos
☐ frutos secos	☐ caramelos, chocolates y chocolatinas	☐ otros: .....

7. sin contar las comidas principales, cuántas veces ...

	nunca	menos de una vez a la semana	1-2 días por semana	3-4 días por semana	(casi) todos los días	varias veces al día
bebes algo mientras juegas con videojuegos:	○	○	○	○	○	○
comes algo mientras juegas con videojuegos:	○	○	○	○	○	○

¿qué comes y bebes mientras juegas con videojuegos? (marca todas las respuestas oportunas de esta lista)

○ normalmente no bebo nada	☐ refrescos light	☐ infusiones
☐ agua	☐ refrescos azucarados	☐ café
☐ leche o productos derivados	☐ cerveza	☐ otras bebidas: .....
☐ zumo de frutas		
○ normalmente no como nada	☐ snack salado (patatas)	☐ bocadillo
☐ fruta	☐ bollería	☐ productos lácteos
☐ frutos secos	☐ caramelos, chocolates y chocolatinas	☐ otros: .....

8. sin contar las comidas principales, cuántas veces ...

	nunca	menos de una vez a la semana	1-2 días por semana	3-4 días por semana	(casi) todos los días	varias veces al día
bebes algo mientras navegas en internet:	○	○	○	○	○	○
comes algo mientras navegas en internet:	○	○	○	○	○	○

¿qué comes y bebes mientras navegas en internet? (marca todas las respuestas oportunas de esta lista)

○ normalmente no bebo nada	☐ refrescos light	☐ infusiones
☐ agua	☐ refrescos azucarados	☐ café
☐ leche o productos derivados	☐ cerveza	☐ otras bebidas: .....
☐ zumo de frutas		
○ normalmente no como nada	☐ snack salado (patatas)	☐ bocadillo
☐ fruta	☐ bollería	☐ productos lácteos
☐ frutos secos	☐ caramelos, chocolates y chocolatinas	☐ otros: .....

Cuestionario sobre el nivel socioeconómico

6750042059

¿Has fumado tabaco alguna vez?

- ○ Sí
- ○ No

¿Con qué frecuencia fumas tabaco actualmente?

- ○ Cada día
- ○ Al menos una vez a la semana, pero no cada día
- ○ Menos de una vez a la semana
- ○ No fumo

¿Cuántos cigarros fumas por semana?

- ○ Ninguno
- ○ Menos de 5
- ○ Entre 5 y 10
- ○ Entre 11 y 20
- ○ Más de 20

¿Cuánto mides descalzo? 1 **m** ☐☐ **cm**

¿Cuánto pesas sin ropa? ☐☐☐,☐

Por favor, indícanos la afirmación más apropiada para tu madre:

- ○ tiene sobrepeso/obesidad
- ○ tiene peso normal
- ○ esta delgada/muy delgada
- ○ no lo sé

Por favor, indicanos la afirmación más apropiada para tu padre:

- ○ tiene sobrepeso/obesidad
- ○ tiene peso normal
- ○ esta delgado/muy delgado
- ○ no lo sé

El término familia se refiere a miembros viviendo juntos en la misma casa: padre, madre, hermanos
Para aquellos que vivan en dos familias, contesta acerca de la familia con la que vivas la mayor parte del tiempo.

¿Con quien vives la mayoría del tiempo?

- ○ con tus dos padres
- ○ con tu madre sóla
- ○ con tu madre y su nuevo compañero
- ○ con tu padre sólo
- ○ con tu padre y su nueva compañera
- ○ con tu madre la mitad del tiempo y tu padre la otra mitad
- ○ con tus abuelos
- ○ con padres adoptivos
- ○ en un centro de acogida
- ○ algún otro lugar

¿Cuantos de tus hermanas y/o hermanos incluyendo hermanastras y/o hermanastros viven en casa (excluyéndote a ti)?

○ 0 ○ 1 ○ 2 ○ 3 ○ 4 ○ más de 4

¿Dispones de tu propia habitación para ti sólo?

○ no ○ si

¿Cuantos coches posee tu familia? (por "familia" queremos decir miembros que viven juntos: padre, madre y hermanos)

○ 0 ○ 1 ○ 2 ○ más de 2

¿Tienes conexión a internet en casa?

○ no ○ si

¿Cómo le va a tu familia económicamente?

- ○ Fantásticamente bien
- ○ Muy bien
- ○ Normal
- ○ No muy bien
- ○ Mal

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Las siguientes preguntas son respecto a tu madre y a tu padre.
Si tienes madre y madrastra o padre y padrastro, contesta en relación a la persona más importante en tu educación.

Por favor indicar el nivel educativo más alto de tu madre y tu padre :

	PADRE	MADRE
educación elemental	○	○
terminó la ESO (BUP)	○	○
terminó Bachiller (COU)	○	○
termino educación superior (universitaria)	○	○

¿Cuál es la ocupación de tu padre ?

- ○ trabaja jornada completa
- ○ trabaja media jornada
- ○ amo de casa
- ○ retirado o enfermo
- ○ aprendiz/estudiante
- ○ en paro
- ○ temporalmente sin trabajar (ej: baja paternal)
- ○ nunca le veo
- ○ falleció
- ○ no lo sé

¿Cuál es la ocupación de tu madre ?

- ○ trabaja jornada completa
- ○ trabaja media jornada
- ○ ama de casa
- ○ retirada o enferma
- ○ aprendiz/estudiante
- ○ en paro
- ○ temporalmente sin trabajar (ej: baja maternal)
- ○ nunca la veo
- ○ falleció
- ○ no lo sé

Por favor, anota el tipo de trabajo que tienen tus padres. Si tienen más de un trabajo, indícalos

	PADRE	MADRE
1. Personal administrativo Presidente, Director de administración pública, Consejo de Administración (Jefe de Departamento o equivalente)	○	○
2. profesiones intelectuales y científicas Personal cualificado (matemático o especialista en ciencias y salud, especialistas técnicos :arquitectos, ingenieros, informáticos, biólogos, famrmacéuticos, médicos, abogados, profesor universitario, psicólogo, sociólogo, etc.)	○	○
3 Profesiones intermedias: Técnicos o peritos y otros trabajos intermedios (electricista, mecánico, enfermero/a, dietista, empleado de oficina, maestro, representante comercial y profesionales asociados)	○	○
4. Administración/Oficinas Banca, contabilidad, seguros, bibliotecarios, etc.	○	○
5. Empresas de negocios Ventas, marketing, publicidad, comunicaciones, etc.	○	○
6. Trabajadores cualificados de agricultura y pesca Granjeros, pescadores, guardabosques, etc.	○	○
7. Artesanos, manufactura y oficios relacionados Peluquero/a, mecánico, operario, artesano, mecánico, operario en industria textil, calzado, etc.	○	○
8. Operarios de maquinaria y montadores Trabajadores industriales y operarios de máquinas, conductores de grúas, etc.	○	○
9. Trabajos y ocupaciones elementales vendedores, empleados del hogar, albañiles, vigilantes de seguridad, limpiadores, etc.	○	○
10. fuerzas armadas	○	○
11. otro nombre (descríbelo detalladamente) **.....**	○	○
12. no trabaja	○	○