FAMILIAL HYPERCHOLESTEROLEMIA IN CHILDREN. FROM DETECTION AND CHARACTERIZATION TO LIFESTYLE CHANGES

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DOCTORAL THESIS
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Supervisors: Lluís Masana Marín and Núria Plana Gil

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I STATE that the present study, entitled "**Familial Hypercholesterolemia in children. From detection and characterization to lifestyle changes**" presented by **Cèlia Rodríguez Borjabad** for the award of the degree of Doctor with international mention, has been carried out under my supervision at the Department **Medicine and Surgery** of this university.

And so that it is registered and has the appropriate effects, we signed this document in Reus, on July, 2021.

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Per tots aquells que han fet això possible,

pels que hi són i pels que, tot i no ser-hi,

sé que estan al meu costat.

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Abbreviations

Abbreviations

Apo A	Apolipoprotein A1
Apo B	Apolipoprotein B100
ApoE	Apoprotein E
BMI	Body mass index
CE	Cholesteryl ester
CH	Carbohydrates
CHD	Cardiovascular heart disease
Ch-P	Children-to-parent pathway
CM	Chylomicrons
CVD	Cardiovascular disease
CVR	Cardiovascular risk
cIMT	Carotid intima-media thickness
DALYsD	Disability-adjusted life years
DHA	Docosahexanoic
DLCN	Dutch lipid clinic network
EAS	European Atherosclerosis Society
ESC	European Society of Cardiology
EPA	Eicosapentanoic
FA	Fatty acid
FC	Free cholesterol
FCHL	Familial combined hyperlipidemia
FFQ	Food frequency questionnaire

FH	Familial hypercholesterolemia
HDL-C	High-density lipoprotein cholesterol
HL	Hepatic lipase
HeFH	Heterozygous familial hypercholesterolemia
HoFH	Homozygous familial hypercholesterolemia
HRQL	Health-related quality of life
IDL	Intermediate-density lipoprotein
IHD	Ischemic heart disease
IQR	Interquartile range
LDL-C	Low-density lipoprotein cholesterol
LDLR	Low-density lipoprotein receptor
LDLRAP1	Low-density lipoprotein receptor adapter protein 1
LLT	Lipid-lowering therapy
Lp(a)	Lipoprotein (a)
LPA	Low physical activity
LPL	Lipoprotein lipase
LRP	Lipoprotein receptor-related protein
LXR	Liver X receptor
MUFA	Monounsaturated fatty acid
NMR	Nuclear magnetic resonance
PA	Physical activity
P-Ch	Parent-to-child pathway
PCSK9	Proprotein convertase subtilisin/kexin type 9
PUFA	Polyunsaturated fatty acid

ROC	receiver operating characteristic
sdLDL	Small dense LDL particles
SFA	Saturated fatty acid
SD	Standard deviation
SMCs	Smooth muscle cells
TG	Triglycerides
TLSC	Therapeutic lifestyle changes
TLSC-IP	Therapeutic lifestyle changes – intensive protocol
YLDs	Years lived with disability
YLLs	Years of life lost
VLDL	Very low-density lipoprotein

Table of contents

Table of contents

1. Presentation and justification

1. PRESENTATION AND JUSTIFICATION

The doctoral thesis project was carried out at the Unit of Vascular Medicine and Metabolism (UVASMET) of the Internal Medicine Service, Sant Joan University Hospital in Reus and the Lipids and Atherosclerosis Research Unit (URLA) of the Department of Medicine and Surgery at the Universtat Rovira i Virgili. Dr Lluís Masana and Dr Núria Plana have supervised the doctoral thesis.

This doctoral thesis deals with three different areas:

- FH screening in children
- New insights into biochemical characterization of metabolic disease
- Implementation of therapeutic lifestyle changes in FH children

Familial hypercholesterolemia (FH) is the most prevalent genetic disorder in paediatric age and is characterised by increased low-density lipoprotein cholesterol (LDL-C) levels in childhood leading to early cardiovascular disease. For this reason, screening methods for early diagnosis and treatment are essential to preventing cholesterol accumulation throughout life and cardiovascular (CV) events.

FH accelerates coronary atherosclerotic disease in individuals in their fourth decades. The high prevalence of the disease means a high risk of cardiovascular disease (CVD), which in turn makes FH a public health problem. Despite the high cardiovascular risk (CVR), most patients are undiagnosed and untreated until advanced ages. Efficient screening strategies are crucial to detect FH in children. Early diagnosis would enable the use of preventive measures and improve prognoses.

We also aim to improve the characterization of the disease so that we can better identify FH candidates and therefore individuals suitable for genetic testing, and so we can study in greater depth the pathophysiological mechanisms of the metabolic alteration. To characterise FH children more precisely, we have used nuclear magnetic resonance (NMR) of plasma. This technique characterises the quantity and quality of the lipoprotein particles beyond lipid concentrations. We have also studied the role of

circulating molecules associated with LDL receptor (LDLR) expression as FH biomarkers. A greater knowledge of the disease will help to improve treatment.

The first line of treatment in children is to establish therapeutic lifestyle changes (TLSC) to prevent cholesterol accumulation and to prevent risk factors such as obesity, hypertension, diabetes mellitus, etc., that would aggravate the situation. It has been widely demonstrated that diet plays a protective role against cardiovascular disease, but it is not fully known how lifestyle affects the lipoprotein profile in FH children. It is important to implement healthy lifestyles at an early age because these have been shown to persist into adulthood. We have also tested the impact of two quite different diets that have shown a beneficial impact on CVD, the Mediterranean diet and the Nordic diet. We have investigated the differences between these two types of diet and how these diets influence FH in children.

Our multidisciplinary unit focuses on preventing, diagnosing and treating this type of illness and on studying the most basic aspect of this disease to know how it develops. In recent years, research into FH in children has gained great importance because the process starts at birth and can be detected in childhood, thus leading to early preventive action. The results of the present thesis are intended to improve understanding of this disease and to be a useful tool in making recommendations for preventing CV.

Although the thesis is built around 9 scientific publications, it is organized into the following traditional sub-sections: the Introduction, which contains information about the detection, characterisation and treatment of FH disease, in particular in children; the Hypotheses and Aims; the Methods section, which describes materials and methods used in each publication; the publications themselves; the Results; a Discussion; and the Conclusions and Bibliography.

2. Introduction

2. INTRODUCTION

2.1. Epidemiological aspects of cardiovascular disease

Cardiovascular disease (CVD) continues to be the leading cause of mortality in Catalonia and throughout the world (1). CVD continues to cause a large proportion of deaths and disability in Europe and places a substantial burden on the health care systems and economies of Europe. There have been significant improvements in recent years through the adoption of many measures to tackle CVD; however, these improvements do not seem to be enough (2). In 2017 the global number of deaths from CVD was 16 million (3).

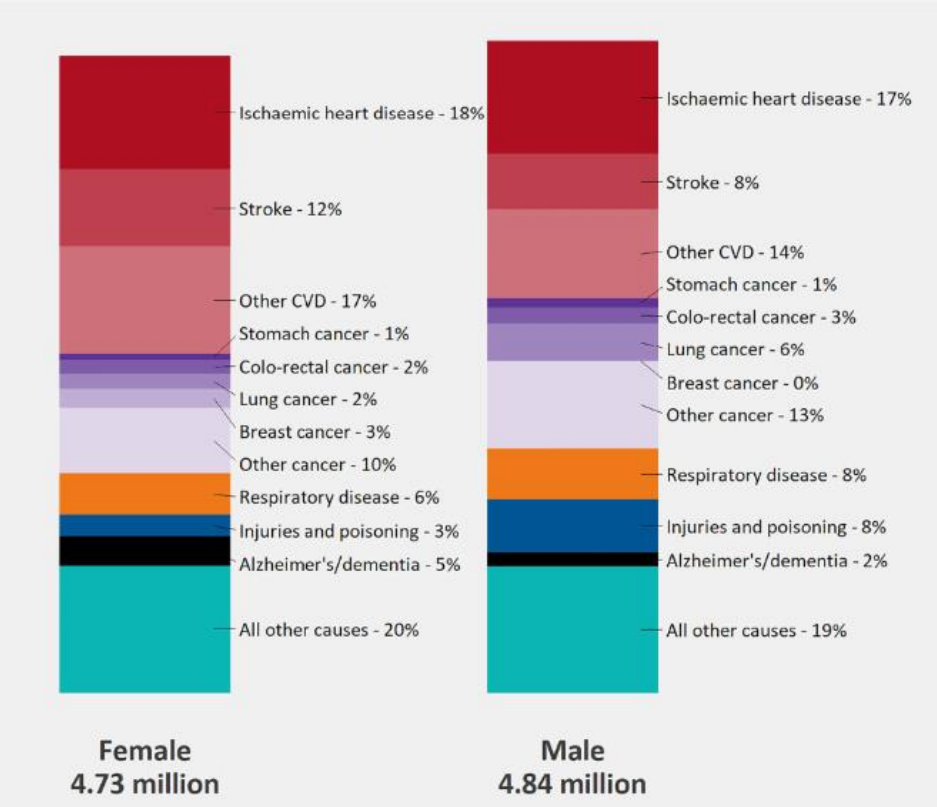

***Figure 1**: Deaths by cause for all ages. Data source: WHO Mortality Database and adapted by Timmis A et al* (4).

In Europe alone, each year CVD causes 2.2 million deaths in women and 1.9 million in men (figure 1) and it accounts for 33% and 29 % of early deaths in men and women, respectively (figure 2) (4).

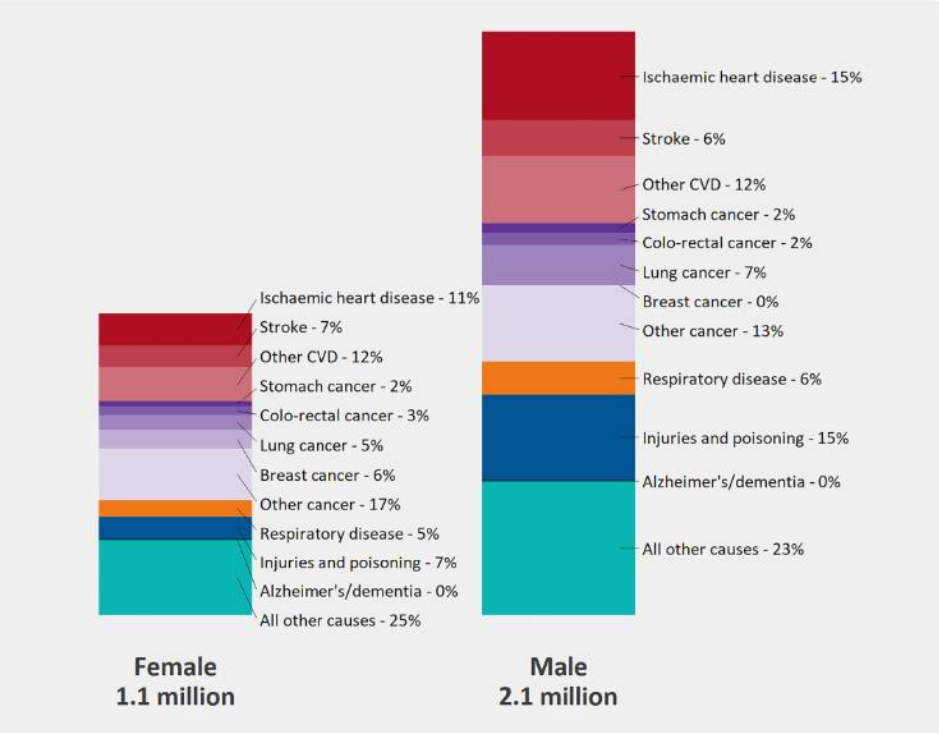

***Figure 2**: Premature deaths by cause for all ages. Data source: WHO Mortality Database and adapted by Timmis A et al* (4).

Additionally, the cost of treating this disease puts a huge burden on the European economy with a cost of almost 111 billion euros annually (5). Moreover, the problem is increasing too; according to data from the World Health Organization (WHO), it is estimated that by 2030 the figure will reach 23.6 million (1). The CVD burden is continuing its decades-long rise for almost all countries outside high-income countries, and alarmingly, in high-income countries the age-standardised rate of CVD has begun to rise in some locations where it was previously declining. There is an urgent need to focus on implementing existing cost-effective policies and interventions if the world is to meet its targets for Sustainable Development Goal 3 and achieve a 30% reduction in premature mortality due to non-infectious diseases (6).

Risk factors are conditions that increase the risk of developing a disease. These factors have previously been investigated through large epidemiological studies (7–10) such as the Framingham Heart Study in 1948, one of the most important studies (11,12). There are two types of risk factors: modifiable and non-modifiable. The non-modifiable factors are age, gender, family history, ethnicity and socioeconomic status. However, we must focus on those factors that can be modified, these being obesity, high LDL-C,

high pressure, diabetes and unhealthy diet, low physical activity (LPA), smoking, and taking drugs (13). High LDL-C remains a major threat to public health, and the overall burden in terms of number of disability-adjusted life years (DALYs), deaths, years lived with disability (YLDs), and years of life lost (YLLs) is increasing globally (6,14,15). The risk associated with high LDL-C is especially high in some locations and deserves immediate public health attention. Health systems and countries may need to focus on new approaches that can reverse these trends. These might include improved government policies on diet and tobacco, school physical activity (PA) programs, and, when needed, the use of lipid-lowering therapy in keeping with contemporary guidelines in which statins are the first choice. The following figure compares the 1990 and 2019 rankings of the CVD burden attributable to modifiable risk factors. It is interesting to note that the positions of nearly all risk factors remain the same (6).

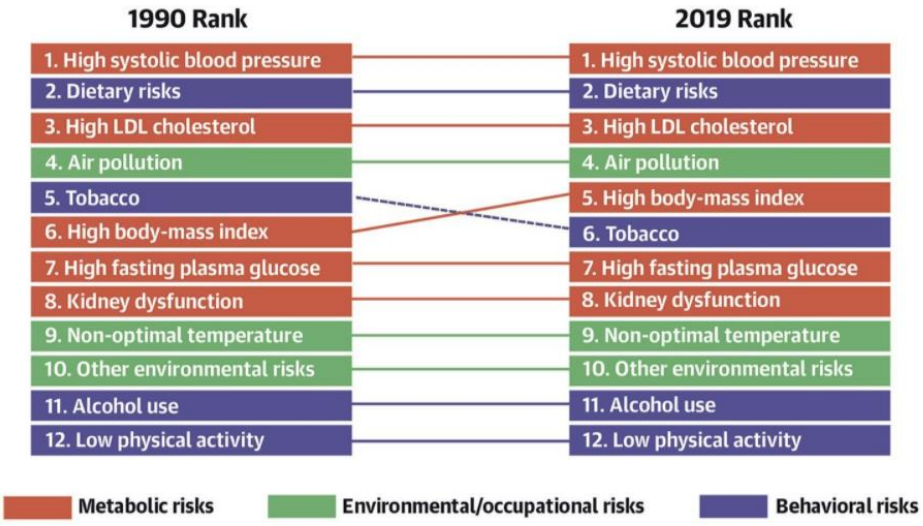

***Figure 3**: CVD burden attributable to modifiable risk factors* (6).

CVD is highly responsive to lifestyle, and therefore represents a preventable disease. The Global Burden of Disease Project recently claimed that unhealthy diet is among the most important contributors to years of life lost due to premature cardiovascular death (16,17). 7.94 million annual deaths and 188 million annual DALYs attributed to dietary risks. Worldwide, the absolute disease burden caused by dietary risks has risen for the last 30 years, regardless of how it is measured (6).

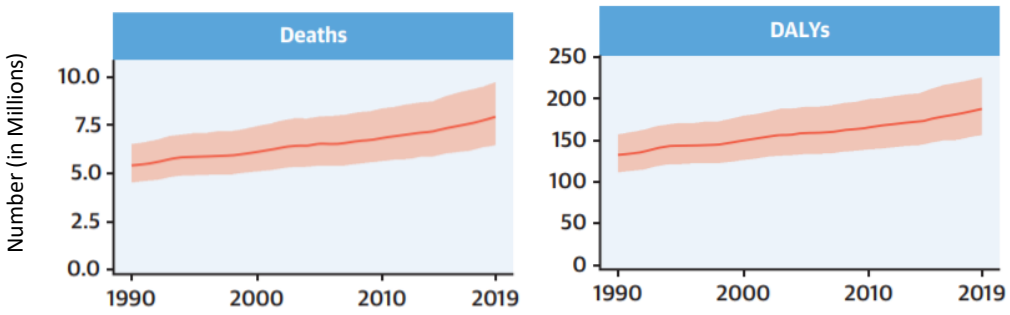

Figure 4: *Total Numbers and Rates of Dietary Risks from 1990-2019. Figure adapted from citation* (6).

Sotos-Prieto and Micha et al. state that poor dietary habits influence CVD significantly (18,19) as have other researchers (20,21). Micha et al. assert that 45.4% of cardiovascular deaths could be attributed to poor dietary habits (19).

Diet is important for reducing mortality, but so too is PA. LPA is an important contributor to premature mortality, morbidity, and DALYs in most countries. Globally, the total number of DALYs due to LPA increased continuously from 8.61 in 1990 to 15.7 million in 2019 (6).

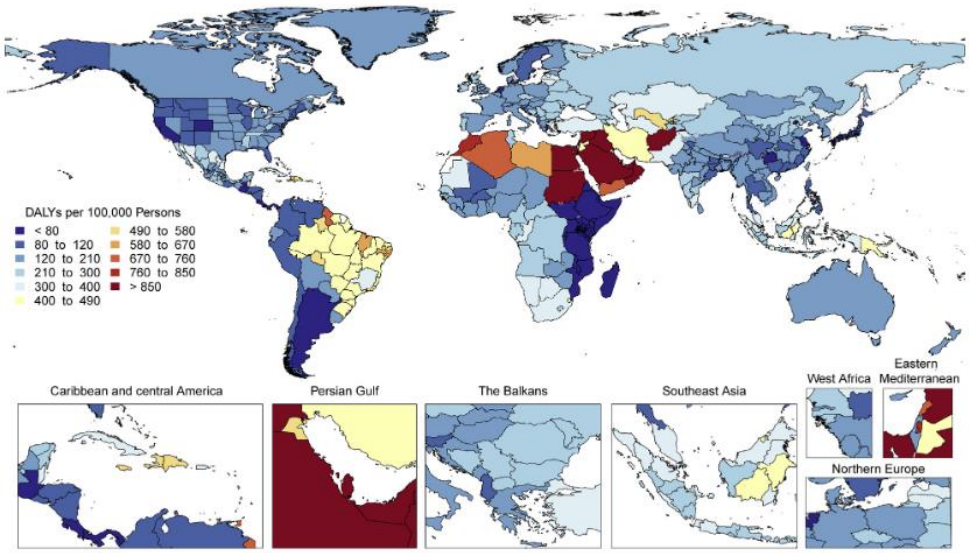

Figure 5*: Map of age-standardised DALYs due LPA in 2019* (6).

LPA remains a major threat to public health. LPA causes multiple pathologies and is considered a pandemic (22–24). Although few health systems have historically focused on LPA, there is increasing awareness and attention being paid to LPA, as exemplified by the 2018 WHO Global Action Plan for PA (25). Increasing PA will depend

on effective partnerships, workplace strategies and collaboration between sectors such as health, education, urban planning, transportation and business (26).

For this reason, it is very important to raise public awareness and modify these risk factors in order to reduce the mortality rate. More global investment in research is needed to address LDL-C related knowledge and therapeutic gaps and thus tackle this persistent global health threat (6).

2.2. Pathophysiology of atherosclerosis

Atherosclerosis is the major cause of CVD. Autopsy studies show that atherosclerosis develops slowly over many years and is a complex process that starts with accumulations of cholesterol and lipids in the intimal space followed by a non-resolving inflammation. This fact produces a complex plaque and, ultimately, clinical disease (figure 6) (15).

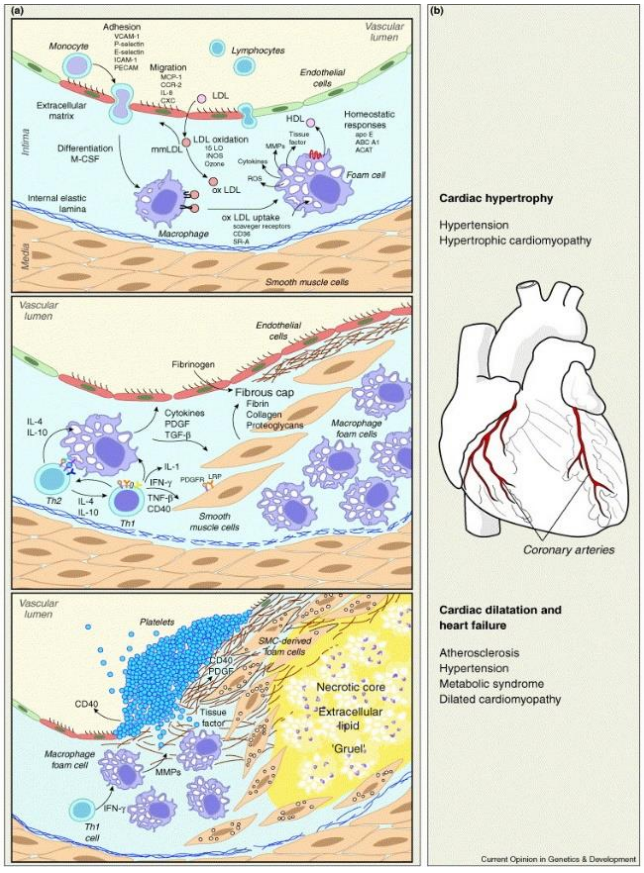

Figure 6: *Evolution of atherosclerosis from fatty streak to the complex plaque and clinical disease.*

Understanding how an atherosclerotic plaque occurs is essential to improving prognosis. A simple description of how the atherosclerotic plaque is formed is the one proposed by Peter Libby (figure 7) (28).

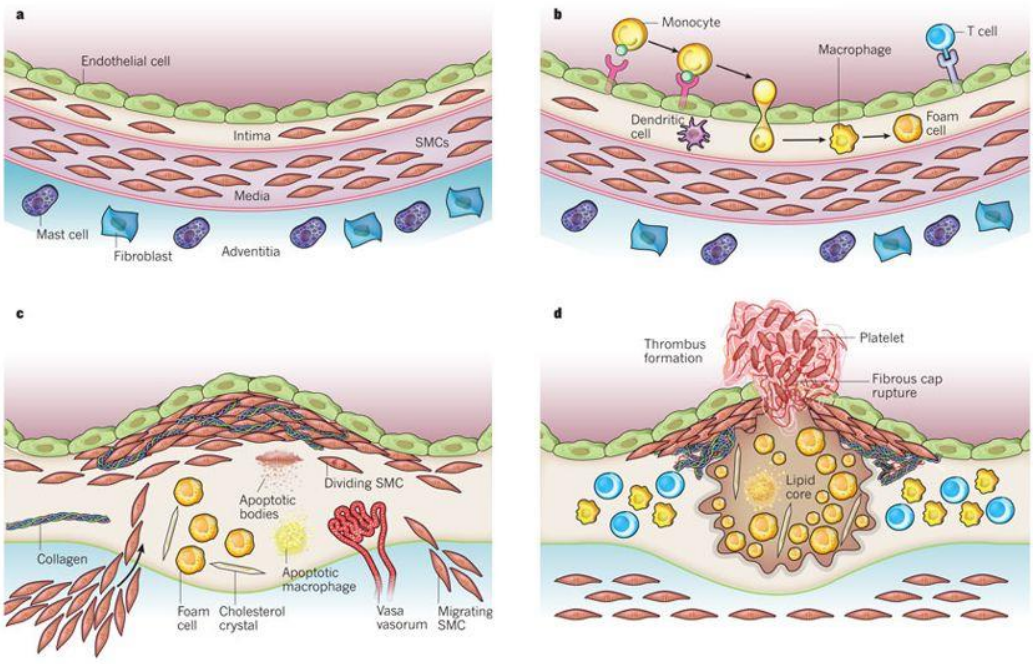

Figure 7: *Different stages in the development of atherosclerotic lesions showed by Peter Libby, et al* (28).

A normal artery contains three different layers. The inner layer is lined with a monolayer of endothelial cells that is in contact with blood overlying a basement membrane. This layer contains resident smooth muscle cells (SMCs). The middle layer contains SMCs embedded in a complex extracellular matrix. Finally, the adventitia, the outer layer of arteries, contains mast cells, nerve endings and microvessels (figure 7a). The first steps of atherosclerosis include: adhesion of blood leukocytes to the endothelial monolayer, directed migration of the bound leukocytes into the intima, maturation of monocytes into macrophages and their uptake of lipid yielding foam cells (figure 7b). The lesions start when the SMCs migrate from the media to the intima layer, and continue with the proliferation of these SMCs and resident intimal SMCs, and the heightened synthesis of extracellular macromolecules (collagen, elastin and proteoglycans) (figure 7c). Plaque macrophages and SMCs can die in advancing lesions, some by apoptosis. Extracellular lipids derived from dead cells can accumulate in the central region of a plaque. Advancing plaques also contain cholesterol crystals and

microvessels. If a plaque's fibrous cap is fractured, different blood coagulation components are exposed to tissue factors in the plaque's interior, triggering the thrombus that extends into the vessel lumen, where it can impede blood flow (figure 7d).

The high cholesterol in plasma results in changes to the arterial endothelial permeability that allow the migration of lipids, especially LDL-C particles, into the arterial wall (29). For this reason, hypercholesterolemia is considered one of the main triggers of atherosclerosis and cholesterol carried by LDL, and TG rich lipoprotein is considered an etiological factor of the disease.

2.3. Lipids and lipoprotein metabolism

Lipoproteins are central particles in the transport and metabolism of exo- and endogenous lipids. Structurally, lipoproteins are complexes of lipids and proteins that are insoluble in water (hydrophobic) (30). For this reason, these lipids must be transported in association with proteins. Lipoproteins are complex particles with a central core containing cholesterol esters and triglycerides (TG) surrounded by free cholesterol (FC), phospholipids, and apolipoproteins, which facilitate lipoprotein formation and function (31).

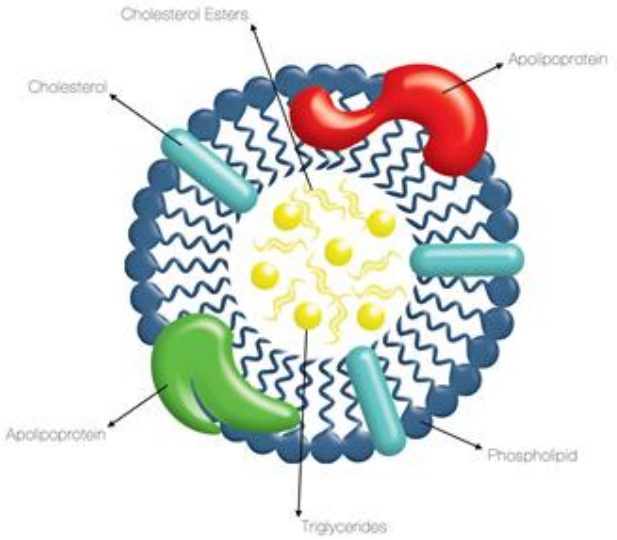

Figure 8. *Structure of a lipoprotein (extracted from wikidoc.org).*

On the basis of size, lipid composition, density and apolipoproteins, plasma lipoproteins can be divided into seven classes: chylomicrons (CM), CM remnants, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), LDL, high-density lipoprotein (HDL), and lipoprotein a (Lp(a)). VLDL, IDL, LDL, and Lp(a) are all pro-atherogenic while HDL is anti-atherogenic. Apolipoproteins have four principal functions including 1) serving a structural role, 2) acting as ligands for lipoprotein receptors, 3) guiding the formation of lipoproteins, and 4) serving as activators or inhibitors of enzymes involved in the metabolism of lipoproteins (32).

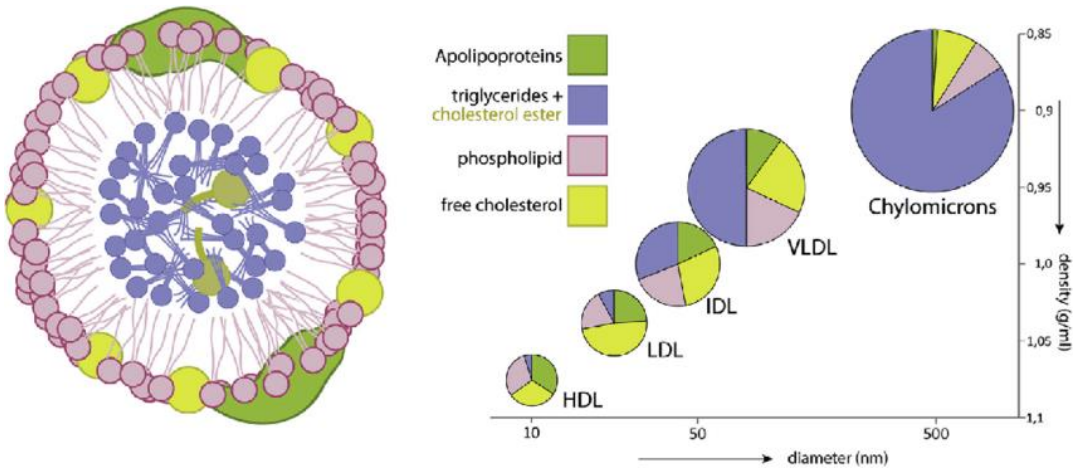

Figure 9: *Lipoproteins are usually classified based on their density by ultracentrifugation into five classes; CM, VLDL, IDL, LDL, and HDL. CM are the largest lipoproteins and HDLs are the smallest* (33).

We ingest lipids mostly in the form of TG, but also some free fatty acids (FA), cholesterol and phospholipids. Gastric and pancreatic lipase and bile acids contribute to the hydrolysis and emulsification of the lipids into micelles, free FA and 2-monoacylglycerol, which then enter the enterocytes by facilitated diffusion. Once inside the enterocytes, the lipids are dependent on CM formation for transport in the blood (30).

The exogenous pathway of lipoprotein metabolism involves the absorption of dietary fat and cholesterol in the intestine, and these lipids are incorporated into CM (34,35). CM are the largest and least dense of the lipoproteins, containing one

apoprotein B (ApoB). CM circulate in the blood and function as a substrate for lipoprotein lipase (LPL) in adipose and muscle tissue where TG from CM is hydrolysed and decreases in size (36). The remaining particles after depletions of CM are called the remnant. This remnant continues circulating until it is removed from circulation by lipoprotein receptor-related protein (LRP) in liver cells (37).

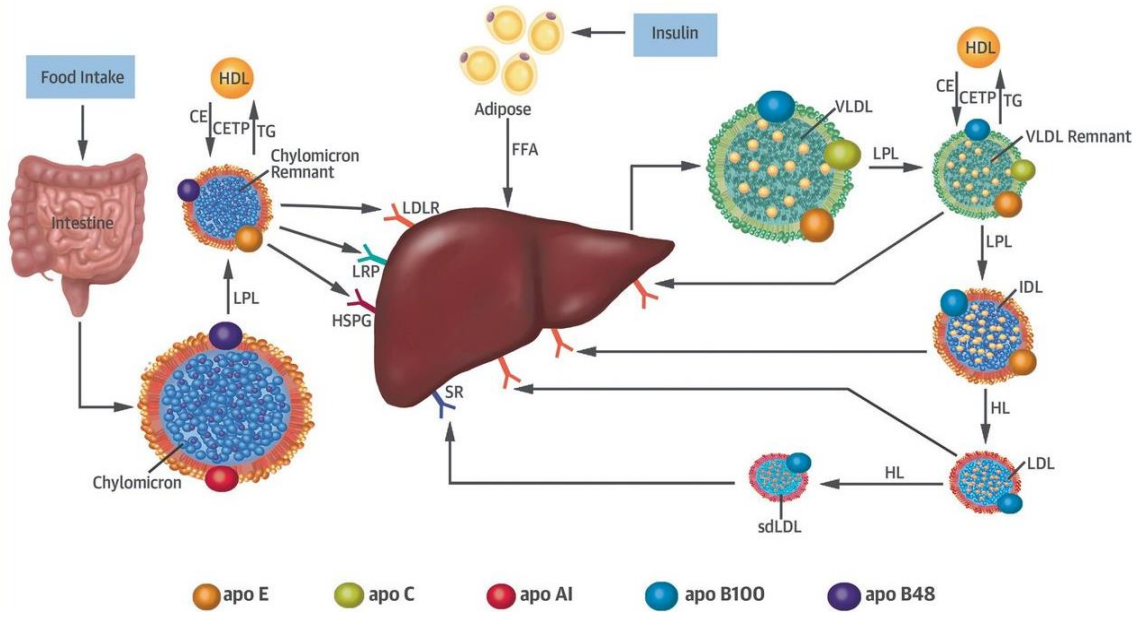

***Figure 10**: Remnant lipoprotein metabolism. The figure is taken from Saeed A, et al.* (37).

Endogenous pathways start in the liver, where cholesterol and TG are incorporated into VLDL, which is released into the blood. VLDL contains one ApoB, TG and cholesteryl esters (CE). VLDL is the first lipoprotein of the endogenous lipoprotein metabolism and is responsible for the distribution of TG to peripheral cells by the LPL enzyme. This LPL enzyme will cleave FA, making it smaller as it circulates and emptying it with TG (36). As the particle now is reduced in size, it is referred to as IDL. IDL is cleared from plasma by the LDLR; However, if the particle remains in the circulation and continues to decrease in size due to further hydrolysis of TG by LPL and hepatic lipase (HL), it became a LDL particle. LDL carries most of the cholesterol in the circulation. LDL contains one ApoB on the outer surface with FC, phospholipids and an inner core enriched with CE and TG. The ApoB has a ligand function for the LDLR. Through the uptake of the LDLR, the LDL-C levels in the blood are reduced. When the concentration

of LDL and ApoB in the blood is elevated, the particles can accumulate in the carotid intima-media thickness (cIMT).

The HDL particle contains one apolipoprotein A (ApoA) and is responsible for the transport of cholesterol from tissue back to the liver in reverse cholesterol transport. HDL metabolism is complex, but one of the roles of HDL is to obtain cholesterol from peripheral tissue and other lipoproteins and transport it to where it is needed most. HDL exchange cholesterol, TG, phospholipids and apolipoproteins with CM, VLDL and LDL during lipolysis as they pass in circulation (38). The overall effect is anti-atherogenic.

Finally, Lp(a) is a LDL that contains apoprotein (a) and may also directly promote atherosclerosis. This apoprotein is characterised by 5 cysteine-rich regions called kringles. One of these regions is homologous with plasminogen and is thought to competitively inhibit fibrinolysis and thus predispose to thrombus formation. For this reason, it is crucial to analyse Lp(a) in patients in order to better estimate the CVR (39,40).

2.3.1. LDLR

LDLR is a crucial molecule for lipoprotein metabolism; it plays a major role in cholesterol metabolism through the receptor-mediated endocytosis of LDLP and regulation of intracellular cholesterol homeostasis (41). LDLR is a single-pass transmembrane protein in the plasma membrane that has a binding domain on the cell exterior for ApoB-100 and a cytosolic domain that binds the AP2 adaptor.

LDLR receptors were defined in the classic studies by Goldstein and Brown, who described a process for transporting large lipoprotein particles (∼24 nm) across the cell membrane (figure11) (42) .

LDLR is synthesised at the endoplasmic reticulum. LDLR is then transported to the Golgi where glycosylation takes place. Mature LDLR is transported to plasma where it will perform its function. The first step in this process is the interaction between the ApoB of LDL in the cysteine-rich receptor-binding domain of LDLR. The LDLR/LDL complex undergoes endocytosis with subsequent delivery to the lysosome where the particle is degraded to its components. LDLP components are targeted for lysosomal

degradation, whereas the LDLR is recycled to the cell surface. Delivery of FC inhibits endogenous cholesterol synthesis and continuous exogenous delivery (43).

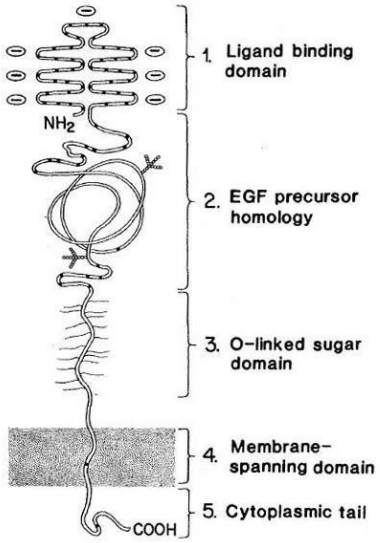

***Figure 11**: Structure of the LDLR receptor proposed by Brown MS and Goldstein JL* (42).

LDLR genetic variants affect different LDLR cycles and result in dysregulation of the cycle (figure 12) (44). Patients with FH have low levels of LDLR and thus a greater risk of atherosclerosis due to the increase in circulating cholesterol and its deposition in the arteries.

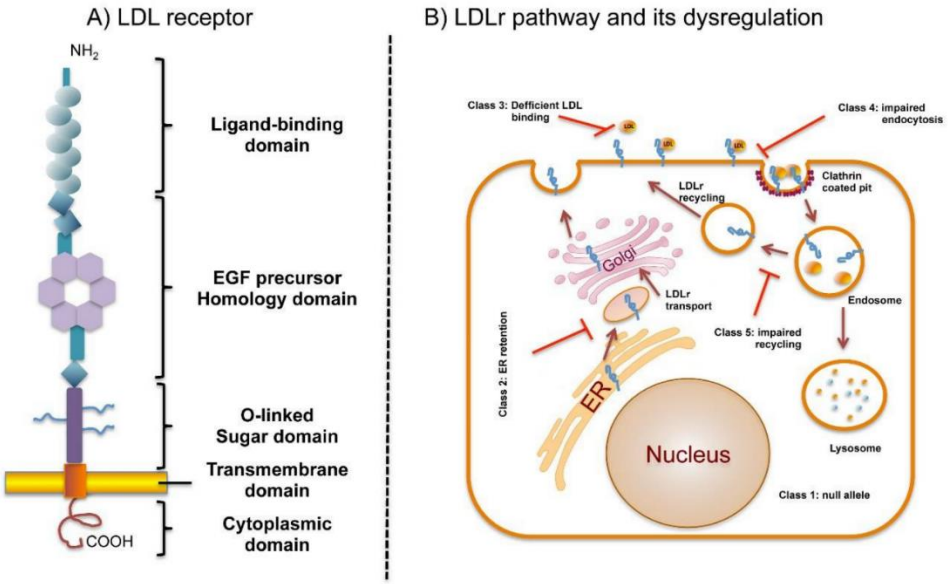

***Figure 12**: Domain organization of LDLR and LDLR pathway and its dysregulation by defective genetic variants. Figure proposed by Benito-Vicente A et al* (44).

2.3.2. Lipoprotein profile assessed by nuclear magnetic resonance (NMR)

Despite, the complexity of lipid and lipoprotein metabolism, the usual clinical approach to assessing lipoprotein profile involves the biochemical determination of total cholesterol, HDL-C and TG and the calculation of LDL-C.

In recent years it has been shown that knowing the amount and size of lipid particles gives us more accurate information about cardiovascular risk. The quantity and quality of lipoprotein particles can be evaluated using a metabolomics technique based on nuclear magnetic resonance (NMR). This technique measures the number and size of lipoprotein particles and then arbitrarily assigns the lipoproteins to different subgroups according particle size.

Metabolomics studies allow us to obtain more information and predict possible adverse effects and NMR technology is well-validated for metabolomics in epidemiology (45,46). Different methods and different companies use this technology (for example Liposcale®(47) and Nightingale® (45)).

Recent guidelines recommend the study of LDL subclasses to improve CVR (48–50). The number of LDLP and, in particular, the smaller LDLP provide a better prediction of CVD than the standard lipid profile (51–54). The smaller LDLP detected by NMR include small and dense LDLP (sdLDLP) defined by density gradient to between 1.034 and 1.063 g/mL (55). sdLDL are important because they are more easily oxidized, they can pass through the membrane easily and they have less affinity for the receptor (56,57). Figure 13 shows all the mechanisms.

Plasma LDL-C may not reflect the real concentration of LDLP. In general, the higher LDL-C, the higher the LDLP. However, some individuals have excess cholesterol per LDLP number and vice versa. These individuals are referred to as discordant. In patients with discordant LDL-C and LDLP values, LDLP offers a better prediction of CVR (58). For this reason, it would be interesting to determine the LDLP profile of patients with a higher CVR, for example patients with FH.

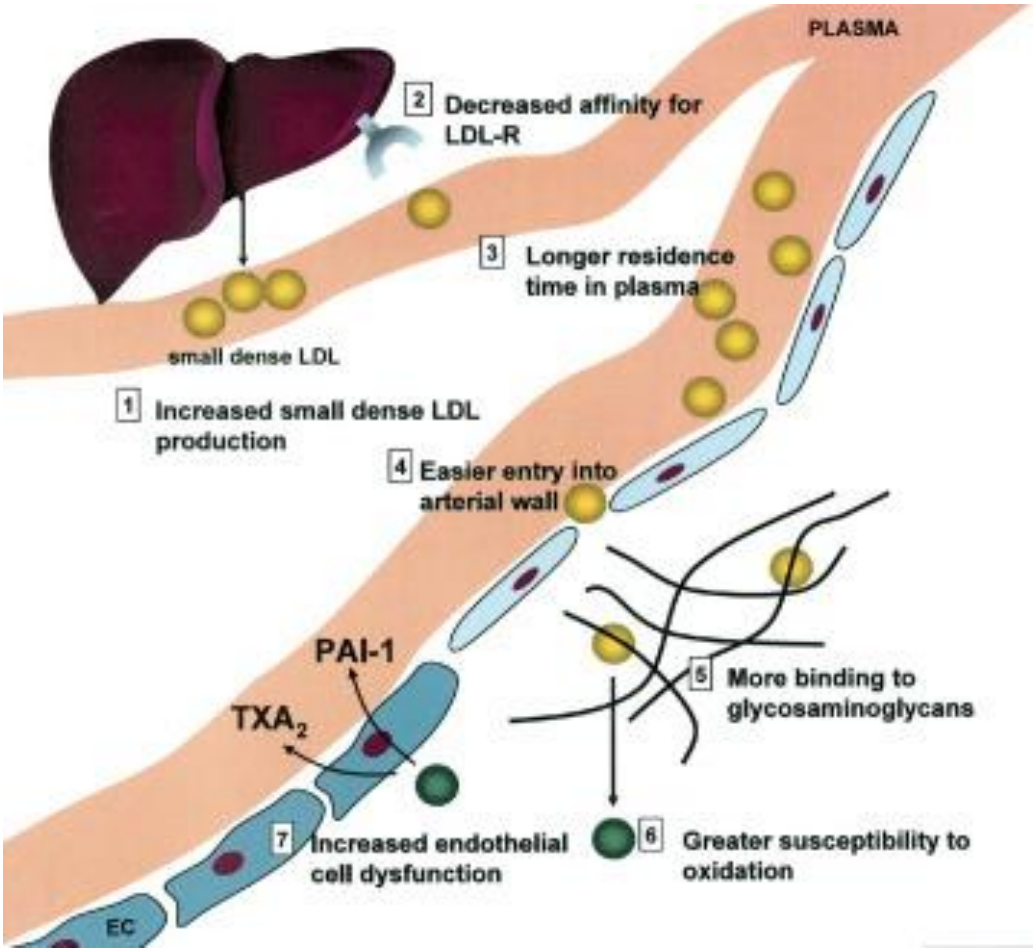

***Figure 13**: Proposed Biochemical and Cellular Mechanisms of sdLDL particles metabolism. The figure is shown in Ikezaki's article* (54)

2.4. Familial hypercholesterolemia in children

2.4.1. Definition and context

FH is the most common autosomal dominant genetic disorder of lipoprotein metabolism. It is transmitted to 50% of offspring (59–61). It was first described by Müller in 1938 (62). Initially, the FH defect was long thought to be caused by oversynthesis of cholesterol (63), and it was not until the mid-1970s that Brown and Goldstein found that the FH defect was due to the absence of a high-affinity receptor for the uptake of LDL-C (64,65). These investigators characterised the LDLR pathway and its implications in other pathways and identified the genetic defect that caused malfunction of the LDLR (42). Since then, the disease has been described as a defect in the cellular capture of LDL-C (42,66–68). In each age period, children with heterozygous FH (HeFH) have, on average, LDL-C levels three times higher than non-affected children (69,70), and this could manifest in premature coronary disease in adulthood (71–73).

FH is classified into two groups: 1) Heterozygous FH (HeFH), which is the most frequent disorder due to a defective allele of the *LDLR* gene or associated protein and which is clinically characterised by the presence of LDL-C above 200 mg/dL; and 2) Homozygous FH (HoFH), which is much stronger and characterised by genetic variants in both the allele of the *LDLR* gene and the associated protein. The HoFH group, usually, encompasses defective alleles of different genes involved in the disease and they are referred to as compound heterozygous and double heterozygous. It is clinically characterized by the presence of very high LDL-C, above 500 mg/dL (66,74–76).

FH is mainly caused by a mutation in the *LDLR* gene, although it can also be due to defects in *ApoB, protein convertase subtilisin-Kexin type 9 (PCSK9)* and *LDLR adapter protein 1 (LDLRAP1)* genes (figure 14) (77,78). The principal mutation is in the *LDLR* (64,76,79). So far, 1700 genetic variants have been described for this gene located on chromosome 9, which account for 93% of the total genetic variants (80,81). Not all genetic variants have the same pathogenicity. Five classes are involved in *LDLR* genetic variants and their implications are described in figure 15 (77).

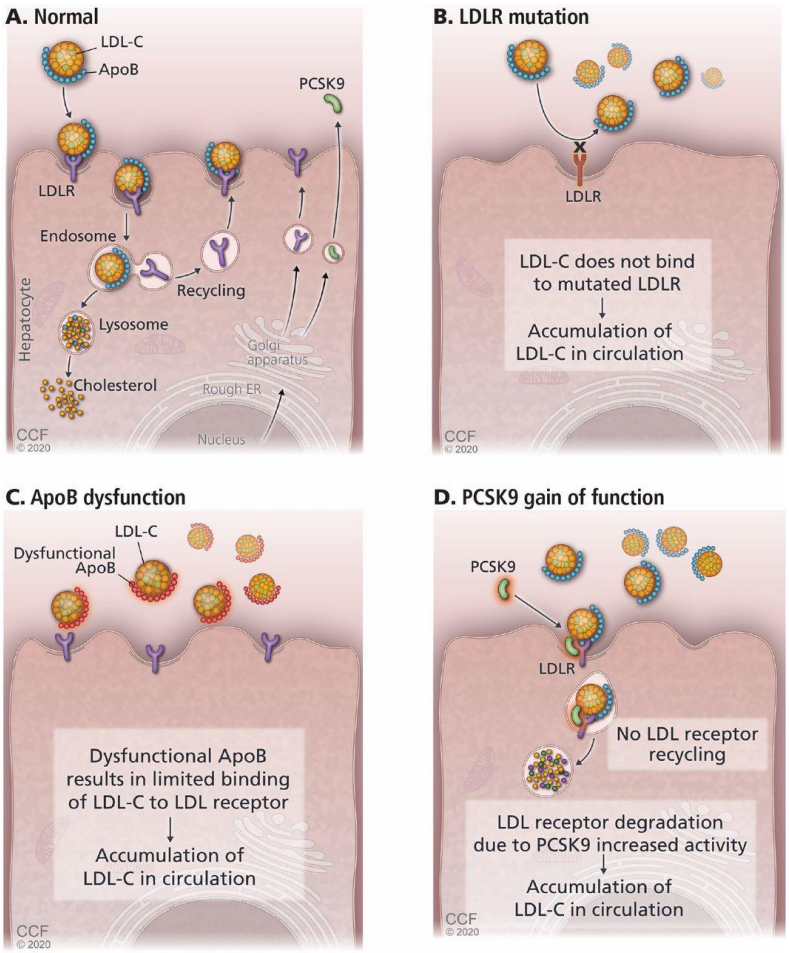

Figure 14. *Main genetic variants causing FH disease. Image extracted of Shah NP et al* (82).

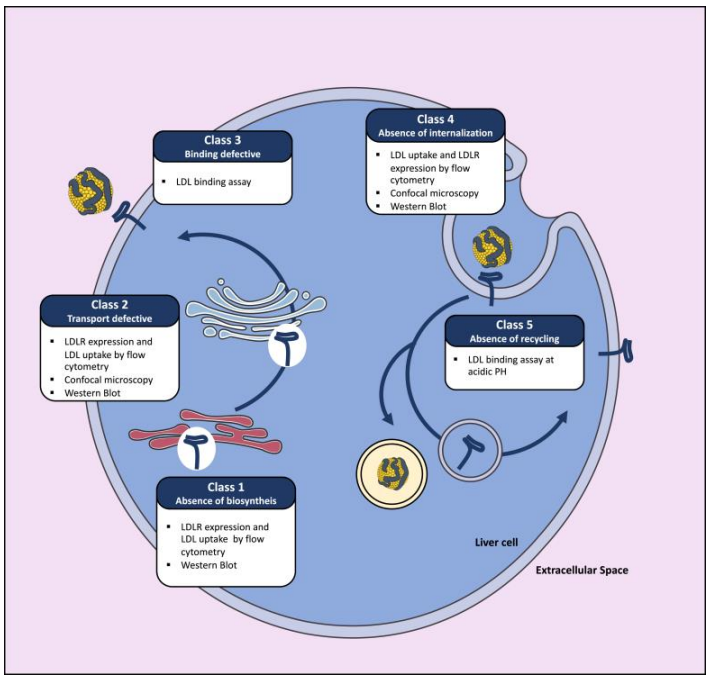

Figure 15. *LDLR class defects. The figure of Chemello K, et al* (77).

To a smaller extent, we find defects in the gene that encodes *ApoB* (83,84) and *PCSK9* (85,86). ApoB is located in the short arm of chromosome 2, which produces a protein with limited binding capacity with LDLR and therefore an increase in LDL, and in the gene that encodes the *PCSK9*, located in the short arm of chromosome 1 and encodes a protein that binds to the RLDL-ApoB complex, thus favouring the intracellular degradation of the receptor. These two genetic variants reappear as 5% and 1% of the total genetic variants, respectively.

Finally, another mutation has been described, *LDLRAP1* (87), which is also found in the short arm of chromosome 1 and which is very uncommon because it is a recessive form and represents less than 1%. Mutations have also been described in the STAP1 gene, in the gene of apoprotein E (ApoE) (88) and other minor determinants (figure 16).

FH-associated genetic variants are classified as null when their functional capacity is less than 2% and defective when their residual functional capacity is between 2 and 20%. In 5-30% of cases, with definite FH phenotype, the causative gene variant cannot be identified (61,70,89,90).

Gene	Inheritance pattern	OMIM number	Proportion of patients with monogenic FH (%)	Mutation types
Major determinants				
LDLR	Autosomal co-dominant	606945	80–85	Splicing, frameshift, copy number variation, nonsense, and missense
APOB	Autosomal co-dominant	107730	5–10	Frameshift, missense, nonsense, and splicing
PCSK9	Autosomal co-dominant	607786	<1	Frameshift and missense
LDLRAP1	Autosomal recessive	605747	<1	Frameshift, missense, and nonsense
Minor determinants				
APOE	Autosomal dominant	107741	<<1	Missense
STAP1	Autosomal dominant	604298	<<1	Missense
LIPA	Autosomal recessive	613497	<<1	Frameshift
ABCG5	Autosomal recessive	605459	<1	Nonsense
ABCG8	Autosomal recessive	605460	<<1	Unproven (only by analogy with *ABCG5*)

Figure 16. *Summary of FH genetic variants described by Berberich AJ et al.* (91).

2.4.2. Epidemiology and prevalence

Until a few years ago, it was estimated that the prevalence of FH was 1:500 in HeFH individuals and 1:1,000,000 individuals in HoFH (92,93). More recent studies have found that the prevalence of HeFH is in fact up to twice as high as previously reported. European data have confirmed that HeFH has an incidence of 1/200-250 and that HoFH has an incidence of 1/160,000-300,000 (66,94–96). Focusing on our study populations, Zamora et al. describe the prevalence of HeFH in Catalonia as 1/217. Other studies around the world describe similar prevalence (97). In populations where there is a certain consanguinity such as Lebanese Christians (98), Ashkenazi Jews of Lithuanian descent (99), Afrikaner South Africans (100), French Canadians (101), Druze (102) and Finns (103), the prevalence increases to 1/100 (104). Differences in prevalence rates in various populations are partly attributed to the inherent genotypic/phenotypic variability of FH and to the discrepancy in the criteria used to identify FH individuals in the studies (105). The following picture shows the prevalence and criteria of FH in different countries (figure 21).

The prevalence rate has also increased for HoFH, reaching values of approximately 1:800,000 based on a German population (106), 1:450,000 in Spain (107), 1:300,000 in the Netherlands (94) and 1:160,000 in Denmark (71).

It is estimated that 35 million people worldwide could present HeFH (71). In Europe it is calculated that there are about 4.5 million FH patients and around 20-25% are children and adolescents and only 6% are diagnosed (71,108). In the Netherlands, for example, the detection rate is much higher (70%) thanks to universal screening programmes.

FH affected people have 10-15 times more chance of developing premature CVD compared to non-affected individuals. It is estimated that 85% of men and 50% of women will experience some coronary events before 65 years of age if they do not receive adequate treatment (109).

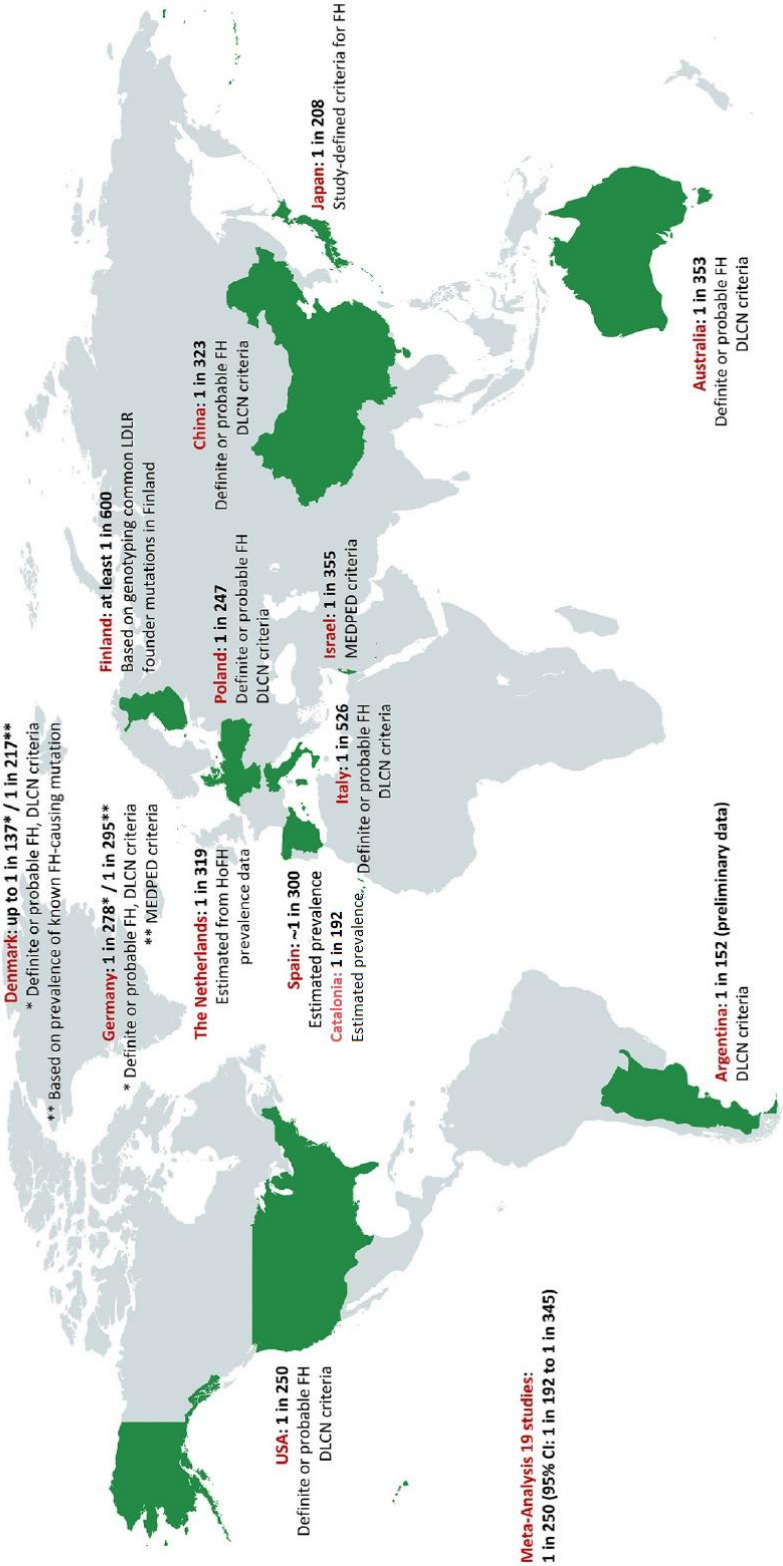

Figure 21. *Overview (non-systematic review) of overall prevalence rates reported from contemporary studies for HeFH in the general population. Image adapted from Vallejo-Vaz A and Kausik R publication* (110).

A systematic review including 104 studies estimates FH prevalence in subjects with ischemic heart disease (IHD), premature IHD, and severe hypercholesterolemia compared with those in the general population. Compared with 1:313 among subjects in the general population, the FH prevalence is 10-fold higher among those with IHD, 20-fold higher among those with premature IHD, and 23-fold higher among those with severe hypercholesterolemia (111) (figure 22).

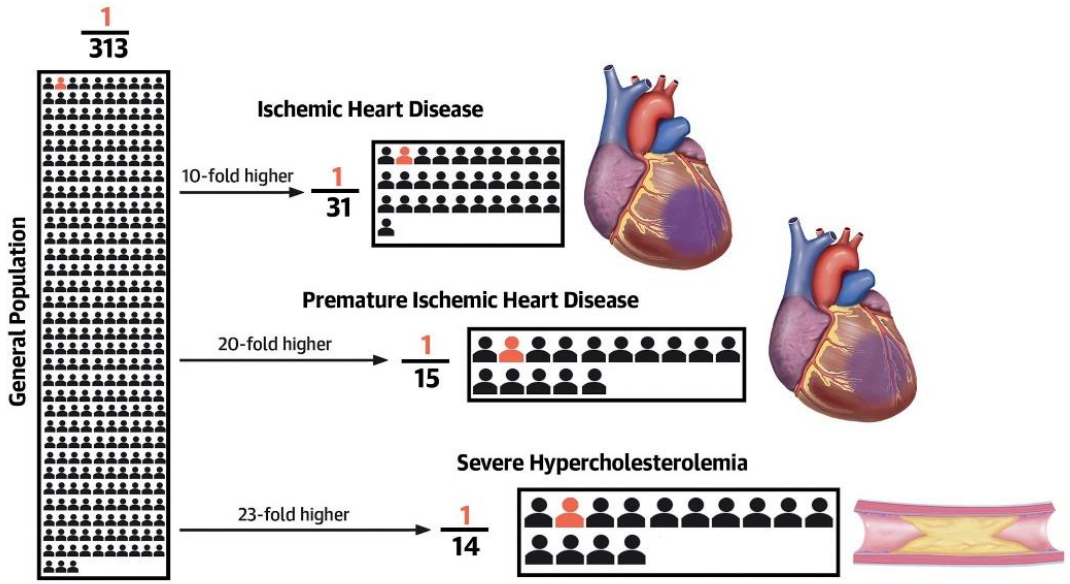

Figure 22. *Prevalence of FH according to CV event. Illustrations from Beheshti, 2020* (111).

The EUROASPIRE study, which included patients with CVD (7,000 subjects), also described an FH prevalence of 8.3% (the percentage being higher among women) (112).

In summary, the prevalence of FH is unknown in 90% of countries in the world and even less so in the case of children with FH. Determining the prevalence in children is key to avoiding CV events in adulthood (66,71,97). Early diagnosis and early treatment are important to improve FH prognosis and evolution in the long term.

2.4.3. Clinical manifestations

FH is characterised by high LDL-C and normal TG (or slightly elevated). Patients with FH usually have twice as much cholesterol as the rest of the population. This accumulation can lead to various physical signs.

In adults, the accumulation of cholesterol can be manifested in the form of tuberous or tendinous xanthomas (figure 23A-B), corneal arch (figure 23C) and xanthelasma (105).

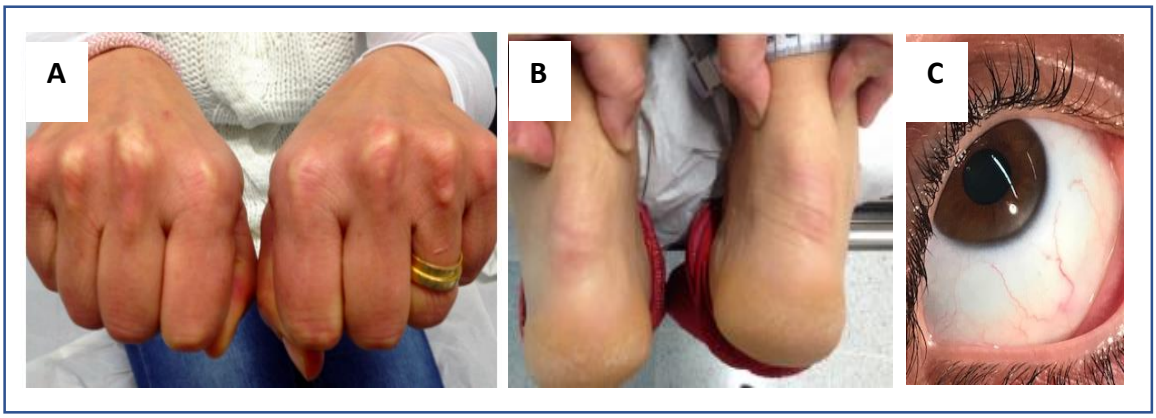

Figure 23. *Images of FH clinical expression. Images provided by Dr. Plana.*

Achilles tendon xanthomas and the corneal arch are included in the "Dutch Lipid Clinic Network" scoring. This score makes it possible to clinically diagnose FH in adults (71).

Xanthomas can appear anywhere on the body but are most commonly found in the extensor tendons of the hands and Achilles tendons. Xanthomas have been described as associated with a three times higher risk of CVD among FH patients (113). This shows that is important to detect xanthomas because these patients need more aggressive lipid-lowering therapy (LLT) (114). However, tuberous and tendon xanthomas are not always detected and are found in less than 30% of cases with a genetic diagnosis. Therefore, their absence does not rule out an FH diagnosis (114–116).

The corneal arch should only be counted in young patients (less than 45 years) because afterwards it can be due to different causes. This sign is associated with carotid artery stenosis too (117).

In children with HeFH, these clinical manifestations are not found because the children have not accumulated enough cholesterol. Even so, echography of the Achilles tendons of FH children have shown that they are thicker than in non-FH children (118).

However, in HoFH children, who have very high LDL-C, those physical signs can already be present even at an early age (figure 24) (70). Children with HoFH are at high risk of developing coronary heart disease and aortic valve disease before the age of 20 if they are not treated intensively (66).

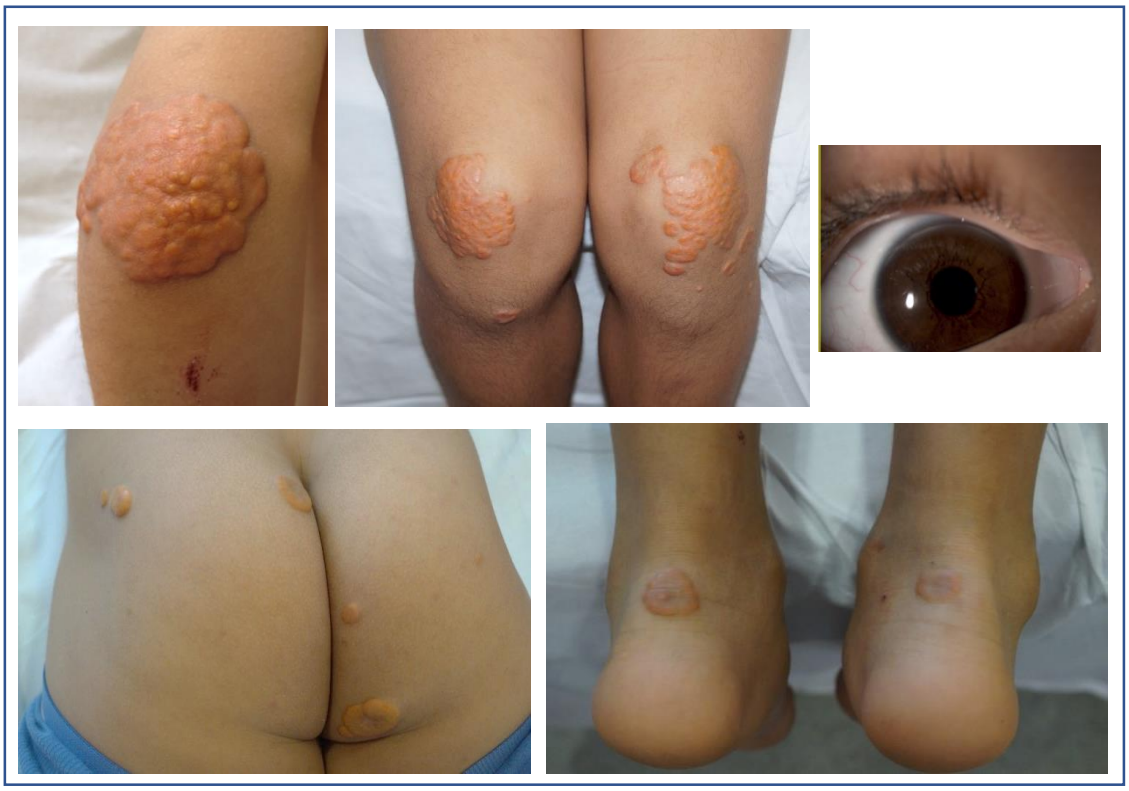

Figure 24. *Images of HoFH clinical expression. Images provided by Dr. Plana and Dr. Ibarretxe.*

The increase in LDL-C not only produces physical signs. The biggest problem with the accumulation of LDL-C is the increase in CVR. FH is the genetic disorder most commonly associated with premature atherosclerotic disease (72).

High cholesterol levels result in changes to the arterial endothelial permeability that allow the migration of lipids into the arterial wall (29). It has been observed that endothelial function is impaired in children with familial hypercholesterolemia (119); in fact, two studies have demonstrated that the atherosclerotic process even dates back to the foetal stage (120,121). In addition to these early functional changes, accumulation of LDL-C in children with familial hypercholesterolemia leads to deterioration of the vascular morphology and gives rise to increased cIMT (122–127). A systematic review shows that FH children (as young as 7 years) had higher cIMT than their unaffected peers

(128). Finally, this accumulation in the arterial wall causes inflammation and the appearance of atherosclerotic plaques and therefore increases CVR (119).

Figure 25 shows the importance of detecting FH in children and starting treatment at an early age to substantially delay the progression of atherosclerosis.

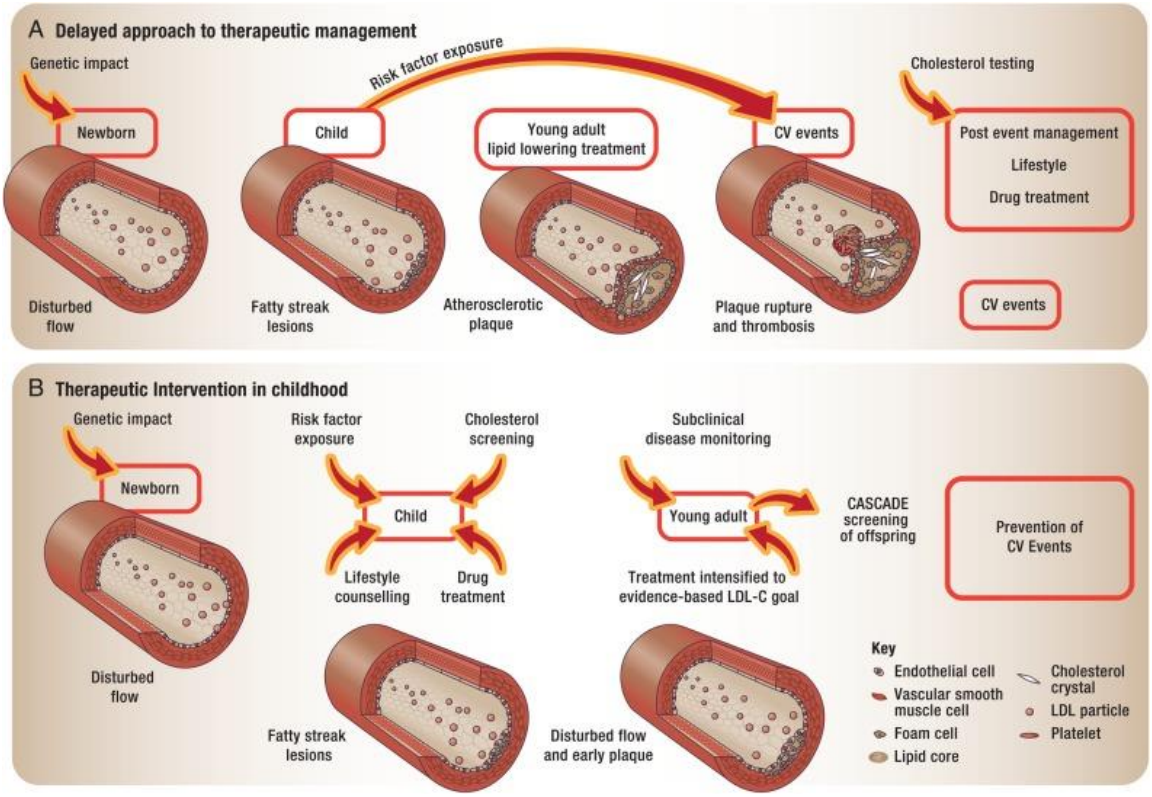

Figure 25. *Development of early atherosclerotic vascular disease in FH children* (66).

2.4.4. Diagnosis

The FH diagnosis is based on either clinical criteria and/or genetic testing. In adults, the clinical criteria recommended by the European Atherosclerosis Society (EAS)/European Society of Cardiology (ESC) are the DLCN. This score is based on family and personal history, physical examination, biochemical data and genetic study. The diagnosis categories are: >8 definite, 6-7 probable, 3-5 possible and <3 unlikely. Unfortunately, this type of score cannot be used in children and should only be applied to parents.

FH may be suspected in children in at least three situations: a child from a family where HeFH has been identified or is suspected (clinical/genetic criteria); a child from a

family with a history of premature CHD (below 55 years in men and 60 years in women), a child with one or both parents displaying primary hypercholesterolaemia or absence of information about parents with levels above the p95 (≥135 mg/dL). This emphasises the importance of assessing family history in terms of cholesterol levels, CHD and confirmed or suspected conditions in all children (66,119). Morais Lopez and Arroyo Díez were described the reference lipid values in the Spanish children population considering LDL-C >130 mg/dL as high (129,130).

The detection of the FH causative mutation is considered the gold standard FH diagnosis (131). However, genetic testing is expensive and not always available. Searching for causative genetic variants is important because in FH-affected families, some individuals have positive genetic variants even with normal LDL-C concentrations. On the other hand, individuals with a definite FH phenotype have no detectable genetic variants. These patients and affected families are now thought to carry a number of small gene variations that lead to high LDL-C levels and are considered polygenic forms of FH.

Therefore, FH can be also diagnosed according to clinical criteria, based on the presence of high LDL-C levels in a child from an FH family according to criteria depicted in table 1.

- Family history of premature CHD plus high LDL-C levels are the two key selective screening criteria.
- Cholesterol testing should be used to make a phenotypic diagnosis.
- An LDL-C level ≥5mmol/L (190 mg/dL) on two successive occasions after a 3-month diet indicates a high probability of FH. A family history of premature CHD in close relative(s) and/or baseline high cholesterol in one parent, together with an LDL-C ≥4mmol/L (160 mg/dL) indicates a high probability of FH. If the parent has a genetic diagnosis, an LDL-C ≥ 3.5mmol/L (130 mg/dL) suggests FH in the child.
- Secondary causes of hypercholesterolaemia should be ruled out.
- DNA testing establishes the diagnosis. If a pathogenic LDLR mutation is identified in a first-degree relative, children may also be genetically tested.
- If a parent died from CHD, a child even with moderate hypercholesterolaemia should be tested genetically for FH and inherited elevation in Lp(a).

Table 1. *Diagnosis of familial hypercholesterolaemia in children and adolescents* (66).

Sometimes the clinical diagnosis in children is difficult due to a phenotypic overlap with multifactorial hypercholesterolemia caused by the interaction between genetic and environmental factors (132,133).

Before suspecting FH in children, it should be noted that secondary causes (table 2) must be ruled out and LDL-C levels should be tested again after TLSC implementation. (66,71).

1. Drugs: amiodarone, corticosteroids, anabolic steroids, cyclosporin, progestogenic phenobarbital, phenytoin, thiazides, etc.
2. Anorexia nervosa.
3. Cholestasis: biliary cirrhosis, biliary laryngitis.
4. Growth hormone deficit.
5. Endocrine diseases: hypothyroidism, hypopituitarism.
6. Renal diseases: Sd. Nephrotic.
7. Idiopathic hypercalcemia.
8. Acute Intermittent Porphyry.
9. Depot diseases: Glucogenosis, Tay-Sach, Gaucher, Niemann Pick.
10. Dietary factors.

Table 2. *Different causes of hypercholesterolemia in childhood.*

2.4.5. New insights into FH detection and biochemical characterization

2.4.5.1. Potential plasma biomarkers of LDLR function

It is well known that LDL-C values in FH individuals sometimes overlap normal concentrations and hinder FH detection (132). Therefore, there is a growing interest in searching for new biomarkers to detect this pathology both in children and adults. Among the putative FH markers, three molecules associated with LDLR expression stand out because their soluble fractions can be detected circulating in plasma, these being the soluble LDLR (sLDLR) itself, IDOL and PCSK9.

LDLR is the main molecule that regulates LDL-C in plasma concentrations (134). This cell surface receptor is expressed primarily in the liver and removes cholesterol-carrying LDL-C from plasma by receptor-mediated endocytosis (42). Genetic variants in LDLR result in a substantial increase in the plasma LDL-C levels and higher CVR.

Moreover, drugs that induce LDLR upregulation decrease the LDL-C concentration, reducing the occurrence of CV events. Several meta-analyses have shown that a reduction in LDL-C of 1 mmol (39 mg/dL) decreases the relative risk by 23% (figure 27) (135). There is soluble fraction of LDLR that can be measured in plasma and the mechanisms controlling its function and expression need to be researched, given its clinical significance.

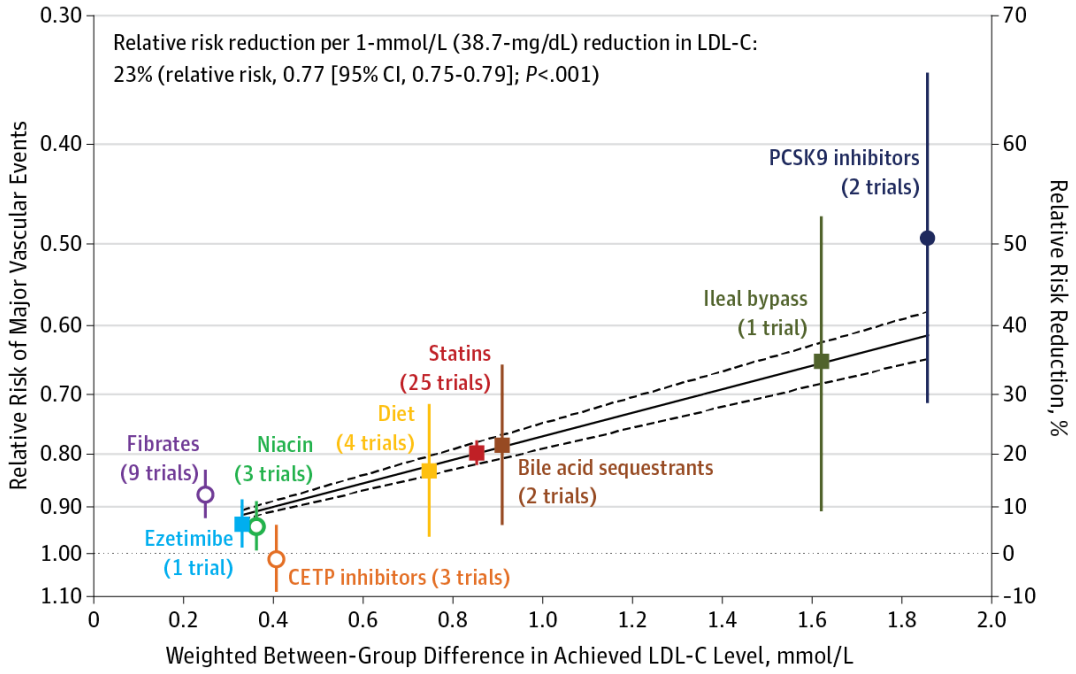

Figure 17. *The importance of lowering LDL-C to reduce relative risk; described by Silverman MG, et al* (135).

IDOL is an E3 ubiquitin ligase that mediates the ubiquitination and degradation of the *LDLR* (136). IDOL expression is controlled at the transcriptional level by the cholesterol-sensing nuclear receptor liver X receptor (LXR). Increased cholesterol in the hepatocytes induces LXR and stimulates the IDOL expressions, thereby limiting further uptake of exogenous cholesterol through the LDLR pathway (137,138). Interestingly, genome-wide association studies have identified IDOL as a locus associated with circulating LDL-C concentrations (139,140), and loss-of-function genetic variant have been associated with low LDL-C (141) (figure 18). IDOL has attracted increasing interest as a putative target for hypercholesterolemia treatment (142).

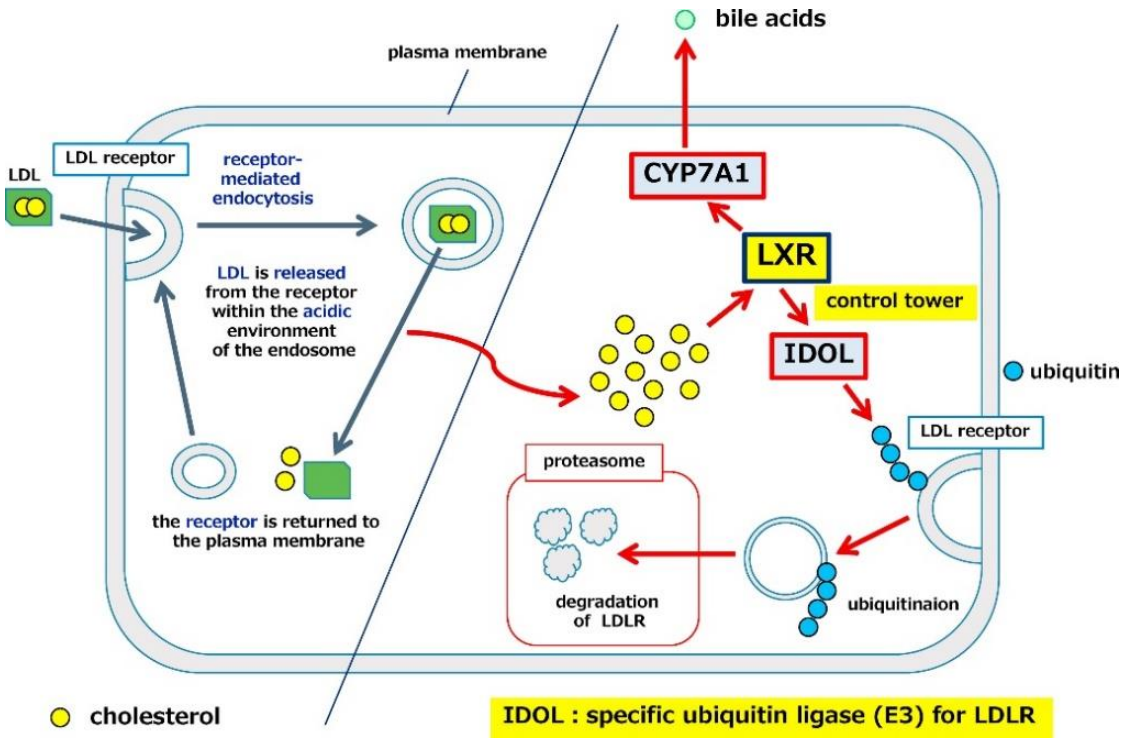

Figure 18. *The CYP7A1 activation by LXR and LDLR degradation by IDOL* (143)

Because LDLR degradation is enhanced by PCSK9, PCSK9 inhibition increases LDL-receptor recycling and LDL uptake (figure 19). PCSK9 has emerged as a central functional regulator of *LDLR*, and its expression parallels that of LDLR according to intracellular cholesterol content (144). PCSK9 binds to LDLR that is internalized with it. PCSK9 precludes LDLR molecule recycling. Inhibition of PCSK9 by monoclonal antibodies leads to an increased number of receptors at the cell surface, resulting in a marked LDL-C reduction (143,145). Consequently, PCSK9 activity decreases the level of *LDLR* in a manner similar to IDOL, albeit through a different mechanism. Similar to IDOL, loss-of-function genetic variants in the PCSK9 gene have been associated with low LDL-C levels in humans (146).

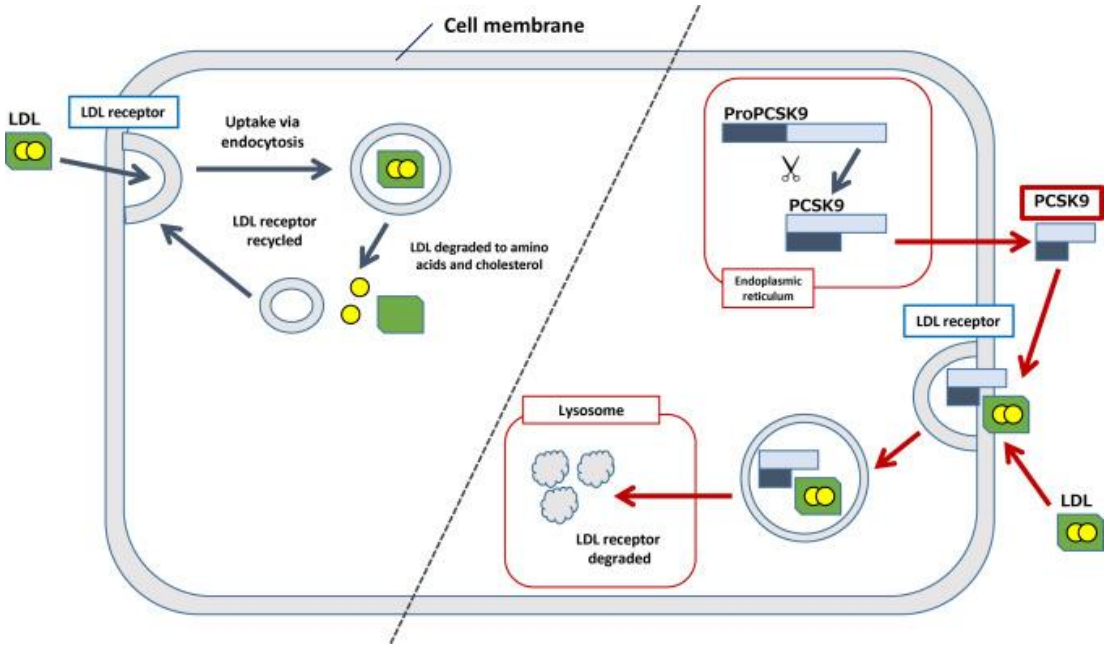

Figure 19. *LDLR pathway and LDL receptor degradation by PCSK9* (143) **.**

2.4.5.2. Lipoprotein particles number and size characterization.

As mentioned in previous sections of the thesis, evaluating the number and size of the particles helps us to better categorize the CVR.

Few studies address the number and size of lipoprotein particles in children with FH. An increase in smaller LDL particles has been reported (147). Other studies have shown alterations in the HDL subfractionst, suggesting impaired reverse cholesterol transport (figure 20) (148).

Studies of particle number and size in the adult population with FH are also scarce. It has also been reported that patients with familial combined hyperlipidaemia (FCHL) had more sdLDL particles than healthy patients (149,150). Interestingly, when this population was compared with FH patients, the ratio of sdLDL/LDL was higher in FCHL (149).

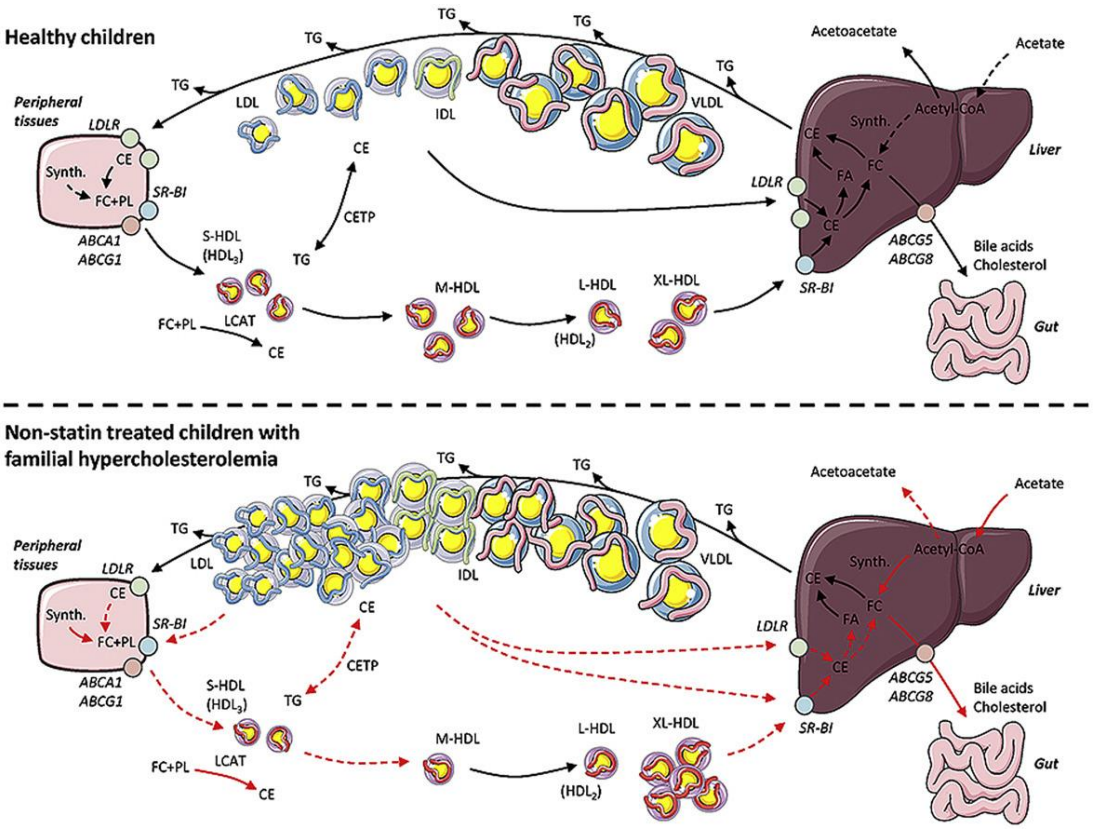

Figure 20. *Graphical abstract of lipid and metabolite profiling of children with and without FH* (148).

2.4.6. Screening

In order to minimise the effect of pubertal activation and diet, the optimal window for screening is between 2 and 10 years of age. During childhood, the LDL values may decrease and some patients may go unnoticed. Lipid values after ten years of age might be altered by pubertal activation, therefore this is not an advisable age for screening (151,152).

It is increasingly recognised that childhood and early adolescence offer the most favourable timeframe for diagnosing FH as well as introducing and maintaining lifelong treatment and management strategies.

It is important to detect children with FH and start treating them early to reduce the risk of suffering a cardiovascular event at an early age, because high LDL-C levels from birth accelerate cholesterol accumulation to the threshold for CVD development (figure 26).

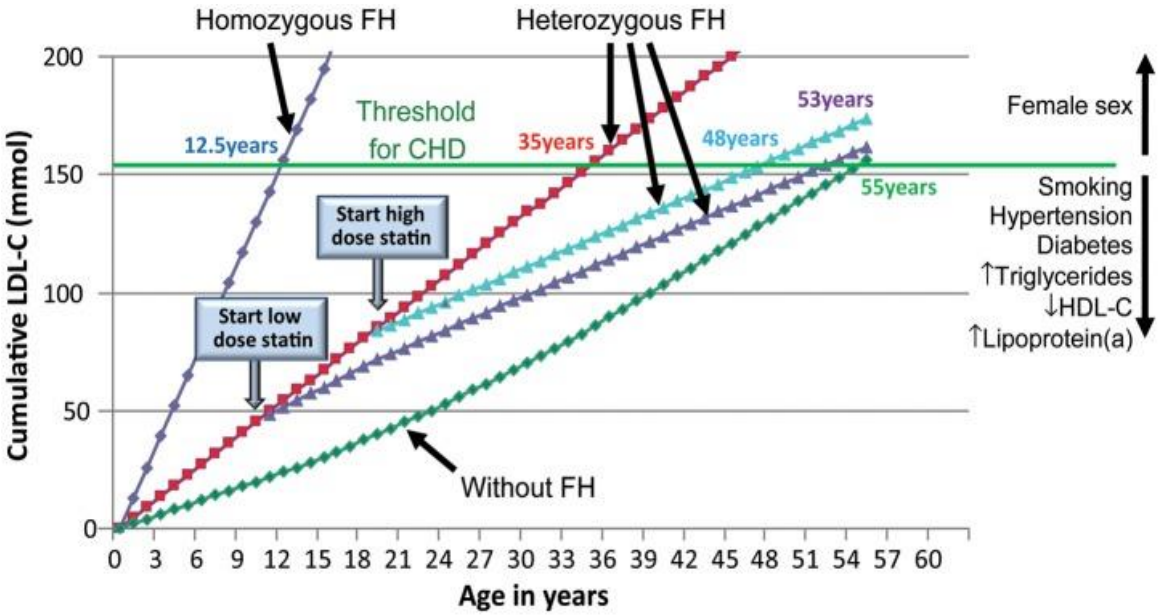

Figure 26. *LDL-C burden in individuals with or without FH as a function of the age of initiation of statin therapy. Figure of Nordestgaard B, et al* (71).

The HeFH is clinically silent during childhood and beyond, thus, it is necessary to design and implement different strategies for detection (153,154). We can distinguish 5 types of screening strategies:

- **Universal screening**: FH meets the WHO guidelines for universal screenings, which are 1. Childhood represents a latent stage of the disease. 2. There is a simple test to diagnose FH that is acceptable to the general population. 3. There is effective treatment, and 4. Case-finding can be made part of routine medical practice (155).

  Universal FH screening is a two-step method. Universal measurement of cholesterol levels between ages 2 and 9 followed by genetic testing in children with total cholesterol or LDL-C above a predefined threshold. Universal FH screening has been shown to detect 90% of children with FH between ages 1-9 with a false positive ratio <1% (156). One country where such screening is applied is Slovenia, children are screened at the age of 5 (157–159). Data from the UK that proposed universal screening at 1-2 years old also show a similar yield (160). The American Academy of Paediatrics propose to cholesterol measurement at

9-11 years and explained that selective screening based on family history is not effective at identifying children with LDL-C in the FH range (161).

- **Oportunistic screening**: This consists of adding the measurement of total cholesterol to any blood test prescribed by the paediatrician for any reason. For example, in Slovakia screening was conducted in children aged 11 to 17 years during cholesterol screening visits (162).
- **Selective screening**: This is based on measuring cholesterol levels in children, with a positive family history for hypercholesterolemia or premature CVD in first- or second-degree relatives (163–165). This kind of screening was proposed by the National Cholesterol Education Programme (73). Austria has also implemented selective screening because parents do not perceive the need for routine medical checks in children (166).
- **Direct cascade screening**: This is a more focused type of selective screening. Once an individual has been genetically identified as FH positive, all first-degree relatives (167) are genetically tested. This type of screening is the most cost-effective strategy for FH (168,169) because the genetic exploration focuses on an already known genetic defect.
- **Reverse cascade screening:** In this strategy the child is the index patient and whose parents are then studied. This method has a high yield when combined with opportunistic screening (156,170,171).

In the literature, several varieties or combinations of screening methods focusing on children from FH families have been recommended on the basis of their cost-benefit ratio (131,172,173).

2.4.7. Therapeutic goals

Although there are various recommendations, in general the expert consensus recommends that FH children should show levels of LDL ≤130 mg/dL or a 30% reduction in LDL-C baseline levels between 10-14 years or 50% between 14-18 years (66,174).

2.4.8. Treatment

The main goal of treatment in familial hypercholesterolemia is early CVD prevention. This prevention is driven by lowering LDL-C, which is the metabolic

consequence of the genetic defect. This must be obtained either by TLSC or putting children on lipid-lowering therapy (LLT).

2.4.8.1. Therapeutic Lifestyle changes

Nutritional advice and promotion of a healthy lifestyle are the milestones of FH treatment in childhood. The aim of the treatment is to teach the child and his or her family about the correct nutritional habits that they need to adopt into adulthood. If adequate lifestyle education is started before pubertal age, they will adopt appropriate habits that will last during the following years (66,168).

Until now, clinical guidelines for treatment of FH patients have insisted on complying with percentages of macronutrients, which are: 30% of total daily energy from lipids, less than 7% of total daily energy from saturated fatty acids, daily cholesterol intake lower than 200 mg, 12–14% of total daily calories from proteins and 55–60% of total energy intake from CH (preferably complex CH) (66,175,176). These guidelines based on macronutrient percentages have now been substituted by recommendations of healthy diet patterns because it is considered better to focus on food choices and not on macronutrients. Diets rich in fruits, vegetables, legumes, nuts, olive oil and complex CH and moderate in meat, fish and dairy products have been described as beneficial for CV health (48,177). Mediterranean (177,178) and Nordic (179,180) diets, both of which contain these types of food, have been suggested to improve CV health.

Healthy diet, PA and avoiding toxic habits are the key to improving the prognosis. Healthy lifestyle forms the basis of FH treatment in children; for this reason, this thesis contains another section on diet information.

2.4.8.2. Lipid lowering therapies

In recent years, many National and European guidelines have been published for identifying and managing children with FH (66,71,72,152,169,181–184).

	Age	Dose	Reduction
STATINS			
Rosuvastatin	> 6 years	6-9 years (5-10 mg/day) > 10 years (10 mg/day)	40-46% 46%
Pitavastatin	> 6 years	6-9 years (1-2 mg/day) > 10 years (4 mg/day)	32-38% 41%
Pravastatin	> 8 years	8-13 years (10-20 mg/day) > 14 years (20-40 mg/day)	25-31% 31-38%
Atorvastatin	> 10 years	(10-20 mg/day)	38-41%
Fluvastatin	> 10 years	10-13 years (20 mg/day) > 14 years (40 mg/day)	25-31%
Simvastatin	> 10 years	(10-40 mg/day)	31-41%
EZETIMIBE			
	> 10 years	(10 mg/day)	20%
RESINS			
Colestiramine	> 6 years	6-10 years (max 4 g/day) > 10 years (max 8 g/day)	10% 20%
Colestipol	> 10 years	2,5 g/day (max 20 g/day)	10-20%

Table 3. *A summary of the recommended treatments in childhood* (185)*. Table adapted by Dr. Plana.*

Nowadays, statin therapy is the primary pharmacological treatment for hypercholesterolaemia in childhood. Statins are key to FH management. Simvastatin, Lovastatin, Atorvastatin, Pravastatin, Fluvastatin and Rosuvastatin are approved in the USA and Europe for use in FH children, and will be prescribed from 6-10 years. The combination of statin plus ezetimibe (a specific inhibitor of cholesterol absorption) has shown high efficacy in both adults and children with FH. Table 3 shows the recommended LLT according to age and necessary LDL reduction. Ramaswami U, et al. proposes an algorithm for FH treatment according to age and LDL-C values (figure 27) (186).

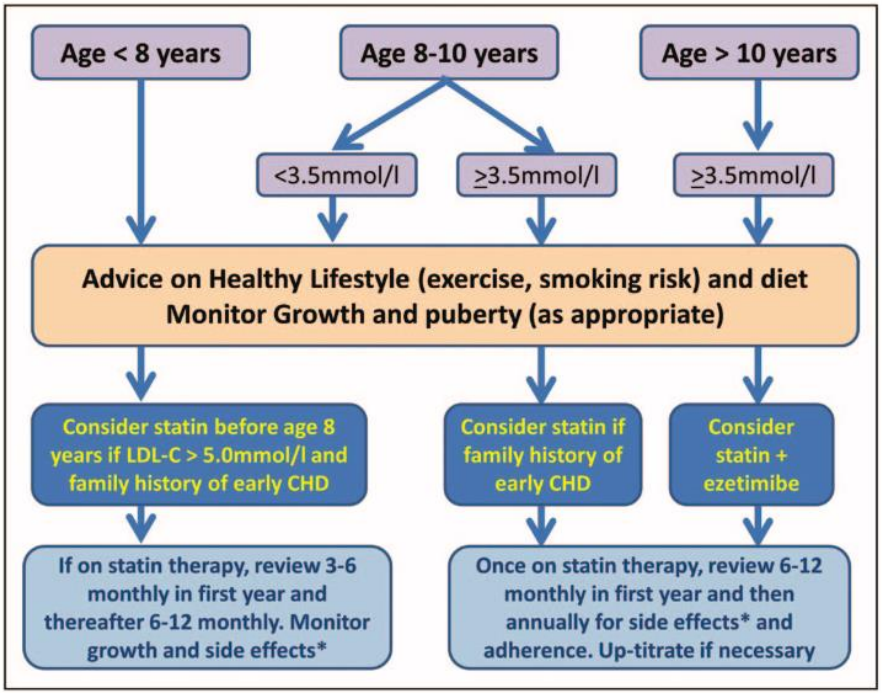

***Figure 27**. Clinical management of children and young people with FH. Algorithm extracted from the Ramawasmi U, et al. article* (186).

The initiation of LLT in FH children is determined by factors such as the child's current LDL-C levels, the age of onset of CHD in relatives, and the presence of other CVR factors. In clinical practice, pharmacological therapy for paediatric patients must always be well evaluated and discussed with the patient and his or her family (66). Parents often find it difficult to accept pharmacological therapy, especially for young children. On the other hand, adolescents might not be interested in their pathology, meaning their compliance with the therapy can be deficient (119).

Adherence to and tolerance of pharmacological therapy must be strictly monitored. Adverse effects to statins are rare in paediatric age, the most common being myopathies and hepatotoxicity. Therefore, liver (hepatic aminotransferases) and muscle (creatine kinase) enzymes should be measured before starting treatment and then periodically monitored.

Different studies show the importance of early treatment. If children receive LLT early, future events will be avoided. For example, Luirink IK, et al. showed Kaplar-Meier curves that demonstrate a reduction in events during adulthood in children who received early treatment compared to their parents who received treatment later (187).

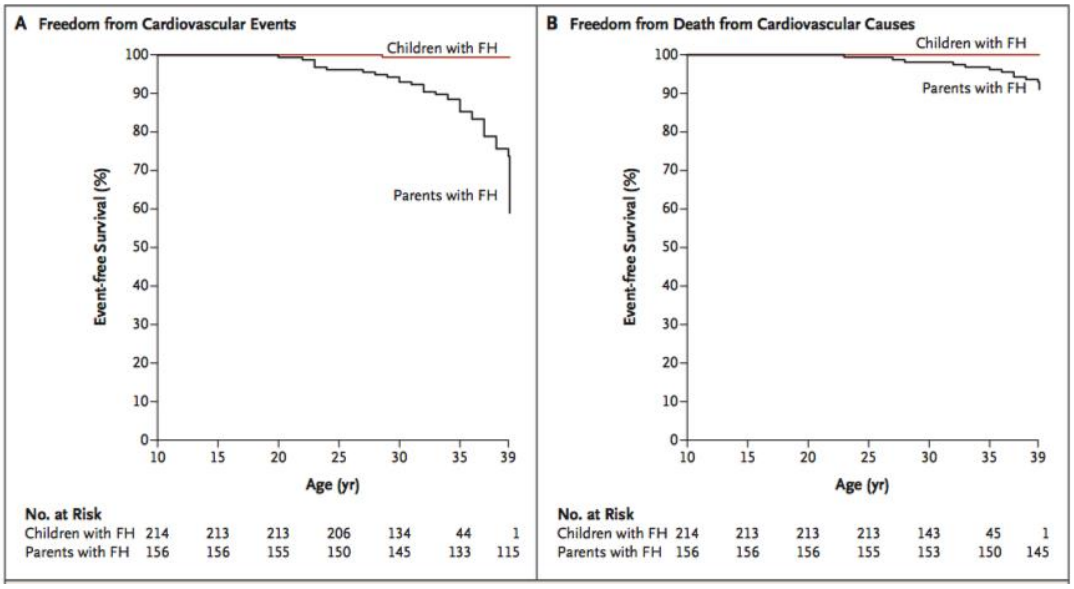

***Figure 28**. Kaplan-Meier curves showing differences in cardiovascular events among parents and children in relation to start of LLT. Image extracted from Luirink IK, et al. article* (187).

Not all countries recommend starting the treatment of FH children at the same time. Although all children should be treated equally (regardless of where they live), different data suggest that many FH children do not receive the full potential benefit of early identification and the recommended appropriate LLT. Guidelines on LDL-C values and treatment are very similar, but the adoption of these recommendations is likely to be influenced by local factors such as clinician and parental preferences and the different health care and reimbursement systems for LLT (188). Figure 29 shows the proportions of children receiving statins by age at follow up per country.

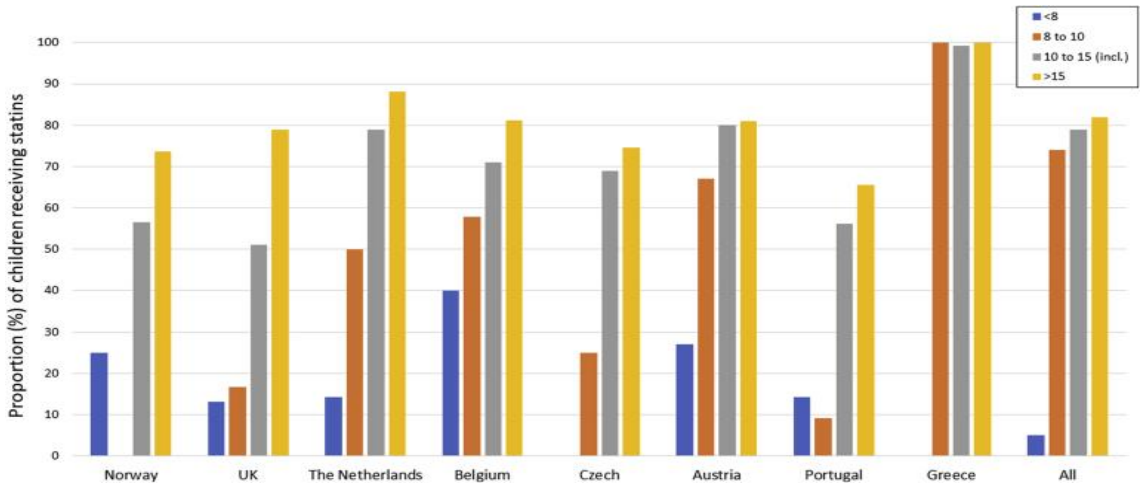

***Figure 29**. Proportions of children receiving statins by age at follow upper country. Figure Image extracted from Ramawasmi U, et al.* (188).

2.5. Healthy lifestyle

2.5.1. Diet and lipids (where all this began).

The physiological action of food has been studied for centuries. Hippocrates (approx. 460 aC - approx. 370 BC) was the first to say "let food be your medicine and medicine be your food", therefore it has long been known that diet plays a key role with chronic diseases.

The influence of diet on the lipid profile has been known since 1913, when Anitschkow and Chalotow demonstrated that a cholesterol-rich diet caused cholesterol accumulation in the arteries and the subsequent onset of an arteriosclerotic plaque (189). Anitschkow continued his research into how this plaque formed in a rabbit and documented it through drawings (figure 30).

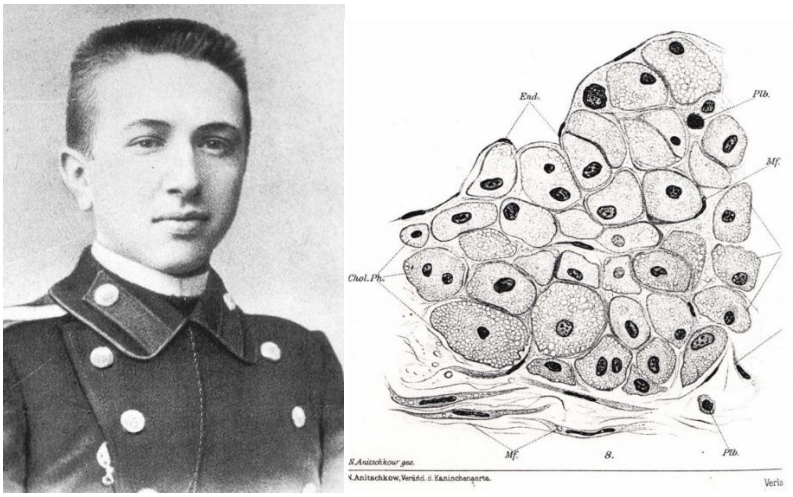

Figure 30. *Anitschkow's drawing of a typical cell foam injury in a rabbit fed a total of 82.7 g of pure cholesterol in sunflower oil for a period of 139 days. Keep in mind that the endothelium on the injury seems to be intact* (190).

Years later, it was discovered that in addition to cholesterol, other macronutrients also have an influence. In the 1960s, Keys, Anderson and Grande observed that saturated fatty acids (SFA) and polyunsaturated (PUFA) had the most influence, whereas monounsaturated fatty acids (MUFA) and CH had no effect. Keys and their group estimated that total cholesterol levels increased by about 24 mg/dL when 10% of the energy contributed by CH was replaced by a mixture of SFA (lauric, myristic and palmitic) (191). However, these three do not act in the same way on cholesterol and it was not until 5 years later when Hegsted D and McGandy together with their respective groups,

conducted experiments with natural oils and fats in which they developed a formula that took into account all the aspects described by Keys to calculate the impact of nutrients on cholesterol concentrations. They found that myristic was the FA with the greatest effect (192). In 1980 Gordon and his team performed epidemiological studies showing that the risk of coronary heart disease is positively related to LDL-C levels and negatively related to HDL-C levels (73).

The first dietary guideline for FH was issued by Muller in 1930 and recommended a low cholesterol diet ("dietary cholesterol and animal fat as necessary restrictions in patients with FH") (193) . Some authors praised Muller's findings and maintained the dietary recommendations (194), despite the fact that there had not been a single randomised controlled trial reporting coronary event benefits from the diet.

Therefore, we can see that diet influences CVR and that this has been well known for years (195–199). Dietary factors influence the development of CVD either directly or through their action on traditional CVR factors, such as plasma lipids, blood pressure, or glucose levels.

Dietary recommendations have evolved considerably in recent years. Initial emphasis was on the percentages of nutrients before the focus shifted more recently to eating patterns (figure 31) (48,200).

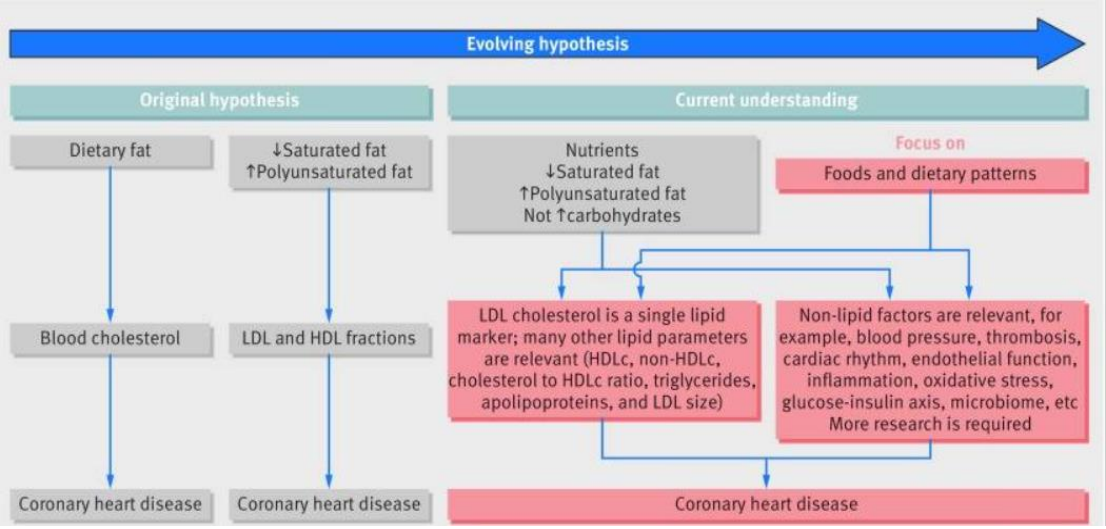

Figure 31. *Evolution of CV recommendations. Image taken from* (200)

The lack of agreement between studies is due both to methodological problems (particularly inadequate sample sizes or short study durations) and the difficulties of evaluating the impact of a single dietary factor independently of any other changes in the diet (200). For this reason, the present thesis collects evidence of both types of recommendations.

2.5.2. Macronutrients

A balanced and healthy diet is key to treating FH and the preventing atherosclerosis (201). Total cholesterol and LDL-C levels can be modulated by dietary intake (202). Studies have shown that dietary adjustments can reduce plasma cholesterol by 20-30% (203) and LDL atherogenicity (204).

The efficacy of lifestyle interventions in children with FH, where LDL-C levels are genetically driven, needs to be fully researched. Some authors have observed that the reduction in LDL-C in patients with FH is lower than in patients without FH (176,201,205,206). It has been observed that the diet can reduce LDL-C levels between 10-15%, the reduction varying considerably according to the type of patient and the type of genetic variant (207,208). In these children, dietary recommendations will be insufficient as a therapeutic treatment. However, they are essential for maintaining appropriate weight and thus preventing additional risk factors that usually persist into adulthood and aggravate the CVR.

Recommendations must start very early, from 2 years of age, under the supervision of a qualified dietitian-nutritionist who helps to promote nutritional therapy in the family setting (209). At this age, children are learning all the time and imitate what they see at home. For this reason, it is very important to educate the whole family to improve adherence to the treatment (119). Other studies have also described how strategies at an early age are more effective (66,210,211).

The main objective of FH treatment is to reduce the risk of arteriosclerotic disease, and for this reason, all patients must undergo a broad programme of lifestyle changes. Good follow-up allows clinicians, in some cases, to reduce the pharmacological dose and control other CVR factors (212). For example, a study carried out on young

Finns followed for 21 years showed that those who followed a healthy diet had better CV health in adulthood, lower concentration of LDL-C and TG and lower cIMT (213). Any such programme must be based on three main objectives: changes in diet, exercise and nutritional behaviour (48,214,215).

2.5.2.1. Fats

The principles of a cholesterol-lowering diet in FH subjects include reductions in the intake of total fat, SFA and cholesterol (175,216). Fat consumption should be limited to <30% of the total calorie intake (157,217). So far, many of the published guidelines specify the need to lower all types of fat and place special emphasis on the importance of lowering the intake of cholesterol (<200-300 mg/day) (218,219). However, nowadays the recommendations focus more on quality than quantity.

The consumption of SFA must be <10% (217); however, some guides are stricter and state that <7% is better (66). Few studies have investigated the association between CVR factors in children and the consumption of foods rich in lipids. An association between increased cholesterol (220,221), obesity (222,223), blood pressure (224) and diabetes (225) has been shown. Torvik K et al. observed that FH children who intake more SFA had more LDL-C and higher ApoB levels (176).

The reduction of SFA and cholesterol in children's diet does not modify their nutritional status, growth or pubertal development (226). We have more information regarding the relationship between lipids and CVR in adults. It has been observed that the CVD risk is reduced by 2-3% by replacing only 1% SFA with PUFA. This does not happen if it is replaced by CH (227). Several meta-analyses have shown that a 2% increase in SFA increases CVR by 23% in the adult population (228), and replacing SFA with PUFA, MUFA or high-quality CH will lower CV events (229).

The intake of MUFA should be around 10%, basically in the form of oleic acid, because it reduces LDL-C and has a favourable effect on HDL-C. The PREDIMED study is the only dietary intervention study testing the impact of the Mediterranean diet on CV events. This study, involving adults at high CVR, demonstrates that the incidence of major CV events was about one third lower among those assigned to a Mediterranean

diet characterized by either high extra-virgin olive oil or nuts, than it was among those assigned to a low-fat diet (figure 32) (230).

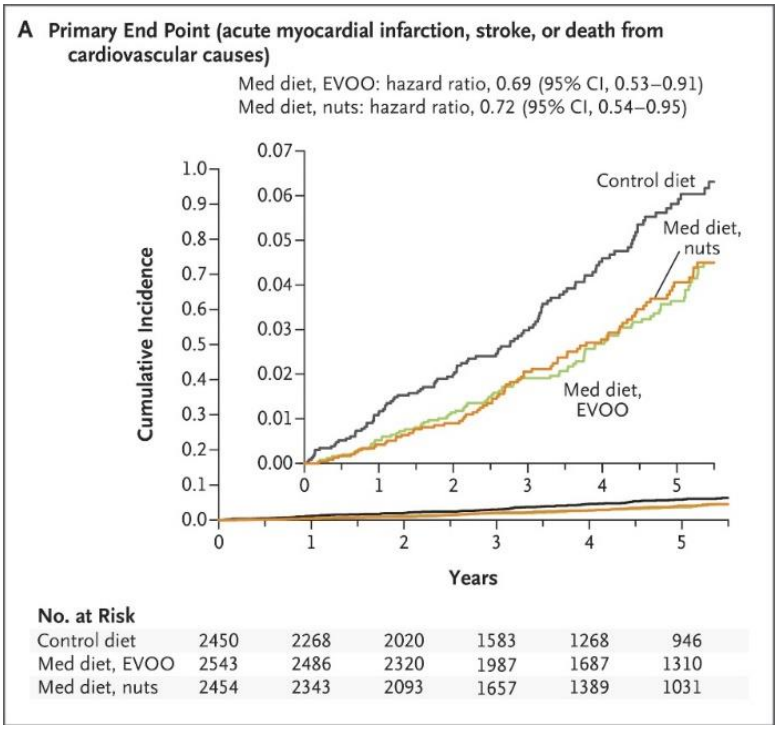

***Figure 32**. Kaplan–Meier Estimates of the Cumulative Incidence of End-Point Events in the Total Study Population. Image from PREDIMED study* (230).

In the case of children, Haro-Mora JJ et al. showed that those who consumed olive oil had lower BMI (OR 0.19 95% CI: 0.04, 0.52) compared to children who consumed a combination of other oils (223).

The trans-FA present in foods made with coconut, palm and palmitic oils and hydrogenated fats used in the production of industrial bakery products, snacks, microwave-ready popcorn and bagged potato chips, among others, should be avoided. The intake of trans fat has been associated with CHD, sudden death from cardiac causes, and diabetes (228).

Given the decades of dietary advice that the lower the total fat content, the healthier the diet, researchers and public health authorities agree on controlling fat-containing food. It is difficult issuing public health guidelines as to which foods should be recommended or limited to reduce the risk of chronic diseases because dietary fats

are typically mixtures of different types of fatty acids (200). Sometimes qualitative changes are disregarded, which is problematic because epidemiological data from infant studies have indicated the importance of quality (231). The new guidelines focus on foods rather than fats (48). The PURE study has analysed the effectiveness of these guidelines. This study of 135,335 adults in 18 countries demonstrates that CH were the macronutrient most strongly associated with chronic diseases. Interestingly, higher SFA intake was associated with a lower risk of stroke (quintile 5 *vs* quintile 1, HR 0.79 [95% CI 0.64–0.98], *p*=0.0498) (figure 33) (232).

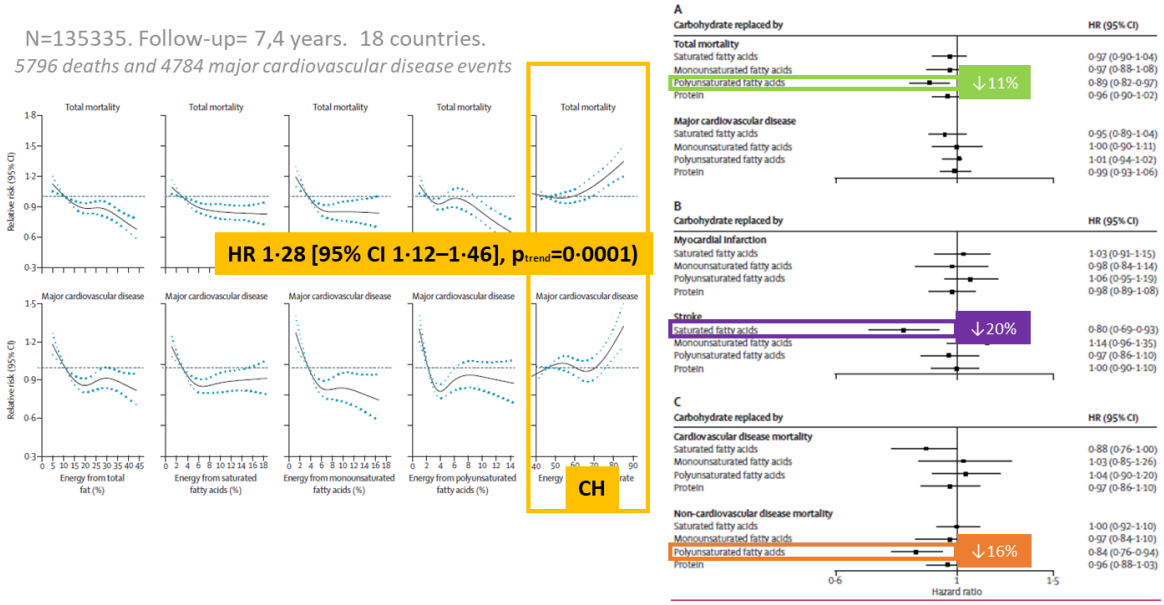

Figure 33. *Modified image of Dehghan M et al. on fat and CH intake in cardiovascular risk* (232).

2.5.2.2. Carbohydrates

CH are the primary energy source for the organism. The group includes fibres, starches and sugars. In addition, they can be classified into two types: complex and simple. Complex carbohydrates are the ones that have been promoted because they provide a lower percentage of calories per mass unit, high fibre content and a slower absorption rate (wholegrain cereals, potatoes, pulses, fruit and vegetables).

Some studies have looked at the association of cereal intake in children. For example, in the United States, it has been observed that cereal consumption for breakfast is related to a lower BMI. Curiously, lower weight and favourable nutrient

profiles were associated with cereal consumption regardless of sugar content (233). The result is interesting because, in paediatric dyslipidaemia, sugar reduction is recommended (234). Other studies (235,236) have shown that the amount of simple sugar and sugar contained in commercialized drinks must be reduced because there is a strong relationship with health. In a recent prospective study in Australian adolescents, the increase in the consumption of sweetened drinks was associated with a higher CVR score in girls (OR 3.2 IC 95% 1.6, 6.2), with less HDL-C in boys and with increased triglyceride levels, BMI and waist perimeter in both sexes (237).

Increasing the intake of whole grains as part of an overall healthy lifestyle may help children to achieve and maintain a healthy weight and is beneficial for their lipid profile (61,66,238). In addition, increased consumption of whole grains is associated with lower CVR (239), although not all authors have observed a statistically significant relationship (240).

The latest European CVR prevention guidelines advise a fibre intake of 30-45 g/day. In children it has been advised to calculated the total grams per day of fibre consumption by adding 5 to the age in years. (66). The effect of fibre intake on CV health is not well studied in this population and the recommended doses are not entirely clear either (241). A higher soluble dietary fibre intake was found to be related to a smaller waist circumference in Latin American children (beta=-0.069, P=0.036) (242). In adults, it has been observed that 7g/day of fibre intake can reduce CVR by 9%, and 10g/day can reduce stroke by 6%, as well as reducing type 2 diabetes mellitus by 6% (203,243,244). The recommended daily dose is 6g/day for children 2-12 years old and 12g/day in children over 12 years old.

In adults, several studies have described an association between wholegrain intake and improved CVR (203,245), so it is vital to boost fibre consumption from a very young age.

2.5.2.3. Protein

The recommendations for protein consumption are independent of the presence of FH. It is recommended that about 15% of energy should come from proteins, mainly

white meats and fish rather than red meats which are richer in SFA. The impact of meat on CV health is difficult to observe since it depends on the type and quality. It has been observed in a group of children between 11 and 18 years of age that the frequency of red meat consumption is associated with an increase in dyslipidaemia (246) and, in turn, in another population of children 6-8 years it has been associated with an increase in metabolic risk (247). However, not all studies observe the same. A Mexican study observed in adolescents a worse LDL-C/HDL-C ratio (248). There are no studies linking proteins with CVR in childhood. Only in the adult population has it been reported that consuming fish more than once a week could reduce the risk of CVD by 16% (249). The Hisayama Study also suggested that a higher dietary protein intake is associated with a reduced risk of stroke in the general population (250).

Two recent studies showed that the daily intake of dairy and low-fat dairy products (43% and 26% respectively) was associated with a lower likelihood of being overweight or having excess body fat after 3 years follow-up, respectively (251). A few studies have observed a positive association between the consumption of milk and dairy products and adiposity in children (252,253); In general, these studies did not find any association in children and adolescents (254). In a study conducted in 2012, lower abdominal obesity was associated with a high level of milk consumption, regardless of the level of PA. Those with low levels of PA and high milk consumption had lower probabilities of abdominal obesity (OR 0.412, 95% CI 0.20 to 0.85), compared to highly active adolescents with low intake of milk (OR 0.928, 95% CI, 0.56, 1.53) (255). And finally, an inverse relationship has also been found between the consumption of milk and DM2 (256), although there are controversies, as is the case in the study by Hoppe C, et al. who observed a positive association with a 75% increase in insulin resistance (257).

2.5.2.4. Salt

Many of the clinical guidelines recommend a maximum intake of 5g/day (2g sodium). In children under 10 years less than 3-4g/day is recommended, while the average consumption of this population is 8.1g/day (258). About 75% comes from processed foods. In the Netherlands, a total of 200 children were analysed and showed an increase in blood pressure significantly associated with salt consumption (259). This

would lead to early hypertension (260). About 60% of children with high blood pressure have hypertension in adulthood. This persistence of high blood pressure has been associated with a higher risk of cIMT increase (261). Therefore, it is important to reduce the amount of salt since a 40% reduction lowers 1.17 mmHg and 1.29 mmHg the systolic and diastolic pressure respectively (262,263).

2.5.3. Food groups and diet patterns

Dietary intervention encourages better combining of multiple foods and nutrients. Therefore, healthy dietary patterns will lead to more beneficial effects than supplementation with a single nutrient, because of the synergistic health effects among the different elements. In fact, as foods are mixtures of different nutrients and other components, it is not appropriate to attribute the health effects of a food to only one of its components. Moreover, if energy intake must be kept constant, eating less of one macronutrient implies necessarily eating more of others. The quality of the replacement (for instance, unsaturated fat vs. highly refined grains) can influence the effect observed, significantly modifying the impact on health of the nutrient replaced. These limitations suggest caution in interpreting the results of randomized controlled trials (RCTs) or even meta-analyses of RCTs in relation to the effect of a single dietary change on CVD (199,200).

The Mediterranean and Nordic diets have been suggested to improve CV health. Mediterranean diet is characterized by a high intake of vegetables and fruits, legumes, olive oil, cereals and nuts, a moderate intake of fish and lean dairy products and a low intake of red meat, with olive oil as a major fat source (264,265). This diet was associated with a 30% CVD relative risk reduction in the PREDIMED study (266). The beneficial effect has not been shown outside of the Mediterranean zone, potentially due to factors such as food availability, food compositions and culinary traditions. It is very important to adapt dietary patterns to a country's culinary traditions to ensure better adherence (267,268). The Nordic diet has emerged as an alternative beneficial diet in Northern Europe and is characterized by a high intake of apples, pears, berries, root and cruciferous vegetables, whole grain and rye bread and cereals, fish (particularly fatty fish), low-fat dairy products, rapeseed oil, potatoes and vegetable fats, among others

(269). In this case, this diet has not been shown to reduce CVD but does have an effect on CV health risk markers (270).

The current body of evidence shows that healthy dietary patterns share similarities, such as a high intake of fibre (whole grains, fruit, vegetables, nuts and legumes), antioxidants, vitamins, minerals, polyphenols, MUFA, PUFA; low intake of salt, refined sugar, saturated and trans fats, low glycaemic CH, sugary drinks and precooked foods. We also have to focus on good education to prevent the consumption of alcohol, tobacco and vaporizers.

2.5.4. Education in nutritional behaviour

Dietary education is the key to achieving healthy dietary patterns. If we raise awareness among patients, they will be able to choose healthier foods. The whole family will be involved in changing habits and this will improve adherence. It must be remembered that a diet for dyslipidaemia must be varied and balanced and must therefore be advisable for the entire population. The recommendations of European expert groups on CV prevention state that, given the cultural diversity of diets in Europe, dietary recommendations have to be translated into practical cooking recipes, taking into account local customs and socio-economic factors. They should also focus on how to prepare food, how to eat and on habits and customs at the table.

In terms of cooking, food should be grilled, steamed, baked or cooked in the microwave rather than fried. And finally, it is important to pay attention to food labels in order to choose the healthiest options. In conclusion, the promotion of healthy habits regarding foods, PA and toxic habits should be a central element of a global strategy aimed at controlling the lipid profile of the population with CVR in general and dyslipidaemia in particular. An optimization of food policies and intervention strategies (individual, family and school) is key to non-pharmacological treatment.

2.5.5. Nutraceuticals

Innovative nutritional strategies have been developed for better handling of dyslipidaemia. In general, so far the evidence concerning the functional foods in this field is incomplete. The main reason is the absence of intervention studies based on a

long-term diet that are relevant to CV prevention. Three groups of substances have been extensively evaluated for use in CV prevention diets: plant sterols/stanols; ω-3 and others.

Different clinical trials have shown that the daily consumption of stanols/plant sterols at a dose of 1.5-3 g/day in children and adolescents with HeFH may be beneficial in reducing levels of LDL-C by between 9-19% (271). Recently, a consensus panel of the EAS has recommended their consumption in patients with FH after 6 years, provided that the intake of vegetables and fruits is sufficient to avoid liposoluble vitamin deficiency (48,272).

Despite the beneficial effect of supplementation with ω-3, data are insufficient for recommendation in children. The contribution of supplements in the form of linoleic acid, eicosapentanoic acid (EPA) or docosahexanoic acid (DHA) is not recommended, both of which are present in fish oil (273). It is recommended to increase the contribution of PUFA by increasing fish consumption three times a week. Supplemental food products with rapeseed oil (274), garlic extracts (275), soy protein (276), psyllium extract (277) and policosanol and red yeast rice (278) are not recommended due to controversies about the results (279,280).

2.5.6. Physical activity

Maintaining an ideal weight and promoting PA are key recommendations. In children it is very difficult to establish causality between physical exercise and the lipid profile for methodological reasons, but it seems that the PA usually increases the levels of HDL-C (281).

Ideally, children should do more than 1h/day of PA and less than 2h of sedentary activities such as playing on computers and sitting in front of the TV (66). The current bibliography highlights the importance of educating children in carrying out an active lifestyle since it has been shown that the thermogenesis has more impact than doing specific hours of active exercise. Children must move and play to grow strong and healthy and avoid a sedentary lifestyle (71,94,282,283).

It has been observed that PA patterns established during childhood will remain throughout life and are associated with higher HDL-C and lower LDL-C (284,285). There is an inverse relationship between PA and dyslipidaemia, and a decrease in LDL-C with PA school programs (286).

2.5.7. Toxic habits

2.5.7.1. Alcohol

The consumption of all kinds of drugs in the school population between 14 and 18 years has fallen considerably in recent years, according to the Survey on the Use of Drugs in Secondary Education Students (ESTUDES study) 2014-2015, prepared by the Government Delegation of the National Drug Plan. However, consumption is still high.

The most consumed daily drink is beer followed by wine. 1.7% have consumed alcoholic beverages every day during the last month and one in three consume alcohol sporadically.

Drinking in moderation, especially red wine, can reduce the risk of CVD, but the results are controversial (169,287), probably due to the J-curve effect of alcohol consumption. We must think about what moderate consumption means, since consumption is very widespread in the Spanish population (figure 34) (288).

It is very important to educate children properly to prevent them from drinking alcohol.

2.5.7.2. Tobacco

The European CV prevention guide proposes different strategies to promote non-smoking (169). The decline in tobacco smoking during the last decade is 60%, according to the report presented by the Spanish Ministry of Health. A total of 38.4% of Spanish adolescents say they have smoked on one occasion and 8.9% smoke daily. Consumption is highest at 14-18 years. Children must be educated to avoid tobacco, with emphasis on the adolescent population because they are at a highly vulnerable stage where their decisions can have consequences for that will affect the rest of their lives.

Tobacco is an additional CVR factor in FH populations (289).

In summary, the promotion of healthy habits and PA and the avoidance of toxic habits should be considered elements in an overall strategy to control lipid values in an FH-affected population. Optimization of food policies and intervention strategies at the individual, family and school levels are key to managing non-pharmacological treatments (168,289).

2.5.8. Psychological status

Chronic diseases can predispose towards lower psychosocial wellbeing. It is important to determine the association between FH and symptoms of anxiety and depression, and health-related quality of life (HRQL). Different studies suggest that patients with FH may report small but measurable differences in anxiety symptoms and mental HRQL (290,291). But this effect has only been studied in adults with FH.

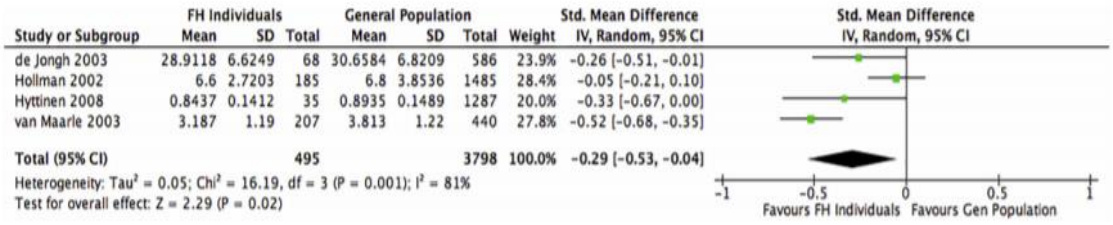

Figure 34.*.Random effects - meta-analysis of FH and anxiety. This image was displayed by Akioyamen L et al* (290).

3. Hypothesis

3. HYPOTHESIS

Familial hypercholesterolemia is the primary genetic condition that leads to early CVD. It is a highly prevalent genetic condition affecting about 1 in 200 people. Although it can be detected in childhood, the clinical diagnosis is usually delayed until middle age, which in turn delays therapy and leads to accelerated atherosclerotic CVD. FH detection in children would allow to preventative actions to be started, thus reducing overall cardiovascular risk. Detecting FH at early ages improves the prognosis of FH; therefore, the overall hypothesis of our work is that:

FH diagnosis in children can be enhanced by the implementation of detection methods that include opportunistic, direct and inverse cascade screening. This methodology will require close collaboration between different levels of health care professionals.

The exploration of new biomarkers associated with lipoprotein metabolism and LDL receptor function could facilitate the detection of children with FH. The circulating lipoprotein profile evaluated by NMR should increase information on the overall alteration of lipid metabolism associated with FH and lead to a better understanding of its alterations.

Early detection of FH, will allow early intervention. Therapeutic lifestyle changes are the first line of intervention, including modifying dietary patterns, PA, and avoiding toxic behaviours throughout life. Techniques and programs specifically targeted and designed for children increase the adoption of long-term healthy habits.

4. Study aims

4. STUDY AIMS

General objective

The present doctoral thesis aims to contribute to improving the detection, diagnosis and treatment of children with FH and consequently to improve their prognosis. This overall aim is achieved through three actions: 1. Establishing an early screening strategy to detect children with FH; 2. Exploring new biomarkers and analysing in greater depth the lipoprotein profile; and 3. Implementing an early treatment based on educational activities aimed at establishing a healthy lifestyle.

Operational objectives

1. **To establish and implement a strategy for detecting FH in children based on opportunistic screening in coordination with primary and hospital care paediatricians, reverse and direct screening cascades**.

2. **To characterise and assess clinical, biochemical and vascular imaging aspects that can help us detect, evaluate and treat children and adolescents with FH.**

    2.1. To describe the main clinical, biochemical and vascular characteristics of children diagnosed with FH.

    2.2. To assess the usefulness of the lipid profile, the concentrations of apoproteins and the biomarkers of LDLR function to improve the detection of FH and the vascular prognosis, according to subclinical data of arteriosclerosis, in children with FH.

    2.3. To investigate the alterations, beyond the standard analysis, in the quantity and quality of the lipoprotein profile evaluated by NMR.

3. **To describe the impact of diet on children with FH and design techniques to implement lifestyle changes in these children.**

3.1. To analyse the clinical and biochemical performance of an educational program focused on improving the lifestyle (diet, PA, smoking) of children with FH.

3.2. To describe the impact of two different diets (Nordic and Mediterranean) on the lipid profile of children with FH.

5. Patients, material and methods

5. PATIENTS, MATERIAL AND METHODS

Detailed information about the material and methods and the patients included in the studies is provided in each paper. Below we will summarize some of the main common aspects of the methodology used.

5.1. Study population

The present study is based on the study of a cohort of FH children (NCT04370899) (aged 4-18 years) that was recruited in the Lipid Unit of the Sant Joan University Hospital (Reus, Spain). For objective 3 (different diets comparison), we have also studied a Norwegian FH children group. Non-FH children were also included as control groups.

The FH children cohort and the non-FH children control group included in our Unit, were recruited through the Program for the Detection of Family Hypercholesterolemia in the Child Population (DECOPIN project). During the period from March 2013 to May 2019, 319 children suspected of having FH were studied. Children were classified as FH if they had a positive genetic test or LDL-C >160 mg/dL and one of the parents had a Dutch Lipid Clinic Network (DLCN) score >8 points, in the case of no available genetic test result. Children who did not meet the FH criteria were included in the non-FH group. None of the children was on lipid-lowering therapy at baseline. The exclusion criteria were chronic renal, hepatic or thyroid disease, type 1 diabetes mellitus, hypercalciuria, eating disorders, autoimmune disease, homozygous FH and other chronic diseases.

For objective 3 a group of 52 children (5-18 y/o) was included in the study (29 FH and 23 controls). This group with positive genetic FH-associated genetic variants was recruited between September and December 2013 (176) from the outpatient Lipid Clinic at Oslo University Hospital (Oslo, Norway). Exclusion criteria were HoFH or other known chronic diseases or conditions other than HeFH. A non-FH control group was recruited from the Stork-child study (2014-2015) from March 2013 to May 2019 (148).

5.2. Screening cascade

We applied a combination of 2 different screening methods to the children: the first one was a combination of opportunistic screening and reverse cascade, and the second one was direct cascade screening. In the first, after detecting suspicious children, we then studied the parents (Children-to-parent pathway, Ch-P), while in the second, we studied the children of already diagnosed parents (parent-to-child pathway P-Ch). To implement the Ch-P pathway, primary care paediatricians were asked to include, in any blood test indicated for children for clinical reasons, lipid profiling to determine LDL-C, or at least, a TC measurement, and if TC was higher than 5.2 mmol/L (> 200 mg/dL), lipid profiling was performed. Children with LDL-C ≥ 4.9 mmol/L (≥ 190 mg/dL) or ≥ 3.5 mmol/L plus one of the following conditions: early CVD in a first- or second-degree relative; a parent with TC > 7.8 mmol/L (and/or on lipid-lowering therapy) or one parent unknown, were sent to the Lipid Unit, or to the hospital paediatric department if secondary dyslipidaemia was suspected (figure 35).

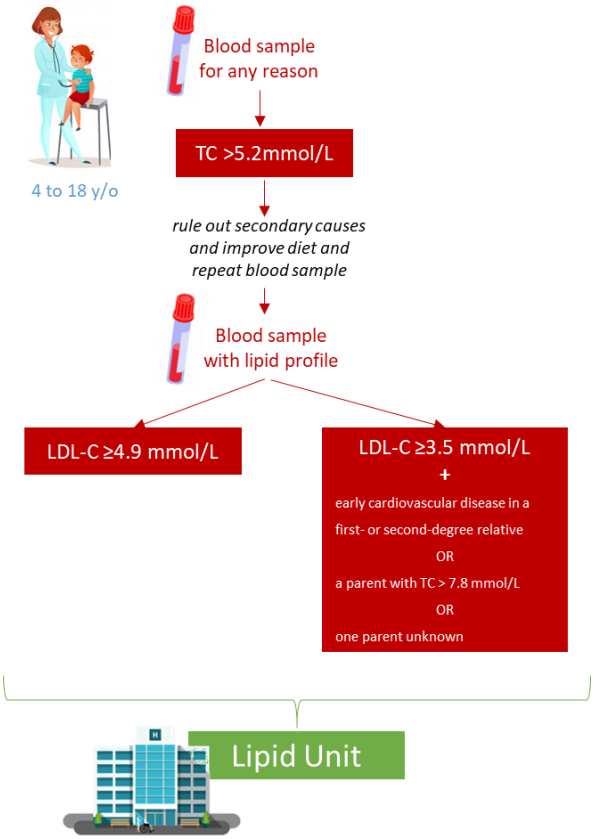

Figure 35: *Illustration on the criteria for referral of paediatrics to the Lipid Unit.*

In the Ch-P protocol, we studied the families and parents, instead of the index child, in order to identify the mandatory vertical transmission. When one of the parents had a DLCN >8 points, a clinical FH diagnosis was established, and genetic testing was performed. In the case of a positive genetic diagnosis, genetic testing for the known genetic variant was carried-out on both the index child and all offspring. The P-Ch pathway consisted of performing a directed genetic study of all offspring of genetically confirmed index cases or a clinical study of children from genetically negative definite FH parents.

5.3. Anthropometry and clinical history

Anthropometry, demography, family history and relevant clinical data were recorded at the basal time and after the 1-year follow-up in the prospective study. BMI z-score was calculated according to the following formula: [(BMI children–BMI 50th percentile of Orbegozo's growth curves)/standard deviation (SD) 50th percentile of Orbegozo's growth curves] (292).

5.4. Biochemical parameters

Standard biochemical analyses were performed in blood samples obtained after overnight fasting. In the case of the Norway cohort, non-fasting blood samples were obtained. TC and triglyceride levels were evaluated using enzymatic colorimetric tests, HDL-C was evaluated using a direct enzymatic colorimetric method, and apolipoprotein levels were measured by immunoturbidimetric assays. LDL-C levels were calculated by the Friedewald equation.

5.5. Full lipoprotein profile by 1H-NMR

5.5.1. Liposcale Test®

The Liposcale Test® (actualized 2018 version) was used to assess the full lipoprotein profile. As previously reported, this method is based on 2D-1H-NMR (29). This method determines the lipid concentration and particle number for the large, medium and small subclasses of the main lipoprotein classes (VLDL, LDL and HDL) and

their size-associated diffusion coefficients. In the updated version, the LDL lipoprotein class has been linearly calibrated to the LDL particles number according to the standard FDA-approved NMR based on the lipoprotein methodology developed by Otvos and colleagues (30) to obtain the best agreement of the absolute LDL particles numbers between the two techniques (31). The variation coefficients for the particle number were between 2% and 4%. The variation coefficients for particle size were lower than 0.3%.

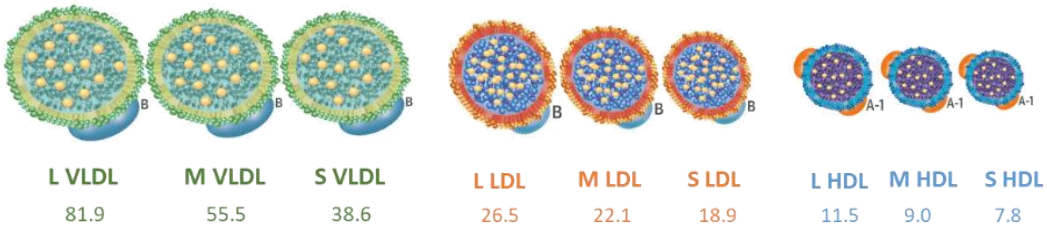

Figure 36: *Illustration of Liposale® NMR test about 9 lipoprotein particles.*

5.5.2. Nightingale's Health®

For objective 3, the Nightingale's Health ® (Finland) method was used to assess the lipoprotein subclasses profile because it was the system already used in the Norwegian cohort. Like the Liposcale®, this method is based on 1H-NMR spectroscopy providing information on lipoprotein particles and a comprehensive lipidomic analysis. The lipoprotein profile is based on the analysis of 14 different lipoprotein subclasses. The method has been extensively described and used in several recent papers (46,148,293–295).

Briefly, the metabolomics profiling includes particle concentration and lipid content of 14 subclasses of lipoproteins.

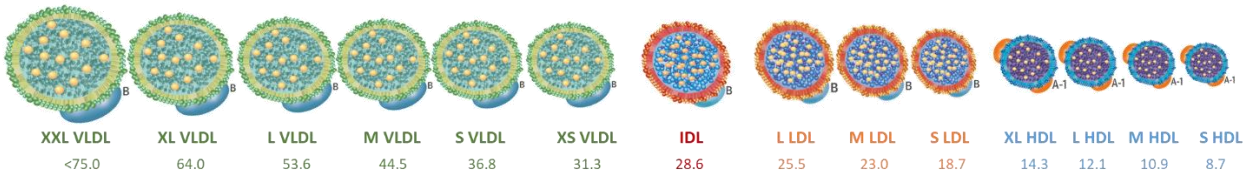

Figure 37: *Illustration of Nightingale® NMR test of 14 lipoprotein particles.*

5.5.3. Comparisons between methods

We compared the lipoprotein subclasses data obtained by both 1H-NMR methods. Although both methods provide similar information, the differences in particle grouping and technical aspects preclude using them interchangeably. Therefore, we have used the Liposcale® test in all papers involving only the Spanish cohort (Objective 2) and the Nightingale method when the Norwegian cohort was involved (Objective 3.2)

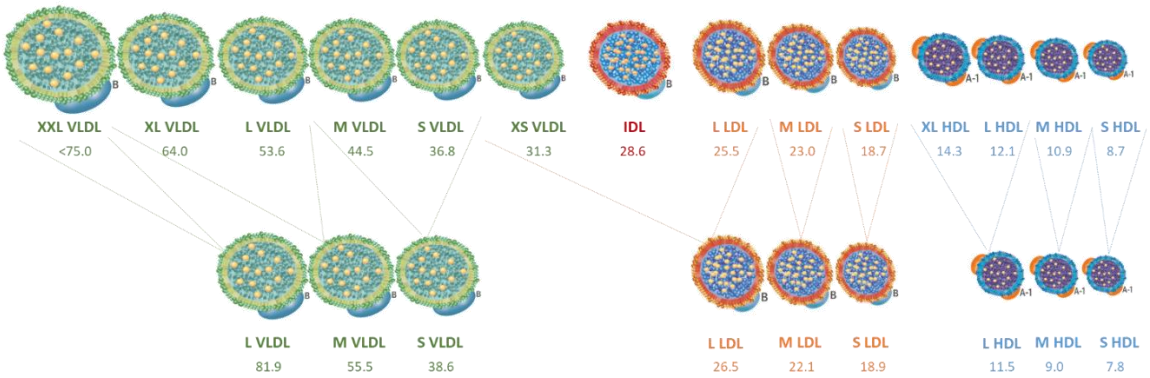

Figure 38: *Illustration of comparisons of Liposale® and Nightingale® NMR test (mean diameter in nm).*

	Catalan cohort	Norwegian cohort
VLDL	Large VLDL (81.9)	Extremely large VLDL (>75.0)
	Medium VLDL (55.5)	Very large VLDL (64.0) Large VLDL (53.6)
	Small VLDL (38.6)	Medium VLDL (44.5) Small VLDL (36.8)
LDL	Large LDL (26.5)	Extra small VLDL (31.3) IDL (28.6) Large LDL (25.5)
	Medium LDL (22.1)	Medium LDL (23.0)
	Small LDL (18.9)	Small LDL (18.7)
HDL	Large HDL(11.5)	Extra large HDL (14.3) Large HDL (12.1)
	Medium HDL (9.0)	Medium HDL (10.9)
	Small HDL (7.8)	Small HDL (8.7)

Table 4. *Table showing medium sizes of different lipoprotein particles of Liposale® and Nightingale® NMR test (mean diameter in nm).*

5.6. Carotid Intima-media Thickness (cIMT)

The cIMT of the right and left common carotid arteries was determined using a MyLab 60X-Vision sonographer (Esaote SpA, Genova, Italy). A 7.5-10 MHz linear transducer and semiautomatic radio frequency software were used (QIMT©, Esaote SpA). The images were obtained and measured by a single operator to reduce observer variability. The measurements of the left and right carotid arteries were averaged to obtain the mean cIMT (296,297).

5.7. Diet information

5.6.1. Data collection on dietary intake

The diet data were collected with a quantitative food frequency questionnaire (FFQ) that included 137 items plus alcohol, as validated in the PREDIMED study (266,298). The participants were asked to report the frequency of all foods and drinks consumed over a year. The frequencies of consumption were reported on an incremental scale with nine levels (never or almost never, 1-3 times per month, once per week, 2-4 times per week, 5-6 times per week, once per day, 2-3 times per day, 4-6 times per day and more than six times per day). Diet data have been reported as food groups and as percentage of macronutrients. For this reason, the reported frequencies of food consumption were converted to the number of intakes per day and multiplied by the weight of the portion size indicated in the questionnaire.

In the Norwegian cohort, the diet data were collected from 4 pre-coded food diaries. This diary was developed and validated by the Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, and has previously been validated in Norwegian children (9-13 years of age) (299–301). The PFDs contain a list of 277 food items, drinks and supplements. The daily intake of energy and nutrients was computed using the AE-14 food database and the “kost beregnings system (KBS)” software (version 7.1, year 2014).

In two cohorts, household units and a validated photographic booklet were used to estimate portion sizes. Specialized dietitians-nutritionists have collected these questionnaires.

5.6.2. Spanish and Norwegian food group comparisons

In order to compare the diet of the 2 cohorts, the aliments were grouped into food groups, so that each group included the same foods in the two cohorts. We defined 55 food groups, 20 main categories and 35 subcategories, according to standard definitions (e.g. milk, vegetables, fruits, meats, fish, etc.). These food groups were defined using the 277 and 137 food items contained in the food frequency questionnaires from Norway and Spain respectively.

The following table shows the main food groups analyzed.

	The group include the following foods:
1.Cereal	Rice, pasta, breakfast cereal and all kind of bread.
1.1.White bread	*Bread with 100% of sifted flour.*
1.2.“Middle" whole grain bread	*Bread <50% total flour.*
1.3.Whole grain bread	*Bread >50% total flour.*
1.4.Cereal breakfast with sugar	--
1.5.Cereal breakfast without sugar	--
2.Potatoes	All kind of potatoes.
2.1.Fried potatoes	*Potato fries and French fries.*
2.2.Other potatoes	
3.Vegetables	Chard, spinach, cabbage, cauliflower, broccoli, lettuce, endives, escarole, tomato, carrot, pumpkin, green beans, eggplant, zucchini, cucumber, pepper, paprika, asparagus, artichoke, leek, celery, onion, garlic and mushrooms.
4.Fruits	Orange, tangerine, grapefruit, banana, apple, pear, strawberry, peach, figs, apricot, nectarine, melon, watermelon, grapes, kiwi and berries.
5.Jam	All kind of jam.
6.Nuts	Almonds, walnuts, pistachios, hazelnut, pinions, cashews and raisins.
7.Olives	--
8.Legumes	Chickpeas, lentils, beans and peas.
9.Meat	Chicken, turkey, rabbit, pork, veal, cow, beef, sheep, lamb and goat.
9.1.Red meat	*Pork, veal, beef, sheep and mutton.*

9.2.White meat	*Chicken, turkey and rabbit.*
9.3.Game	*Goose, moose, deer, boar, quail…*
9.4.Processed red meat	*Turkey and chicken ham.*
9.5.Processed white meat	*Salami, serrano ham, liver pate, bacon, hamburger.*
10.Fish	All the types of fish: fresh fish, smoked fish, canned fish and seafood.
10.1.Fat fish	Salmon, herring, mackerel, tuna, sardine…
10.2.Sellfish	Prawns, mussels, clams, oysters…
10.3.Lean fish	Hake, cod, sole, monkfish, grouper, sea bream…
11.Eggs	Eggs and omelettes.
12.Total dairy	Milk, yoghurt and cheese.
12.1.Milk	*Whole, low fat and skimmed milk.*
12.1.1.Whole milk	--
12.1.2.Low fat milk	--
12.1.3.Skimmed milk	--
12.2.Yoghur	*All kind of yoghurt*
12.3.Cheese	*All kind of cheese.*
12.3.1.Low-fat cheese	*Cheeses with less than 30% fat.*
12.3.2.High-fat cheese	*Cheeses with more than 30% fat.*
13.Fat dairy products	Cream, sour cream, ice cream and dairy desserts.
14.Total fat cooking	Butter, margarine, rapeseed oil, olive oil, soy oil, sunflower oil, corn oil and lard.
14.1.Butter	--
14.2.Margarine	*Normal margarine.*
14.2.1.Low-fat margarine	*Margarines with PUFA*
14.3.Rapeseed oil	--
14.4.Olive oil	--
14.5.Others oils and fats	*Sunflower, soybean oil and lard*
15.Dressings	Ketchup, mayonnaise and mustard.
16.Sweet products	Sugar, honey, chocolate, cocoa powder, cakes, pastries and cookies.
17.Snacks	Chips potatoes, pop corn, toasted corn, nachos, etc.
18.Precooked food and fast food	Pizza, kebab, taco, wraps, casserole dishes, spring rolls, etc.
18.1.Pizza	--
18.2.Prepared soups	*Soups that are already prepared.*
19.Beverages	Natural juices or smoothies, juice and soft drinks with sugar and with artificial sweeteners, coffee and tea.
18.1.With sugar	*Juice and soft drinks with sugar.*
18.2.Artificial-sweeteners	*Soft drink with artificial sweeteners.*
18.3.Natural fruit juices	*Natural juices or smoothies without sugar.*
18.4.Tea/coffee	*Coffee and tea.*
20.Alcohol	Wine, beer and alcohol drinks.

Table 5: *Food groups including foods of Mediterranean and Nordic diet.*

5.6.3. Intervention diet (TLSC-IP)

The TLSC intervention protocol (TLSC-IP) consisted of nutritionist visits during which children and their families were taught to eat healthily, using simple and healthy culinary techniques and meal plans. They were also motivated to perform physical exercise and avoid alcohol and tobacco consumption. At the end of the clinical visits, each participant was provided with a personalised lifestyle recommendation report consisting of tailored advice to bring the diet in line with international guidelines according personal deviations.

The TLSC-IP is divided into three parts: 1. workshops, 2. magazines and 3. a blog.

Workshops: these consisted of 6 educational activities over 6 months (1 activity/month). Each workshop lasted 1 hour. The 6 workshops were: 1. Cooking is fun. 2. Catching foods. 3. Let's move on! 4. A healthy breakfast. 5. The journey of the senses. 6. A celebration of colour. With all of these items we cover different aspects of food and PA. More detailed information about the workshops is given in table 6.

	Name of activity	Description
1	**Cooking is fun**	Practical workshop on healthy habits when cooking. The workshop explained the importance of using healthy cooking techniques (baking, steaming, en papillote, boiling, etc.), of mixing different food groups and colours and of making more appetizing dishes with foods that you don't like very much. A chef with experience in leading cooking workshops led the event and demonstrated how to prepare a healthy dish (healthy crepe with vegetable spaghetti, almonds and fresh low-fat cheese). There was an emphasis on hygiene (the importance of washing food and washing hands well). While the children cooked, parents were taught to fill in a 3-day diary.
2	**Catching food**	The children played traditional board games adapted to deal with concepts regarding lifestyle changes. Those over 8 years played "Trivial Pursuit" and those under 8 years played "The Game of the Goose." -In the version of Trivial Pursuit, the six different colours represented different food groups: carbohydrates, fruits, vegetables, dairy products, proteins and extras. When the children landed on a square, they had to take a card of that

		colour and complete a random test (do mime, make something with plasticine, draw, answer a riddle or a true/false question). All of the answers were a food from one of the different food groups. -In the Game of the Goose the children learnt that could move forward round the board if they landed on fruits, fish, legumes and vegetables, but if they landed on foods such as sugary drinks or jelly beans, they had to move back. The squares usually occupied by the Goose had two children playing sports, and each time they landed on one of these squares, the children could throw again. In this way, the children made a positive association between playing sports and progressing in the game (and therefore in life).
3	**Let's move on**	Using traditional games, parents and children increased their PA. In addition to moving, some of the games reinforced a good body posture
4	**Healthy breakfast**	Through playful games, they learned to make a healthy breakfast using the main food groups (dairy, cereals and fruit). We also taught them about phytosterols and about the importance of having breakfast at home and at school.
5	**The journey of the senses**	The children were blindfolded and experienced different foods with their other 4 senses (taste, touch, hearing and smell). The aim of the workshop to encourage children to try new things
6	**A celebration of colour**	A cooking workshop and crafts involving fruits were held. The children had to create succulent dishes from fruit to encourage people to increase their intake. In this activity, we showed that celebrations do not always have to feature cakes and that guests can be offered fruit instead.

Table 6: *Information about workshops given to children with hypercholesterolemia in the intensive TLSC group.*

All the material used in the workshops was prepared by the multidisciplinary team of the Clinical Unit and was thus adapted to the children of the Unit. The material was adapted to the ages and cultural backgrounds of all children who participated in the workshops. Materials and activities were offered for children aged 5-8 years and for children aged 8-12.

The workshops were held in a spacious room to accommodate children and families in a pleasant and relaxing environment. All family members who wished to participate with their children were invited.

Magazine: After each workshop, the children were given a fun magazine containing the main concepts of the workshops. The magazine included different games to improve their awareness and encourage the correct choice of foods and lifestyle.

Blog: In addition, a closed blog was created for sharing experiences and photos from the workshops, questions, recipes and different competitions to encourage families to participate.

5.7. Physical activity

PA was assessed using the Minnesota leisure-time PA questionnaire. In accordance with international guidelines, we quantified the number of hours or minutes of PA per week (60-61).

5.8. Statistical analysis

The results are expressed as the mean ± SD for normally distributed data, as the median (interquartile range (IQR)) for data that were not normally distributed and as frequencies for categorical data. Kolmogorov-Smirnov tests were used to ensure that the data had a normal distribution. T-tests and ANOVA were used to determine significant differences between groups when the data were normally distributed and Mann-Whitney and Kruskall-Wallis tests were used to detect significant differences when the data were not normally distributed and chi-square test was used for categorical variables. The ANCOVA test was used to observe differences between age-adjusted cohorts.

Binary correlations were evaluated using Pearson's test for normally distributed data and Spearman's test for data that were not normally distributed. A multiple linear regression analysis was performed to studied the associations of diet with the normal lipid profile and full lipoprotein profile.

The paired T-test were used in normal variables and Wilcoxon test in non-parametric variables to determine the difference after one year intervention.

To assess the relationships between lipid variables and patient provenance, we carried out a series of multivariate models. To do so, we divided our dataset into a training set consisting of 80% of the patients in which models were constructed and a test set consisting of the other 20% of the patients to evaluate model performance. The models included three types: logistic regressions regularized via elastic net, random forests and boosted models. For each scenario, we trained several versions of each type of model, adjusted their parameters via 5-fold cross-validation and chose the model that performed best. This model was then evaluated on the test set, and its performance is the one that we report.

To assess the effect of the predictive variables on the target, we carried out a dual approach. Random forests and boosted models proved to be more accurate models for such complex scenarios (302) and furthermore allowed us to assess the relative importance of each variable against all others via out-of-bag accuracy before and after variable permutation (303). Both models are slightly different in variable selection but close enough to be able to combine them to draw meaningful conclusions. A receiver operating characteristic (ROC) curve based on a model including the particle number of all lipoprotein subclasses was performed to estimate the differences between both cohorts.

All analyses were performed using the SPSS 25.0 statistical package for Windows (SPSS, IBM®, Chicago, IL) and the R statistical program version 4.0 (R Core Team, 2014). A *p*-value <0.05 was considered statistically significant in all analyses.

5.9. Ethical aspects

The papers in the present thesis all comply with the 1975 Declaration of Helsinki and the study protocols were approved by the Ethics Committee of the “Pere Virgili” Health Research Institute of Reus-Tarragona and Oslo University. Written informed consent was obtained from all the children and from parents when the children were under 16 years of age.

6. Results

6. RESULTS

The overall results are contained in the following scientific articles:

Objective 1

To establish and implement a strategy for detecting FH in children based on opportunistic screening in coordination with primary and hospital care paediatricians, reverse and direct screening cascades.

ARTICLE 1

*Ibarretxe D, **Rodríguez-Borjabad C**, Feliu A, Bilbao JL, Masana L, Plana N. Detecting familial hypercholesterolemia earlier in life by actively searching for affected children: The DECOPIN project. Atherosclerosis.2018;278:210-216.*

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Detecting familial hypercholesterolemia earlier in life by actively searching for affected children:The DECOPIN project

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HIGHLIGHTS

- Detection of familial hypercholesterolemia (FH) in children remains a major challenge.
- To implement strategies for FH detection in children is necessary.
- Active children to parent and parent to children FH screening pathways increase early FH detection.
- Close collaboration with paediatricians provides high-performance detection method.

ARTICLE INFO

Keywords:
Familial hypercholesterolemia
Children FH
Opportunistic screening
Reverse cascade screening
Direct cascade screening

ABSTRACT

Background and aims: Familial hypercholesterolemia (FH) is underdiagnosed in children. We assessed a combination of two screening methods. The first method was to detect hypercholesteraemic children and then study the parents (Ch-P pathway), and the second one was to study the offspring of FH-affected parents (P-Ch pathway).
Methods: In the Ch-P path, primary care paediatricians were asked to include lipid profiling or, at least, total cholesterol (TC) and then lipid profiling if TC was higher than 5.2 mmol/L in any clinically indicated blood test. Children with LDL-C ≥ 3.5 mmol/L, plus either a family history of early cardiovascular disease or one parent with severe hypercholesterolemia, were referred to the lipid unit where the parents, rather than their children, were studied. In parents with definite, clinical FH, a genetic study was performed. Focused genetic testing was performed on all offspring of genetically positive parents. The P-Ch path consisted of the active study of children from definite FH adults.
Results: Fifty-nine paediatricians covering a total population of 63,616 children agreed to participate in the project. Of the 216 children (122 Ch-P and 94 P-Ch) who were ultimately referred to the lipid unit, 87 children with FH (84% genetically positive) were identified. Additionally, 41 parents (from 40 families) were newly diagnosed with FH (63% genetically positive). Forty-nine different mutations were detected: 46 in the *LDLR*, 2 in the *PCSK9* and 1 in *APOB* gene.
Conclusions: The implementation of active strategies to detect FH in children, in close collaboration with primary care paediatricians, provides a high-performance method for early FH detection.

1. Introduction

In Europe, it is estimated that approximately 4.5 million individuals are affected by familial hypercholesterolemia (FH), of whom 20–25% are children and adolescents [1]. Of those, less than 10% are diagnosed. FH is underdiagnosed, and moreover, it is generally detected late in adults [2], precluding any early management of lifestyle education during childhood or early pharmacological therapy if necessary [1]. According to the Safeheart cohort data, only 68.2% of FH patients younger than 18 y/o are on statins, and only 41.5% of patients had LDL-C < 3.4 mmol/L [3].

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Unlike adults, the Dutch Lipid Clinic Network (DLCN) [4] and other clinical criteria for diagnosis cannot be applied in people younger than 18 years. Diagnosis in children should preferably be established by the detection of the FH causative mutation, which is considered the gold standard [5]. However, genetic testing is expensive and not always available. In that case, FH can be diagnosed according to phenotype criteria, such as the presence of very high LDL-C levels in a child from an FH family.

Because heterozygous FH (HeFH) is clinically silent during childhood and beyond, it is necessary to design and implement different strategies for detection [6,7]. Among these, the universal screening [8] of cholesterol concentrations or genetic mutations has been proposed [9]. On the other hand, several varieties of selective screening focusing on children from FH families have been recommended, considering they are more balanced cost-benefit methods [5,10,11]. In other words, there is no global consensus on FH detection strategies either in adults or children. Different countries apply screening methods based on opinions of local expert groups or scientific societies. For example, in the United States, selective screening is recommended beginning at the age of 2 years, and universal screening at 9–11 years [12,13], whereas in most European countries, selective cascade screening based on genetic testing is recommended [14–16]. Therefore, the detection of FH in children remains a major challenge [17].

The objective of the present study is to evaluate an active search strategy in identifying FH children based on two parallel strategies. One implies the collaboration between specialized lipid units and primary care paediatricians. When one suspected case is detected, a child-to-parent study pathway is activated. The second pathway is based on the activation of a direct cascade screening from definite FH-parents.

2. Materials and methods

2.1. Study design

During the period from July 2015 to December 2017, we applied a combination of 2 different screening methods in children: the first one was a combination of opportunistic screening and reverse cascade, and the second one was a direct cascade screening. In the first one, after detecting suspicious children, we studied the parents first (Children-to-parent pathway- Ch-P), while in the second, we studied the children from already diagnosed parents (parent-to-child pathway, P-Ch). To implement the Ch-P pathway, primary care paediatricians were asked to include, in any blood test indicated for children for clinical reasons, lipid profiling to determine LDL-C, or at least, a total cholesterol (TC) measurement, and if TC was higher than 5.2 mmol/L (for conversion to mg/dL multiply by 38.665), lipid profiling was performed. Children with LDL-C ≥ 4.9 mmol/L or ≥3.5 mmol/L plus one of the following conditions: early cardiovascular disease in a first- or second-degree relative; a parent with TC > 7.8 mmol/L (and/or on lipid lowering therapy) or one parent unknown, were sent to the Lipid Unit, or previously, to the hospital paediatric department if secondary dyslipidaemia was suspected. In the Ch-P protocol, we studied the families and parents, instead of the index child, in order to identify the mandatory vertical transmission. When one of the parents had a DLCN > 8, a clinical FH diagnosis was established, and genetic testing was performed. In the case of a positive genetic diagnosis, genetic testing for the known mutation was carried-out on both the index child and all offspring (Fig. 1). The P-Ch consisted of performing a directed genetic study of all offspring of genetically confirmed index cases or a clinical study of children from genetically negative definite FH parents (Fig. 2). The Hospital Ethics Committee approved the study, and all parents provided their written consent to participate in the study. The study complied with the Declaration of Helsinki.

2.2. Clinical evaluation and diagnosis in parents and children

In the Ch-P pathway, parents underwent personal and family anamnesis and directed physical examination (searching for corneal arcus and tendinous xanthomas). Lipid profiling was performed if it was not available in the last two years, and the DLCN index was calculated.

In children, anamnesis and complete physical examination, including anthropometry data and a new lipid profile, were performed.

The FH diagnosis was established both in children and adults by a positive genetic test. Alternatively, the FH clinical diagnosis was established in adults with a DLCN > 8 (definite diagnosis). In children, the FH clinical diagnosis was established if they had an LDL-C > 3.9 mmol/L, and one of the parents had definite FH.

2.3. Genetic testing

In parents with definite FH, the presence of FH-associated mutations was studied by next-generation sequencing (NGS) (Liponext, SEQPRO LIPO RS, Roche Diagnostics). The Liponext detects mutations in *LDLR, APOB, PCSK9, APOE, STAP1 (ADH) and LDLRAP1 (ARH)* genes, and copy-number variation in *LDLR*. A genetic study, focused on the known mutation, was performed for all offspring of positive cases detected from the Ch-P path or already-known FH patients detected in the P-Ch path.

2.4. Statistical analyses

The descriptive results are expressed as the mean ± SD for normally distributed data, the median (interquartile range, IQR) for data that were not normally distributed and the frequencies for categorical data. Because only descriptive results are shown, no additional statistical analyses were performed.

3. Results

3.1. Child-to-parent pathway

A total of 59 primary care paediatricians from the hospital reference zone agreed to collaborate. These paediatricians treat a total population of 63,616 children. Approximately 13,000 TC tests and 3.540 complete lipid profiles were performed. In total, 127 children (3.6% of those with full lipid profile performed) fulfilled the hospital derivation criteria. Seventeen were excluded from the protocol due to several reasons (Fig. 1); thus, 110 children were considered index cases. Forty-one parents, from 40 different families, out of 220 had a DLCN > 8. The genetic study was carried out in these 41 parents, with a positive result in 26 (63%), two from the same family. A directed genetic study was carried out in "all" offspring of genetically positive parents. In total, 32 offspring from these 25 genetically positive FH families plus 8 children from 8 families with at least one unavailable parent (4 dead and 5 unknown, and two from the same family) were studied. Twenty-nine children were genetically positive for FH (28 from positive FH families (87.5%) and 1 from families with some unknown parent), and among them were 2 homozygous individuals from the same family. Additionally, 9 children from genetically negative, but clinically definite FH parents were considered FH because of clinical criteria (definite FH parent + LDL-C > 3.9 mmol/L). Therefore, by the Ch-P pathway, from 110 index children studied in the lipid clinic, a total of 79 new FH cases (38 children, 76% genetically positive, and 41 adults, 63% genetically positive) were detected (Fig. 1).

A girl affected with acid lysosomal lipase deficiency was diagnosed among the non-FH children.

3.2. Parent-to-child pathway

In the context of this project, 94 offspring from 61 FH patients (65% genetically positive), treated and controlled in our unit, were studied. From the 65 offspring of genetically positive families, 44 had a detected mutation (67.7%). Five out of 29 children from genetically negative FH families were considered to have clinical FH.

Overall, out of 216 children studied from Ch-P and P-Ch pathways, 87 children with FH (84% genetically positive) were detected. Additionally,

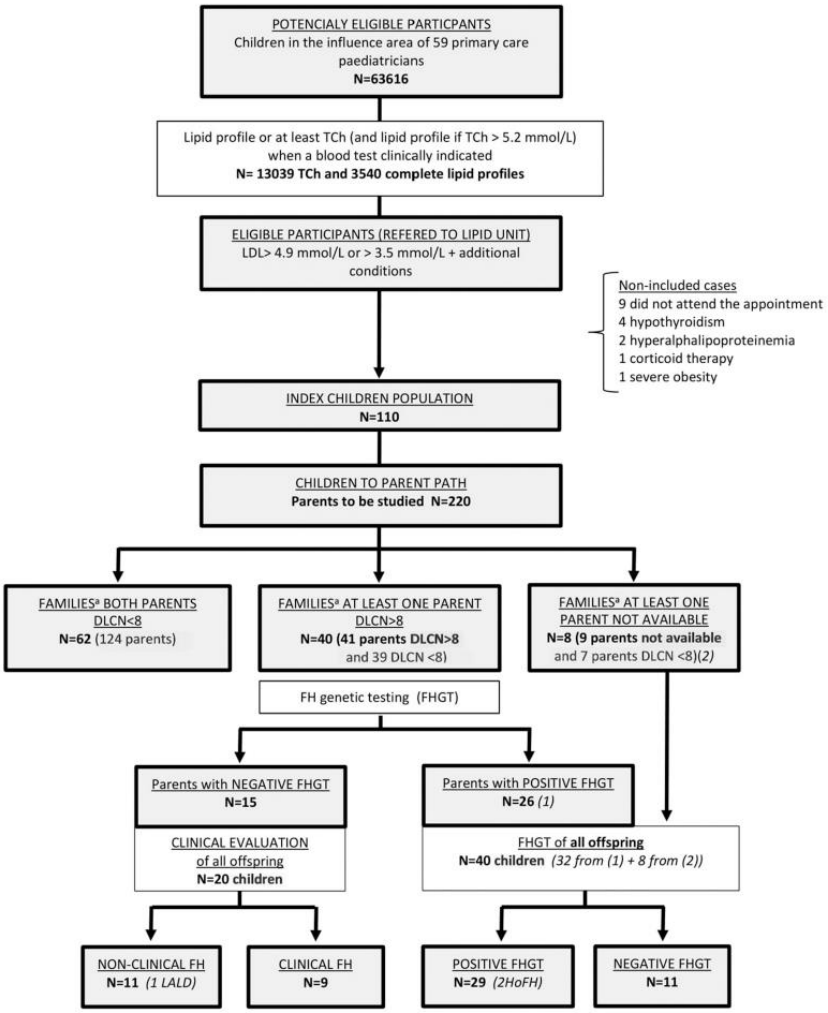

Fig. 1. Child-to-parent pathway.
[a] We studied 220 parents from 110 index children. We sorted the families according to the presence of at least 1 parent with DLCN > 8 and families with at least 1 non-available parent. Two parents from the same family had DLCN > 8 and two parents from 1 family were not available.

41 parents (40 families because in one family both parents were affected) were newly diagnosed with FH (63% genetically positive) (Fig. 2).

The clinical and biochemical characteristics of FH and non-FH children, sorted according the diagnostic pathway (P-Ch or Ch-P), are shown in Table 1.

We identified 49 different mutations: 46 in the *LDLR*, 2 in the *PCSK9* and 1 in *APOB* gene (Table 2).

Seven out of 73 FH children with a positive mutation had an LDL-C < 3.5 mmol/L; in other words, approximately 10% of genetically defined FH did not have the expected phenotype.

4. Discussion

We report the impact of implementing an active FH search in children by combining two different screening strategies, Ch-P and P-Ch pathways.

While in the P-Ch pathway we studied offspring from definitive FH adults, in the Ch-P pathway, the first step was the opportunistic lipid measurement by paediatricians. We cannot exclude that the clinical indication for blood sampling would influence lipid levels in plasma. However, in general, blood testing was indicated as part of a global health screening or for minor health problems and cholesterol concentrations are quite constant in these circumstances. On the other hand, those children investigated in our unit had at least a new lipid profile measured in basal conditions.

Ultimately, 216 children were studied in our unit, and 87 of them had FH (84% genetically positive). Moreover, through the Ch-P pathway, 41 parents from 40 different families were newly diagnosed, for a total of 128 newly detected FH cases.

This screening resulted in being highly efficient in detecting genetically positive FH children. From 105 genetic tests performed in children, 73 were positive (70%). This percentage is well above that obtained from other strategies. The universal genetic screening, studied by others, will detect approximately 4 positive cases and 996 negative results per 1000 studies, according to the accepted prevalence of 1/250 [9,18]. Therefore, methods designed to perform genetic tests in selected

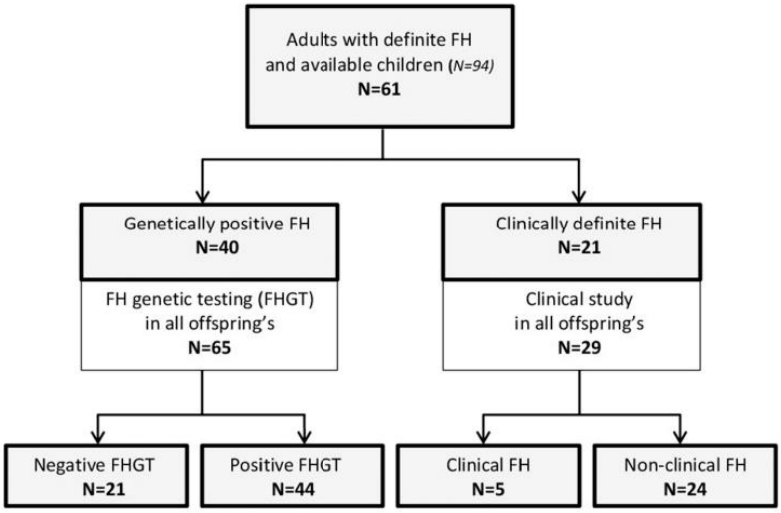

Fig. 2. Parent-to-child pathway.

children seem to be more cost-effective [5,10]. Among them, the Slovenian experience [8], based on the universal screening of cholesterol levels at the age of 5, is highly illustrative. By performing genetic testing on children with very high TC and/or a familial history of cardiovascular disease, they obtain a positivity rate of 57%. Even among clinically definite FH [19], the proportion of positive genetic tests is below 70%; therefore, ours could be considered a high-yield strategy. An interesting aspect of our Ch-P pathway is that it is not exclusively based on LDL-C levels in children but also takes into account vertical transmission [20]. We performed 41 genetic tests on parents and obtained 26 positive results (63%) and then performed 32 genetic studies on children (all offspring) and obtained 28 (87.5%) positive results, two of them HoFH. Another important aspect is that in both Ch-P and P-Ch pathways, the genetic study in children can be focused on the parents' mutation saving time and money. This consideration is of particular importance in countries such as ours, where there is not a majority mutation responsible for the disease. We have detected 49 different mutations in 73 genetically positive FH children. None of them was responsible for more than 5 FH cases.

Our Ch-P strategy was implemented in collaboration with paediatricians, which was welcomed because it could be integrated into paediatricians' daily activities rather than added as an extra task for physicians to complete.

On the other hand, through the P-Ch pathway, we activated the direct cascade screening. Surprisingly, the number of relatives, both adults and children, of definitive FH patients assessed for FH is unacceptably low. There are several reasons, including organization mismatches due to the dependence of relatives on different health areas and physicians, and immigrant population, hindering the study of family members. Our data clearly show that a centralized management of family studies provides high performance. From our P-Ch pathway, we detected FH mutations in forty-four out of 65 (68%) offspring of

Table 1
Characteristics of the studied children sorted by screening pathway and diagnosis.

	Parent to Child pathway (N = 94)		Child to Parent pathway (N = 119)[a]	
	FH (N = 49)	Non- FH (N = 45)	FH (N = 36)	Non- FH (N = 83)
Age	10 ± 4	11 ± 3	9 ± 3	10 ± 3
Gender (female, %)	40.8	44.4	54.1	46.6
Diagnosis age	9 ± 4	11 ± 3	8 ± 3	10 ± 3
Weight (kg)	40.6 ± 18.5	45.3 ± 16.5	36.3 ± 16.4	38.6 ± 16.0
Height (cm)	139.5 ± 30.1	150.1 ± 17.9	135.9 ± 18.6	140.5 ± 17.4
BMI score	0.16 ± 0.90	0.03 ± 0.94	0.28 ± 0.99	0.06 ± 1.06
Waist circumference (cm)	63.0 ± 11.0	66.0 ± 10.0	63.0 ± 14.0	63.0 ± 14.0
SBP (mmHg)	110 ± 13	113 ± 12	106 ± 12	110 ± 11
DBP (mmHg)	65 ± 9	63 ± 8	63 ± 11	64 ±
TC (mmol/L)	7.1 ± 1.4	4.6 ± 0.7	6.7 ± 1.2	5.2 ± 0.8
LDL-C (mmol/L)	5.0 ± 1.3	2.6 ± 0.6	4.8 ± 1.2	3.1 ± 0.7
HDL-C (mmol/L)	1.7 ± 0.4	1.7 ± 0.4	1.5 ± 0.3	1.8 ± 0.5
TG (mmol/L)	0.6 (0.5–0.9)	0.6 (0.4–0.8)	0.9 (0.6–1.1)	0.7 (0.6–0.9)
Apo A1 (mg/dL)	147 ± 26	156 ± 23	146 ± 25	159 ± 30
Apo B100 (mg/dL)	136 ± 29	79 ± 16	137 ± 31	97 ± 18
Lp (a) (nmol/L)	36.0 (15.0–158.4)	20.0 (7.0–48.0)	47.0 (16.0–139.2)	36.0 (14.0–165.0)

FH, familial hypercholesterolemia. BMI score, body mass index score; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglycerides; Apo A1, apolipoprotein A1; Apo B100, apolipoprotein B100; Lp (a), lipoprotein (a).
Data are expressed as mean ± SD for normally distributed data, median (IQR) for not normally distributed data or percentages for categorical data.
[a] Two homozygous FH not included in the FH group. One girl with lysosomal acid lipase deficiency not included in the non-FH group.

Table 2
Detected mutations in children.

Gene change	Location	N	Protein change	Pathogenicity
LDLR				
Regulatory region				
c.-135C > G	Promoter	1		Yes
Missense/in frame				
c.1A > G	exon 1	1	p.Met1Val	Yes
c.241C > T	exon 3	1	p.Arg81Cys	Yes
c.274C > G[a]	exon 3	1	p.Gln92Glu	No
c.283T > G	exon 3	1	p.Cys95Gly	Yes
c.327C > G	exon 4	1	p.Cys109Trp	Yes
c.347G > A	exon 4	1	p.Cys116Tyr	Yes
c.518G > A	exon 4	1	p.Cys173Tyr	Yes
c.533A > G	exon 4	2	p.Asp178Gly	Yes
c.796G > A	exon 5	1	p.Asp266Asn	Yes
c.829G > A[a]	exon 6	2	p.Glu277Lys	No
c.1136G > A	exon 8	1	p.Cys379Tyr	Yes
c.1156G > T	exon 8	1	p.Asp386Tyr	Yes
c.1361C > A	exon 10	2	p.Thr454Asn	Yes
c.1414G > T	exon 10	2	p.Asp472Tyr	Yes
c.1567G > A	exon 10	1	p.Val523Met	Yes
c.1618G > A	exon 11	3	p.Ala540Thr	Yes
[c.1690A > C; c.2393_2401del][b]	[exon 11; 17]	1	[p.Asn564His; p.Lys799_Phe801del]	Yes
c.1775G > A	exon 12	1	p.Gly592Glu	Yes
c.1816G > A	exon 12	1	p.Ala606Thr	possibly
c.1951G > A	exon 13	1	p.Asp651Asn	Yes
c.1952A > T	exon 13	1	p.Asp651Val	Yes
c.2051C > A	exon 14	2	p.Ala684Asp	Yes
c.2099A > G	exon 14	1	p.Asp700Gly	Yes
c.2389G > A	exon 16	1	p.Val797Met	Yes
c.2475C > A	exon 17	4	p.Asn825Lys	Yes
Nonsense/Frameshift				
c.12G > A[a]	exon 1	4	p.Trp 4*	Yes
c.97C > T	exon 2	1	p.Gln 33*	Yes
c.460C > T	exon 4	1	p.Gln 154*	Yes
c.518 del	exon 4	1	p.Cys173Serfs*33	Yes
c.593C > A	exon 4	3	p.Ser 198*	Yes
c.682G > T[c]	exon 4	5	p.Glu 228*	Yes
c.925_931del	exon 6	2	p.Pro309LysfX59	Yes
c.1048C > T	exon 7	1	p.Arg 350*	Yes
c.1448_1451dup	exon 10	1	p.Ile484Metfs*53	Yes
c.2416 dup	exon 17	1	p.Val806Glyfs11	Yes
Intronic/Splicing				
c.313+1G > C[a]	intron 3	1	splicing	Yes
c.313+2T > C	intron 3	1	splicing	Yes
c.1187-10G > A	exon 9	1	splicing	Yes
c.1358+1G > A	exon 9	2	splicing	Yes
c.1845+1G > C	exon 12	1	splicing	Yes
c.2389+4A > G	intron 16	1	intronic	Yes
c.2390-1G > C	exon 17	5	splicing	Yes
Rearrangements				
dup exons 4-5		1		Yes
dup exons 4-15		2		Yes
del exon 12		2		yes
APOB				
c.10580G > A	exon 26	2	p.Arg3527Gln	yes
PCSK9				
c.60_65dup	exon 1	2	p.Leu22_Leu23dup	yes
c.1486C > T	exon 9	1	p.Arg496Trp	yes

[a] Three patients carry two mutations in the same allele of the *LDLR* gene. One child: c.274C > G + c.313+1G > C and two children: c.829G > A + c.12G > A.
[b] These variants usually segregate together (counted as one change).
[c] Two homozygous children.

genetically positive FH patients, again using a mutation-directed search strategy.

Although our study is mainly based on genetic diagnosis, we also included patients with clinical FH diagnosis. Although these patients are currently considered to have a serious form of polygenic hypercholesterolemia, they deserve the same clinical management of patients with a monogenic form [21]. Current guidelines recommend that family studies of genetically negative FH patients not be performed [2]; however, the clinical evaluation of 49 offspring from clinical FH parents identified 14 additional clinical FH children (a parent with definite FH plus LDL-c > 3.9 mmol/L) suitable for lipid-lowering recommendations. Interestingly, clinical FH diagnosis in children was established in only 28.5% of the studied offspring, emphasizing that a second lipid profile after at least three months of diet is always needed in a child to distinguish highly probable from indeterminate FH.

Moreover, 7 positive FH children had normal LDL-C (10%). Wald et al. observed that 33% of genetically detected FH children had LDL-C < 95th percentile [18], reinforcing the utility of proactive protocols to detect affected offspring among members of FH families beyond TC values.

Despite the success of our Ch-P strategy, we detected only approximately one-third of the predicted 254 FH cases in 63,616 children. One of the reasons is that only one-third of children underwent a TC and/or a lipid profile measurement during this period of time. Actively maintaining this protocol will increase the detection rate; however, we agree that our data underline the need for additional protocols. Universal cholesterol screening during childhood associated with our Ch-P pathway could improve the currently unacceptable FH detection rate, and despite some doubts about the long-term clinical impact [22] of these early detection protocols, they will help to improve the lifelong prognosis of this disease.

The study has several limitations. The total number of children included is relatively low, although this number also represents the suspected children from a population of 63,616 individuals. We have also included patients with only the clinical criteria of FH, even when the genetic study was negative, which could be considered inappropriate. However, in clinical practice, these patients are considered to have FH, and they have a genetic cause of hypercholesterolemia, probably polygenic, and should be managed as FH according to current guidelines [21].

In conclusion, we recommend that in addition to an active search of affected children from FH families, implementing a Ch-P strategy preceded by an opportunistic detection or universal screening of hypercholesterolemia in childhood, in collaboration with paediatricians, will provide a highly efficient strategy for the early detection of FH.

Conflicts of interest

D.I. has received lecture fees from Sanofi, MSD and Rubio. C.RB. has received lecture fees from MSD. L.M. has received lecture and advisory board fees from Amgen, Sanofi and MSD. N.P. has received lecture fees from Amgen, MSD, Ferrer, Rubio and Alexion. The other authors have indicated they have no potential conflicts of interest to disclose.

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References

[1] A. Wiegman, S.S. Gidding, G.F. Watts, M.J. Chapman, H.N. Ginsberg, M. Cuchel, et al., Familial hypercholesterolemia in children and adolescents: gaining decades of life by optimizing detection and treatment, Eur. Heart J. 36 (2015) 2425–2437.
[2] B.G. Nordestgaard, M.J. Chapman, S.E. Humphries, H.N. Ginsberg, L. Masana, O.S. Descamps, et al., Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease Consensus Statement of the European Atherosclerosis Society, Eur. Heart J. 34 (2013) 3478–3490.
[3] A. Saltijeral, L. Pérez de Isla, R. Alonso, O. Muñiz, J.L. Díaz-Díaz, F. Fuentes, et al., SAFEHEART Investigators, attainment of LDL cholesterol treatment goals in children and adolescents with familial hypercholesterolemia. The SAFEHEART follow-up registry, Rev. Española Cardiol. 70 (2017) 444–450.
[4] World Health Organisation, Familial Hypercholesterolaemia (FH): Report of a Second WHO Consultation, World Health Organization, Geneva, 1999.
[5] M. Kerr, R. Pears, Z. Miedzybrodzka, K. Haralambos, M. Cather, M. Watson, et al., Cost effectiveness of cascade testing for familial hypercholesterolaemia, based on data from familial hypercholesterolaemia services in the UK, Eur. Heart J. 38 (2017) 1832–1839.

[6] A.C. Martin, S.S. Gidding, A. Wiegman, G.F. Watts, Knowns and unknowns in the care of pediatric familial hypercholesterolemia, J. Lipid Res. 58 (2017) 1765–1776.
[7] G. Langslet, Screening methods in the diagnosis and assessment of children and adolescents with familial hypercholesterolemia, Expert Rev. Cardiovasc Ther. 11 (2013) 1061–1066.
[8] G. Klančar, U. Grošelj, J. Kovač, N. Bratanič, N. Bratina, K.T. Podkrajšek, et al., Universal screening for familial hypercholesterolemia in children, J. Am. Coll. Cardiol. 66 (2015) 1250–1257.
[9] J.W. Knowles, D.J. Rader, M.J. Khoury, Cascade screening for familial hypercholesterolemia and the use of genetic testing, J. Am. Med. Assoc. 318 (2017) 381–382.
[10] R. Pears, M. Griffin, M. Futema, S.E. Humphries, Improving the cost-effectiveness equation of cascade testing for familial hypercholesterolaemia, Curr. Opin. Lipidol. 26 (2015) 162–168.
[11] M. Farnier, F. Civeira, O. Descamps and the FH expert working group, How to implement clinical guidelines to optimize familial hypercholesterolaemia diagnosis and treatment, Atherosclerosis Suppl. 26 (2017) 25–35.
[12] Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report, Pediatrics 128 (2011) S213–S256.
[13] D.P. Wilson, S. Davis, S. Matches, D. Shah, V. Leung-Pineda, M. Mou, et al., Universal cholesterol screening of children in community-based ambulatory pediatric clinics, J. Clin. Lipidol. 9 (2015) S88–S92.
[14] M.A.W. Umans-Eckenhausen, J.C. Defesche, E.J.G. Sijbrands, R.L.J.M. Scheerder, J.J.P. Kastelein, Review of first 5 years of screening for familial hypercholesterolaemia in The Netherlands, Lancet 357 (2001) 165–168.
[15] D.M. Kusters, C. de Beaufort, K. Widhalm, O. Guardamagna, N. Bratina, L. Ose, A. Wiegman, Paediatric screening for hypercholesterolaemia in Europe, Arch. Dis. Child. 97 (2012) 272–276.
[16] L. Masana, F. Civeira, J. Pedro-botet, I. De Castro, M. Pocoví, N. Plana, et al., Expert consensus on the detection and clinical management of familial hypercholesterolemia, Clín. Invest. Arterioscler. 25 (2015) 182–193.
[17] C. Defesche, Defining the challenges of FH Screening for familial hypercholesterolemia, J. Clin. Lipidol. 4 (2010) 338–341.
[18] D.S. Wald, J.P. Bestwick, J.K. Morris, K. Whyte, L. Jenkins, N.J. Wald, Child-parent familial hypercholesterolemia screening in primary care, N. Engl. J. Med. 375 (2016) 1628–1637.
[19] L. Palacios, L. Grandoso, N. Cuevas, E. Olano-Martín, A. Martinez, D. Tejedor, et al., Molecular characterization of familial hypercholesterolemia in Spain, Atherosclerosis 221 (2012) 137–142.
[20] O.S. Descamps, S. Tenoutasse, X. Stephenne, I. Gies, V. Beauloye, M.-C. Lebrethon, et al., Management of familial hypercholesterolemia in children and young adults: consensus paper developed by a panel of lipidologists, cardiologists, paediatricians, nutritionists, gastroenterologists, general practitioners and a patient organization, Atherosclerosis 218 (2011) 272–280.
[21] A.L. Catapano, I. Graham, G. De Backer, O. Wiklund, M.J. Chapman, H. Drexel, et al., ESC/EAS guidelines for the management of dyslipidaemias, Eur. Heart J. 37 (2016) (2016) 2999–3058l.
[22] P. Lozano, N.B. Henrikson, C.C. Morrison, J. Dunn, M. Nguyen, P.R. Blasi PR, et al., Lipid screening in childhood and adolescence for detection of multifactorial dyslipidemia: evidence report and systematic review for the US preventive services task force, J. Am. Med. Assoc. 316 (2016) 634–644.

Objective 2

To characterise and assess clinical, biochemical and vascular imaging aspects that can help us detect, evaluate and treat children and adolescents with FH.

2.1. To describe the main clinical, biochemical and vascular characteristics of children diagnosed with FH.

ARTICLE 2

Plana N, ***Rodríguez-Borjabad C****, Ibarretxe D, Masana L. Familial hypercholesterolemia in childhood and adolescents: A hidden reality. Clin Invest Arterioscl. 2017;29:129-40.*

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REVIEW ARTICLE

Familial hypercholesterolemia in childhood and adolescents: A hidden reality☆

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KEYWORDS
Familial hypercholesterolemia;
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Treatment

Abstract Familial hypercholesterolemia (FH) is the most common genetic disorder in childhood, but in most cases is not detected. High levels of low-density lipoprotein cholesterol are present since the child's birth and this fact will suppose silent development of early atherosclerosis. In cases of homozygous FH, the coronary disease will appear before 20s and in cases of heterozygous FH will occur in middle age. Despite published data, there is not agreement about how and when perform the screening. Familial history of early cardiovascular disease plus presence of hypercholesterolemia in parents is crucial for detection and diagnosis. Actually, it is topic of discussion that it is necessary to achieve therapeutic goals from an early age to improve prognosis. Lifestyle changes are the first line therapy. Statins are the lipid-lowering drugs of choice but the optimal age to start therapy it is still controversial. In this article, current recommendations of expert consensus guidelines about the management and new line therapies of child and adolescents are reviewed.

PALABRAS CLAVE
Hipercolesterolemia familiar;
Niños;
Cribado;
Diagnóstico;
Tratamiento

Hipercolesterolemia familiar en la infancia y la adolescencia: una realidad oculta

Resumen La hipercolesterolemia familiar (HF) es el trastorno genético más prevalente en edad pediátrica; sin embargo, en la inmensa mayoría de los casos pasa totalmente desapercibida. La elevación del colesterol ligado a las lipoproteínas de baja densidad presente desde el nacimiento comportará el desarrollo silente de arteriosclerosis de forma precoz. Este hecho podrá manifestarse en forma de enfermedad coronaria antes de los 20 años en la HF

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homocigota o en la edad media de la vida en la HF heterocigota. A pesar de las evidencias científicas, no existe un acuerdo común de cómo y cuándo se debe hacer el cribado, hecho que se pone de manifiesto al revisar las diferentes guías de consenso de expertos. La historia familiar de enfermedad cardiovascular prematura, junto con la presencia de hipercolesterolemia en uno de los progenitores, es crucial en la detección y el diagnóstico. Alcanzar los objetivos terapéuticos desde edades tempranas es un elemento clave en el pronóstico, aunque sigue siendo un tema de amplio debate. El primer eslabón del tratamiento siempre serán las recomendaciones de hábitos de vida cardiosaludables. En la actualidad, existe controversia sobre a qué edad se debe iniciar el tratamiento farmacológico, siendo las estatinas el fármaco de primera elección. En este artículo se revisan las recomendaciones actuales de las guías de consenso de expertos en el manejo del niño y adolescente con HF, así como las nuevas terapias emergentes.

Introduction

Familial hypercholesterolaemia (FH) is a genetic autosomal dominant disorder, which means that there is a 50% chance of parent-to-child transmission. It is primarily caused by mutations in the gene that encodes the low-density lipoprotein receptor. At present, over 1700 different gene mutations have been described, representing over 90% of all FH cases. To a lesser extent, defects in the gene encoding Apolipoprotein B (ApoB) and the gene encoding *proprotein convertase subtilisin/kexin type 9* (PCSK9) have been described, representing approximately 5% and 1% of cases, respectively. Clinically, these are expressed in the same way and only genetic testing can help us tell them apart. Nevertheless, in around 5–30% of current cases with the FH phenotype, it is not possible to identify the gene responsible for this disease.[1]

There are two types of FH: heterozygous (HeFH), which is the most common hereditary disorder with a prevalence of 1/250–500 individuals, and homozygous (HoFH), with a prevalence of 1/160,000–300,000 individuals, according to the most recent data published on the European population.[2,3] Zamora et al. analysed the electronic records of 2,764,917 patients in Catalonia using the Big Data methodology. Using the cut-off level of low-density lipoprotein cholesterol (LDL-C), described previously in the Spanish population, 14,274 were found to have a FH-compatible phenotype. These data indicate a HeFH prevalence of 1/256 subjects in the Spanish population.[4] Sánchez reported a HoFH prevalence of 1/495,000 subjects after analysing 16,744 genetic studies performed in Spain between 1996 and 2015.[5]

In Europe, it is estimated that the number of affected patients is around 4.5 million, of which 20–25% are children and adolescents. The fact that less than 10% are diagnosed is a problem of huge clinical significance. In countries with intensive genetic screening programmes, like the Netherlands, percentages of detection exceed 70%. In Spain, the rate of FH patients who have undergone testing and been diagnosed is around 6%.[6] Individuals with FH are 100 times more likely to develop premature cardiovascular disease (PCVD) than unaffected individuals.[7] It is estimated that 85% of men and 50% of women will present with some form of coronary event before the age of 65 if they do not receive adequate treatment.[8] Children with HoFH have an increased risk of developing coronary heart disease before 20 years of age if they do not receive intensive treatment.[2,9] As such, early diagnosis and treatment are important for prognosis and long-term outcome.

Patients affected by FH present high plasma concentrations of LDL-C, which may be detected from birth. Children with HeFH have LDL-C levels that are three-times higher than unaffected individuals[10] and the condition may manifest as premature coronary heart disease in adulthood.[2]

In spite of all this, there is no clinical expression in childhood, thus necessitating the design and application of different strategies for its detection. The clinical criteria of the *Dutch Lipid Clinic Network* are not applicable to patients under the age of 18.[11] In children, diagnostic suspicion should be established based on high LDL-C levels, family history of hypercholesterolaemia and/or PCVD.

The ideal age for detection is currently considered to be around 8–10 years, as this is the age of maximum discrimination. During adolescence, total cholesterol (TC) and LDL-C values decrease by around 10–20%, thus making this stage of life less sensitive for the performance of screening.[12,13] Recently, Eissa et al. reported that lipid profiles vary considerably depending on the stage of pubertal development. They propose that this should be considered when screening is indicated.[14] Pang et al. studied 1602 healthy adolescents between the ages of 14 and 17, defining FH according to LDL-C levels and family history of hypercholesterolaemia and/or PCVD. The prevalence of FH was 1/267 adolescents, with the authors underlining the absence of PCVD in parents, but the presence of PCVD in grandparents.[15]

Various studies have been published in which it has been observed that carotid artery intima-media thickness is higher in children with the FH phenotype than in normolipaemic children, as it is directly related to LDL-C levels. This significant difference in carotid artery intima-media thickness was observed in children from 7 years of age.[2] The presence of coronary calcifications has also been observed in 25% of HeFH patients aged 11–23 years, and especially in the aortic artery of the majority of adolescents with HoFH.

Detecting FH in the paediatric population is still a major challenge, as the vast majority are undiagnosed, thus leading to a delay in the onset of treatment, both in terms of lifestyle changes and drugs, which may cause coronary risk to increase in this population during middle age.

The LDL-C cut-off level, the age at screening or the criteria for suspecting the condition in a parent are currently a source of dispute; this review thus describes different screening strategies for improving detection and diagnosis in children with FH. In turn, this review includes up-to-date recommendations on lifestyle changes and pharmacological treatment.

Children and adolescents with familial hypercholesterolaemia

Why perform screening?

Screening in childhood and adolescence is completely justified:

- Hypercholesterolaemia increases the risk of accelerated arteriosclerosis development and, as a result, PCVD.
- Screening may help to identify this high-risk population.
- At present, safe and effective pharmacological treatments are available that may slow down or even reverse arteriosclerosis, thereby decreasing cardiovascular (CV) risk in these children.

However, despite the evidence, there is currently no consensus. If we compare the strategies recommended on different continents, we can observe differences between them. Moreover, there are even discrepancies between the countries within Europe.[7]

Types of screening strategies

There are different strategies—which are not mutually exclusive—for diagnosing new cases among the paediatric population.

Universal screening: consists of the routine measurement of TC levels in children of a certain age. This method has been proven to detect 90% of children with FH between the ages of 1–9, with a false positive ratio of less than1%.[16] An example of this type of screening is the model applied in Slovenia at 5 years of age.[17]

Failing that, opportunistic screening is recommended, i.e. the inclusion of the TC assessment in any blood test prescribed by the paediatrician between 2 and 9 years of age.

Selective screening: consists of the measurement of TC levels in children with a family history of either PCVD or hypercholesterolaemia in one of the parents. This type of screening was recommended by the first paediatric expert panel, the *National Cholesterol Education Program*,[17] by the *American Heart Association*[18] and the *American Academy of Pediatrics*.[19] However, it has become apparent that, on applying this screening method, between 30% and 60% of children with FH pass by undetected.

Direct cascade screening: if we know the genetic mutation responsible for the parent's FH, genetic testing is extended to all first-degree relatives, including children. This type of genetic screening boasts 100% sensitivity and specificity in family studies and is recommended as it is the technique that offers the best cost-effectiveness. A clear example of this type of screening is the model applied in the Netherlands from 1994 to the end of 2014.[20,21] In the United Kingdom, guidelines recommend performing genetic testing in adult individuals and extending cascade screening to children over 10 years of age.[7]

In Spain, the SAFEHEART study detected a total of 1984 new relatives with FH from 768 probands. The authors highlight that, despite being a hereditary disorder, 25% of the detected relatives did not know that they were carriers of the disease and 20% did not receive treatment.[22]

Data from the study by Oliva et al. suggest that familial cascade screening with genetic testing and subsequent statin treatment is cost-effective.[23]

Reverse cascade screening: where testing is initiated in the parents after detecting hypercholesterolaemia in the child. If one parent presents a score ≥ 6 in the clinical criteria of the *Dutch Lipid Clinic Network*, genetic testing will be requested. If the mutation is detected, genetic testing will be performed on the child. In contrast, if the result is negative, the child will not undergo testing, but he/she may be diagnosed with FH if LDL-C levels exceed the 95th percentile. Various guidelines recommend performing this type of screening, but it is underused in clinical practice. Table 1 depicts the up-to-date guidelines and the types of screening they recommend.

Screening strategies in Spain

Various FH reviews and consensuses have been published in recent years.

The *Asociación Española de Pediatría* [Spanish Association of Paediatrics] published guidelines for treating children with hypercholesterolaemia[24] that can be summarised as follows:

- Universal screening is not recommended.
- Selective screening is recommended if there is a history of PCVD in first- and second-degree relatives and/or a TC value >240 mg/dL in one parent.
- The ideal screening age is between 2 and 10 years.

The *Sociedad Española de Arteriosclerosis* [Spanish Arteriosclerosis Society] published an expert consensus document[11]:

- Universal screening in all children between 8 and 10 years of age.
- Direct cascade screening of first-degree relatives of patients with a genetic diagnosis of FH, irrespective of TC levels. If the genetic test comes back negative, LDL-C levels should be determined in all first-degree relatives.
- Reverse cascade screening of first-degree relatives of children with LDL-C levels >135 mg/dL or established genetic testing.
- Selective screening of children with a family history of PCVD and/or hypercholesterolaemia.

The *Fundación de Hipercolesterolemia Familiar* [Familial Hypercholesterolaemia Foundation] published an expert consensus document[25]:

Table 1 Summary of the main guidelines on familial hypercholesterolaemia in childhood and adolescence.

Society	Country	Screening	Diagnosis	Target LDL-C	Start of treatment	Year
National Institute for Health and Clinical Excellence	United Kingdom	Direct cascade	Based on the Simon Broome criteria TC > 260 mg/dL or LDL-C > 155 mg/dL in patients < 16 years of age TC > 290 mg/dL or LDL-C > 190 mg/dL in patients > 16 years of age	50% reduction	10 years and above	2008
Spanish Association of Paediatrics' therapeutic approach to FH	Spain	Selective screening from 2 years of age in obese patients, patients with a history of premature coronary heart disease or parents with FH	Not specified		From 10 years of age and in cases where LDL is >500 mg/dL at 8 years of age	2009
Belgian consensus for FH treatment in children and young adults	Belgium	Non-universal screening Selective screening > 2 years of age Screening if risk factors present at >2 years of age	LDL-C > 200 mg/dL LDL-C > 160 mg/dL + family history LDL-C > 135 mg/dL with familial mutation	LDL-C < 130 mg/dL between 10 and 14 years of age and/or 30% reduction LDL-C < 130 mg/dL between 14 and 18 years of age and/or 50% reduction	10 years and above	2011
Paediatric guidelines on cardiovascular risk in children and adolescents	USA	Selective screening in patients ≥2 years of age Universal screening in patients aged 9–11 years, with TC, HDL-C and non-HDL-C or full lipid profile Universal screening in patients aged 17–21 years, with full lipid profile	Children or adolescents with fasting LDL-C > 160 mg/dL or >190 mg/dL of non-HDL-C. These patients must have relatives with the condition	50% reduction in LDL-C or LDL-C < 130 mg/dL	10 years and above	2011
Paediatric FH screening in Europe	Europe	Universal screening in patients aged 1–9 years, during the vaccination phase	Not specified, although the importance of detecting the mutation in parents is explained	Not specified	8 years and above	2012

Table 1 (*Continued*)

Society	Country	Screening	Diagnosis	Target LDL-C	Start of treatment	Year
Consensus of the European Atherosclerosis Society and the European Society of Cardiology	Europe	Direct cascade if a family member has FH, a TC level > 230 mg/dL, a history of premature coronary heart disease, tendinous xanthomas or premature sudden cardiac death Universal cascade screening is recommended, as performed in Slovenia	In patients <10 years of age with LDL-C levels > 160 mg/dL with a family history of PCVD or a parent with hypercholesterolaemia If the parent tests positive for the FH mutation and the child's LDL-C is >135 mg/dL The criteria of the *Dutch Lipid Clinic Network* are used	Maintain LDL-C levels <135 mg/dL	Between 8 and 10 years of age	2013
Expert consensus of the European Atherosclerosis Society specific to children and adolescents (European Society of Cardiology)	Europe	Consider universal screening Direct cascade screening from 5 years of age or before if homozygosis is suspected	LDL-C ≥ 190 mg/dL or LDL-C ≥ 160 mg/dL with a family history of PCVD and/or a positive genetic test LDL-C ≥ 130 mg/dL and a positive family genetic test **Children diagnosed with FH should have their Lp(a) measured so their risk may be stratified*	In children between 8 and 10 years of age, reduce LDL-C by 50% compared to pre-treatment levels Children ≥ 10 years, especially if there are additional CV risk factors or LDL-C levels < 130 mg/dL	Between 8 and 10 years of age	2015
Primary Care consensus document for FH	Spain	Selective screening before 8 years of age TC should be tested from 2 years of age if there is family history of FH If a relative presents a known mutation, perform genetic testing if LDL-C levels are >150 mg/dL	LDL-C > 190 mg/dL or LDL-C > 150 mg/dL where there is a family history of PCVD and/or hypercholesterolaemia in one of the parents and/or a genetic confirmation in one of the parents	LDL-C < 160 mg/dL in patients under 14 years and LDL-C < 130 mg/dL from 14 years and above, except in the event of other CVRFs or a history of PCVD in the affected parent, as provisions may be stricter in these cases	10 years and above	2015
Scientific statement on FH from the American Heart Association	USA	Universal screening between 6 and 12 years of age Indirect cascade	LDL-C > 160 mg/dL + family history and/or genetic mutation	LDL-C < 100 or 130 mg/dL or achieve a 50% reduction	Between 8 and 10 years of age	2015
Review on the diagnosis and treatment of FH	London	Direct cascade using the proband Opportunistic cascade	LDL-C > 155 mg/dL, having ruled out secondary causes	Maintain LDL-C levels < 135 mg/dL	Not specified	2015

Table 1 (*Continued*)

Society	Country	Screening	Diagnosis	Target LDL-C	Start of treatment	Year
Optimisation of FH treatment in children and adolescents	Netherlands	Universal screening Selective screening based on family history	LDL-C > 190 mg/dL on 2 occasions after a 3-month diet period Family history of PCVD and/or high cholesterol in first-degree relatives together with LDL-C > 160 mg/dL In case of a mutation in a first-degree relative and if the patient presents with an LDL-C level > 130 mg/dL	50% reduction Children aged 8–10 years with LDL-C < 154 mg/dL Children > 10 years of age with LDL-C < 135 mg/dL	Between 8 and 10 years of age	2015
Paediatric guidelines on managing FH from the National Lipid Association expert panel	USA	Familial cascade	LDL-C > 160 mg/dL	50% reduction in LDL-C or <100	Between 8 and 10 years of age	2016

- Direct cascade screening from 2 years of age when one of the parents is diagnosed and, if possible, before 8 years of age.
- Suspect FH in children with LDL-C levels > 190 mg/dL or LDL-C > 150 mg/dL where there is a family history of PCVD and/or hypercholesterolaemia in one of the parents and/or a genetic confirmation in one of them.

Suspicion and diagnosis

The clinical diagnosis of FH in childhood can be difficult as a phenotypic overlap with polygenic hypercholesterolaemia can sometimes be observed. The child's diagnosis should preferably be genetic; however, this is not always possible. In this case, we will rely on the child's phenotypic expression and any family history of PCVD and/or hypercholesterolaemia indicating FH.

At present, in our field clinical suspicion will be based on family histories and TC levels. Table 2 shows the lipid profile considered to be within the normal and pathological range according to age.

In light of TC levels ≥ 200 mg/dL, a second test is recommended within a maximum period of 3 months, requesting a full lipid profile. It would be advisable to also use the second blood draw to rule out secondary causes of hypercholesterolaemia in childhood (Table 3). If TC values ≥ 200 mg/dL and LDL-C levels ≥ 130 mg/dL are confirmed and secondary causes have been ruled out, we should recommend a period

Table 2 Reference lipid profile in childhood and adolescence.

	Acceptable	Limit	High
Total cholesterol	<170	170–199	≥200
LDL-C	<110	110–129	≥130
Triglycerides			
0–9 years	<75	75–99	≥100
10–19 years	<90	90–129	≥130
HDL-C	>45	40–45	–
ApoB	<90	90–109	≥110

Data expressed as mg/dL.
Source: Moráis-López et al.[24]

Table 3 Secondary causes of hypercholesterolaemia in childhood.

Drugs: amiodarone, corticosteroids, anabolic steroids, cyclosporin, phenobarbital, progestogens, phenytoin, thiazides, etc.
Anorexia nervosa
Cholestasis: biliary cirrhosis, biliary atresia
Growth hormone deficiency
Endocrine disorders: hypothyroidism, hypopituitarism
Kidney diseases: nephrotic syndrome
Idiopathic hypercalcaemia
Acute intermittent porphyria
Deposition diseases: glycogenosis, Tay-Sachs, Gaucher, Niemann-Pick
Dietary factors

Table 4 Suspicion criteria for familial hypercholesterolaemia in childhood and adolescence.

Child with LDL-C ≥130 mg/dL and one of the following:
History of PCVD in first-degree relatives <55 years of age and second-degree relatives <50 years
Parent with total cholesterol levels >300 mg/dL or receiving lipid-lowering treatment
Lack of information on the parents
One parent affected by FH with a clinical or genetic diagnosis

of dieting for no less than 6 months. After this period, if LDL-C levels ≥ 130 mg/dL persist in an additional test, we should suspect FH if accompanied by another condition (Table 4). If the child's test is the result of selective screening, his/her full lipid profile will be requested immediately.

If LDL-C levels are ≥190 mg/dL, obtained over 2 consecutive tests with a 3-month gap, the probability of finding a causative mutation for FH is very high. If LDL-C levels are ≥160 mg/dL after a period of dieting and there is a history of PCVD in first-degree relatives (men < 55 years, women < 60 years) or second-degree relatives (men < 45 years, women < 50 years) and/or hypercholesterolaemia (c-LDL ≥ 190 mg/dL) or one of the parents is receiving a lipid-lowering pharmacological treatment, there is a high probability of them being a carrier of a causative mutation for FH. If the causative mutation has been detected in one of the parents and the child has LDL-C levels ≥ 130 mg/dL, he/she is also very likely to carry this mutation. If the parent has died due to PCVD and the child presents with LDL-C levels ≥ 130 mg/dL, genetic testing should be attempted.

Genetic testing is recommended. However, at present this is not possible in the entire Spanish population and there are large differences between autonomous communities, with very different circumstances and realities. A clear example of this is that, while in the Canary Islands and Murcia, genetic testing is not subsidised, in Castile and León, there is no form of restriction.[26]

LDL-C levels in children with HeFH range between 190 and 500 mg/dL while, in those with HoFH, this figure ranges between 500 and 1000 mg/dL, together with the presence of tuberous xanthomas and arcus senilis before the age of 10. However, an overlap of LDL-C levels of between 300 and 500 mg/dL can be seen in both forms.[27]

Recently, a computer program has been designed that calculates the probability of detecting a mutation in a patient with suspected FH and which aims to increase detection in young people. This tool is based on the data collected over a 20-year period in the Dutch FH detection programme (http://vasculaironderzoekamc.nl/fh-calculator/).[28]

Therapeutic objectives

There is no evidence that indicates therapeutic objectives in children with FH. Table 1 summarises the different therapeutic objectives in relation to the various guidelines published in recent years.

Therapeutic recommendations

It is important to initiate therapy early in children with FH.[29]

Lifestyle changes

A balanced and healthy diet is key to FH treatment and in order to prevent arteriosclerosis. It has been observed that diet may reduce LDL-C levels by 10–15%, although this can vary significantly according to the type of patient and type of mutation.[24,25] It is worth noting that, in these children, dietetic recommendations alone are not going to be sufficient to achieve the therapeutic objectives; nonetheless, maintaining an appropriate weight remains important in order to avoid adding further CV risk factors.[30]

These recommendations should be indicated from 2 years of age under the supervision of a qualified dietician/nutritionist who helps to reinforce nutrition therapy in the family setting in order to achieve better treatment adherence.[31]

Fat

Fat consumption should be limited to <30% of the total calorie intake. Until recently, many of the guidelines published specified the need to lower all types of fat and to place special emphasis on the importance of limiting cholesterol intake (<200–300 mg/day). However, more stress is now being placed on quality over quantity.

The intake of saturated fatty acids should be <10%; however, some guidelines are stricter and state it is best to stay below 7%. Reducing saturated fat and cholesterol in children's diets has not been shown to alter their nutritional status, growth or pubertal development.[32] Their monounsaturated fat intake should be kept at around 10%, principally in the form of oleic acid. The *trans* fats found in processed foods should be avoided.

Different clinical trials have shown that the daily consumption of plant stanols and sterols at doses of 1.5–3 g/day in children and adolescents with HeFH can be beneficial in reducing LDL-C levels by approximately 9–19%.[33] Recently, the European Atherosclerosis Society published a consensus panel which recommends consumption of plant stanols and sterols in FH patients from 6 years of age, as long as their fruit and vegetable intake is sufficient in order to avoid a fat-soluble vitamin deficiency.[34]

None of the following types of supplement is recommended: linoleic acid, omega 3, rapeseed oil,[35] soy protein, garlic extracts[36] or cereals containing psyllium extract.[37]

Carbohydrates

Carbohydrates include fibres, starches and sugars. They can also be categorised into two types: simple and complex. Complex carbohydrates should be promoted as they have a lower percentage of calories and a high fibre content (whole grains, pasta, rice, bread, potatoes, legumes, fruit and vegetables).

The latest European guidelines on cardiovascular disease prevention advise a fibre intake of 30–45 g/day. The consumption of water-soluble fibre in the form of fortified cereals can be added to diets that are low in fat or saturated

fat, and the recommended daily dose is 6 g/day for children aged 2–12 and 12 g/day for those over 12.

The intake of simple sugars should also be reduced, along with the sugar content in soft drinks.[38,39]

Proteins

A protein intake of around 15% should be advised. Preference should be given to the consumption of white meat and fish.

Salt

Many of the clinical guidelines recommend a maximum intake of 5 g/day (2 g of sodium). In children under 10 years of age, less than 3–4 g/day is advisable; however, the average consumption among this age group is 8.1 g/day.[40] Around 75% comes from processed foods.

Alcohol

Among the school population aged between 14 and 18 years, the most widely consumed drink during the week is beer; at the weekends, spirits and mixers are most common.

It is important to teach children not to consume alcohol.

Physical exercise

Maintaining an ideal weight and encouraging physical exercise are key factors among the set of measures to be followed.

Ideally, children should exercise for over an hour every day, and should spend less than 2 h performing sedentary activities such as playing on consoles or computers and/or watching TV.[4]

It has been observed that physical exercise patterns established during childhood remain lifelong habits and are associated with increased high-density lipoprotein cholesterol and reduced LDL-C.[41,42]

Smoking

The European CV prevention guidelines propose different strategies for promoting smoking cessation.[13] Tobacco consumption has decreased by 60% in the last decade according to the report issued by the Spanish Ministry of Health; nevertheless, 38.4% of Spanish teenagers admit to having smoked at least once and 8.9% do so on a daily basis.

Smoking is another CV risk factor and this risk is even higher in patients with FH.[43]

Pharmacological treatment

Efficacy and tolerance of statins

Statins are the drugs of choice in the pharmacological management of children with FH. They will always be preceded by a period of dietary treatment. Pharmacological treatment should start before puberty as this improves control and parental adherence, with the child's acceptance also being greater.

Statins act by competitively inhibiting the 3-HMG-CoA reductase enzyme, leading to a decrease in intrahepatic cholesterol synthesis and an increase in LDL receptor synthesis.

Various expert consensus guidelines recommend starting statin treatment at 8–10 years of age in children with HeFH, and at the time of diagnosis in children with HoFH, before 5 years of age and no later than 8.[27]

The statins shown below are currently approved by the *US Food and Drug Administration* and the *European Medicines Agency* (Table 5). Pitavastatin has not been approved in children, although a recently-published study demonstrates its safety in patients aged 6–17 years.[44] Various studies have shown the short-term safety and efficacy of statins. A follow-up study was recently published on a cohort of 214 children with FH treated with pravastatin for 10 months,

Table 5 Pharmacological treatment of children and adolescents with familial hypercholesterolaemia.

Fármacos	Age	Dose/day	LDL-C reduction, %
Resins			
Cholestyramine	>6 years	4–8 g	10–20
Colestipol	>6 years	5–15 g	10–15
Colesevelam	>10 years	1875–3750 g	6–12
Statins			
Pravastatin	>8–13 years	10–20 mg	24–30
	14–18 years	10–40 mg	24–36
Fluvastatin	>9–13 years	10–20 mg	14–20
	14–18 years	10–40 mg	14–26
Simvastatin	>10 years	10–40 mg	30–41
Atorvastatin	>10 years	10–20 mg	36–41
Rosuvastatin	6–9 years	5–10 mg	41–47
	>10 years	5–20 mg	41–52
Absorption inhibitors			
Ezetimibe	>10 years	10 mg	20
Anti-PCSK9			
Evolocumab	>12 years	140 mg/14 days	25–30

Table 6 Recommendations before and after statin use in children.

Prior to starting the treatment, determine the patient's liver transaminases, CPK, creatinine and glucose
After starting the treatment, monitor the patient's lipid profile and liver transaminases
Monitor CPK if the patient reports myalgia
Monitor glucose in patients receiving high statin doses and who are obese
Growth and pubertal development check
Yearly check once the therapeutic objective has been met

demonstrating that the long-term safety does not differ from that reported in adult patients.[45]

Treatment should be started if LDL-C levels are ≥190 mg/dL or ≥160 mg/dL if accompanied by one of the following conditions: history of PCVD in a first-degree relative; other diseases suffered by the child that involve elevated CV risk (diabetes, metabolic syndrome, hypertension, lupus erythematosus, organ transplant and Kawasaki disease); or in the event of high CV risk factors (obesity, smoking, increased Lp(a) and homocysteinaemia).[13]

When statin treatment is initiated, the lowest recommended dose should be chosen, which can then be adjusted according to response and tolerance. In any case, combination therapy with other drugs (ezetimibe or resins) may be necessary in order to achieve the therapeutic objectives. Table 6 shows the parameters to be monitored. Although side effects are uncommon, the following have been reported: cramps, muscle pain, gastrointestinal effects and elevated transaminases. No growth hormone or sex hormone abnormalities has been reported.[46]

If transaminases are observed to be three times the upper limit of normal, the dose should be reduced or withdrawn and an additional follow-up check should be arranged. If the child undertakes intensive exercise, we recommend stopping exercise at least three days before blood draws in order to avoid elevated CPK levels secondary to exercise.

In children that require pharmacological treatment due to other concomitant diseases, it is important to recognise that cytochrome P450 uses the drug to be metabolised. The decision regarding the type of statin to be chosen for initiating treatment will depend on this fact. If the child presents with kidney failure, the statin of choice shall be atorvastatin, as its excretion by the kidneys is minimal. The paediatrician should be aware of potential drug interactions with statins and must avoid prescribing routine drugs like macrolides.

Girls of childbearing age should be advised on different methods of contraception and must be informed that accidental pregnancies are to be avoided. In case of accidental pregnancy, pharmacological treatment must be immediately suspended.

Efficacy and tolerance of ezetimibe

Ezetimibe is a drug that acts by selectively inhibiting cholesterol and plant sterol absorption in the small intestine, without affecting the absorption of fat-soluble vitamins or other substances.

In general, it is a second-line, lipid-lowering drug that is usually administered in combination with a statin. It is indicated in monotherapy in the event of statin intolerance. Experience of the drug in children is somewhat limited; the studies that have been conducted have only evaluated its short-and medium-term safety and efficacy and, as such, more studies are needed to assess its long-term profile[47,48] (Table 5).

Efficacy and tolerance of resins

Ion-exchange resins act by inhibiting bile acid absorption in the intestine.[49]

There are a number of resins that are currently available on the market (Table 5). However, gastrointestinal intolerance and difficult ingestion can lead to discontinuation of treatment. In an attempt to reduce this problem, dissolving the powder or granules in fruit juice and taking them before meals is recommended. It must be remembered that the drug cannot be administered together with other treatments (1 h before or 4 h after). Prolonged treatments may alter the absorption of fat-soluble vitamins (A, D, E and K) and folic acid, so supplements may be required. The most common side effect is constipation, so a fibre-rich diet will be recommended.

Colesevelam is the newest resin to be placed on the market. Its safety and efficacy have been assessed in children with HeFH between the ages of 10 and 17 years. Due to its presentation, it has a seemingly greater tolerance than the other resins.[50,51]

Efficacy and tolerability of PCSK9 inhibitors

PCSK9 is a protein segregated by the hepatocytes, which intervenes in regulating cholesterol metabolism.[52]

PCSK9 inhibition reduces the number of receptors that are due to be degraded and thus increases their density on the cell surface, with the subsequent reduction of plasma cholesterol.

Anti-PCSK9 antibodies are a new group of drugs that have already proven their excellent short-term safety and efficacy. At present, evolocumab and alirocumab have been approved in Spain and are indicated for the FH population in which therapeutic objectives are not met at the maximum tolerated dose of lipid-lowering drugs.[53,54]

Children with HoFH have also been included in clinical trials with evolocumab. Additional LDL-C reductions have been observed in children carrying defective genes; however, no effect was observed on carriers of receptor-negative mutations.[55,56] PCSK9 inhibitors will undoubtedly be a therapeutic option for a very specific group of children with HoFH, which could even lead to fewer LDL apheresis sessions for those administered this treatment (Table 5).

Future therapeutic innovations

Lomitapide

Lomitapide is an oral inhibitor of the microsomal triglyceride transfer protein present in the endoplasmic reticulum

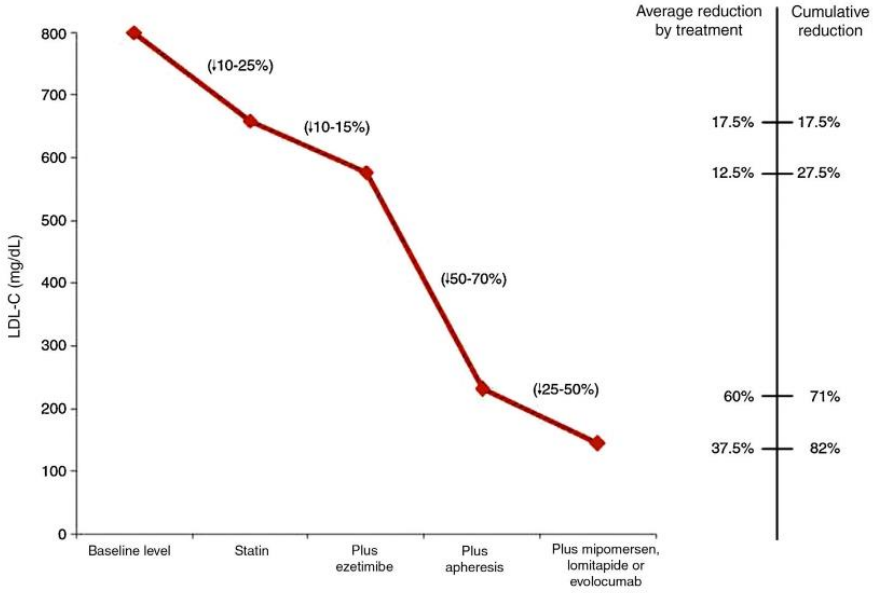

Figure 1 LDL cholesterol reduction according to the treatment administered to children with homozygous familial hypercholesterolaemia.[27]

of hepatocytes and enterocytes. It has been approved by the *US Food and Drug Administration* and the *European Medicines Agency* for the treatment of HoFH. Compared to the standard therapy, one study found lomitapide to reduce the LDL-C concentration by up to a further 50% after 12 months of follow-up.[57] Side effects are very common and primarily of a gastrointestinal nature. Elevated transaminases and the onset of hepatic steatosis have been reported, which must therefore be monitored throughout treatment.

In Spain, the first clinical experience with lomitapide has been published.[58] There are still no data available on children, although it is approved on a ''compassionate use'' basis.

Mipomersen

Mipomersen is an antisense oligonucleotide that inhibits the mRNA transcription of ApoB. The reduction in ApoB synthesis gives rise to a decrease in intrahepatic VLDL and, consequently, LDL-C. A long-term study was recently published where treatment with this drug was associated with a reduction in CV events in patients with FH.[59]

It has not been approved by the *European Medicines Agency* due to the onset of numerous adverse effects, principally comprising local reactions at the injection site and elevated transaminases.[60]

Homozygous familial hypercholesterolaemia

HoFH is the form with the most severe expression. If they do not receive treatment, most children will go on to develop arteriosclerosis before 20 years of age and their life expectancy will not exceed 30 years.[27]

Starting treatment as early as possible is recommended in an attempt to delay the onset of CV disease. The therapeutic objective is the same in children with HeFH; however, in the event of established CV disease, the objective should be LDL-C levels<70 mg/dL. These objectives are very hard to achieve. An oral lipid-lowering treatment should be administered as early as possible from 2 years of age, combining statins with ezetimibe or resins. Despite this, the LDL-C reductions observed will be very mild.

Most children will need to receive LDL apheresis. This is a method of extracorporeal ApoB-lipoprotein elimination, obtaining further LDL-C reductions of 55–70%. This reduction is temporary, so weekly or fortnightly sessions should be performed. It is recommended that apheresis be initiated in children below 5 years of age and never later than 8. LDL apheresis is well tolerated, with adverse effects reported in less than 5% of the procedures. One problem in younger children is venous access. On occasions, fitting a central access device will be necessary, with complications in the form of infections and thrombosis having been reported.

Fig. 1 shows the LDL-C reductions observed according to the treatment administered to children with HoFH.[27]

Children with HoFH should be transferred to specialist units for treatment.

Conclusions

FH is a hereditary genetic disorder with a high CV risk. In order to prevent morbidity and mortality in later life, detection should be initiated in childhood. We believe that the paediatrician plays a vital role and universal screening in children under the age of 10 years would be advisable, although this practice is not accepted across the board at

the present time. As an alternative, we recommend opportunistic screening. Direct cascade and selective screening should be performed in routine clinical practice. Reverse cascade screening has also proven to be an effective tool, and TC should be assessed with all blood draws performed. Healthy lifestyle habits should be encouraged in children from a young age and pharmacological treatment initiated whenever indicated. The performance of genetic testing is important in order to stratify risk, but also as a method of family adherence.

Children with FH should be transferred to specialist referral units with the capacity to perform genetic testing and experience in managing such cases. Children with HoFH will require intensive pharmacological treatment at maximum doses alongside LDL apheresis.

Ethical responsibilities

Protection of people and animals. The authors declare that no experiments were conducted on human beings or animals for this research.

Data confidentiality. The authors declare that no patient data is contained in this article.

Right to privacy and informed consent. The authors declare that no patient data is contained in this article.

Conflicts of interest

The authors declare that they have no conflicts of interest.

References

1. Motazacker MM, Pirruccello J, Huijgen R, Do R, Gabriel S, Peter J, et al. Advances in genetics show the need for extending screening strategies for autosomal dominant hypercholesterolaemia. Eur Heart J. 2012;33:1360–6.
2. Wiegman A, Gidding SS, Watts GF, Chapman MJ, Ginsberg HN, Cuchel M, et al. Familial hypercholesterolaemia in children and adolescents: gaining decades of life by optimizing detection and treatment. Eur Heart J. 2015;36:2425–37.
3. Sjouke B, Kusters DM, Kindt I, Besseling J, Defesche JC, Sijbrands EJ, et al. Homozygous autosomal dominant hypercholesterolaemia in the Netherlands: prevalence, genotype–phenotype relationship, and clinical outcome. Eur Heart J. 2015;36:560–5.
4. Zamora A, Ramos R, Comas-Cufí M, Vila A, Plana N, Masana L. Aproximación a la epidemiologia de la hipercolesterolemia familiar en base a la evidencia en la práctica clínica real. Granada: Sociedad Española de Arteriosclerosis; 2016.
5. Sánchez RM. Hipercolesterolemia familiar homozigota en el registro de dislipemias de la SEA. Granada: Sociedad Española de Arteriosclerosis; 2016.
6. Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. Eur Heart J. 2013;34:3478–90.
7. Kusters DM, de Beaufort C, Widhalm K, Guardamagna O, Bratina N, Ose L, et al. Paediatric screening for hypercholesterolaemia in Europe. Arch Dis Child. 2012;97:272–6.
8. Civeira F, International Panel on Management of Familial Hypercholesterolemia. Guidelines for the diagnosis and management of heterozygous familial hypercholesterolemia. Atherosclerosis. 2004;173:55–68.
9. Benn M, Watts GF, Tybjaerg-Hansen A, Nordestgaard B. Familial hypercholesterolemia in the Danish general population: prevalence, coronary artery disease, and cholesterol-lowering medication. J Clin Endocrinol Metab. 2012;97:3956–64.
10. Ramaswami U, Cooper J, Humphries SE, FH Paediatric Register Steering Group. The UK Paediatric Familial Hypercholesterolaemia Register: preliminary data. Arch Dis Child. 2016, http://dx.doi.org/10.1136/archdischild-2015-308570 [in press].
11. Masana L, Civeira F, Pedro-Botet J, de Castro I, Pocoví M, Plana N, et al. Expert consensus on the detection and clinical management of familial hypercholesterolemia. Clin Investig Arterioscler. 2013;25:182–93.
12. Daniels SR, Gidding SS, de Ferranti SD. Pediatric aspects of familial hypercholesterolemias: recommendations from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. J Clin Lipidol. 2011;5 Suppl.:S30–7.
13. Descamps OS, Tenoutasse S, Stephenne X, Gies I, Beauloye V, Lebrethon MC, et al. Management of familial hypercholesterolemia in children and young adults: consensus paper developed by a panel of lipidologists, cardiologists, paediatricians, nutricionists, gastroenterologists, general practitioners and a patient organization. Atherosclerosis. 2011;218:272–80.
14. Eissa MA, Mihalopoulos NL, Holubkov R, Dai S, Labarthe DR. Changes in fasting lipids during puberty. J Pediatr. 2016;170:199–205.
15. Pang J, Martin AC, Mori TA, Beilin LJ, Watts GF. Prevalence of familial hypercholesterolemia in adolescents: potential value of universal screening? J Pediatr. 2016;170:315–6.
16. Wald DS, Bestwick JP, Wald NJ. Child–parent screening for familial hypercholesterolemia: screening needs a fresh approach. BMJ. 2007;335:1007–8.
17. National Cholesterol Education Program: report of the Expert Panel of Blood Cholesterol Levels in children and adolescents. Pediatrics. 1992;89:525–84.
18. Kavey RE, Daniels SR, Lauer RM, Atkins DL, Hayman LL, Taubert K. American Heart Association guidelines for primary prevention of atherosclerotic cardiovascular disease beginning in childhood. Circulation. 2003;107:1562–6.
19. Haney EM, Huffman LH, Bougatsos C, Freeman M, Steiner RD, Nelson HD. Screening and treatment for lipid disorders in children and adolescents: systematic evidence review for the US Preventive Services Task Force. Pediatrics. 2007;120:e189–214.
20. Langslet G, Ose L. Screening methods in the diagnosis and assessment of children and adolescents with familial hypercholesterolemia. Expert Rev Cardiovasc Ther. 2013;11:1061–6.
21. Galema-Boers JM, Versmissen J, Roeters van Lennep HW, Dusault-Wijkstra JE, Williams M, Roeters van Lennep JE. Cascade screening of familial hypercholesterolemia must go on. Atherosclerosis. 2015;242:415–7.
22. Perez de Isla L, Alonso R, Watts GF, Mata N, Saltijeral-Cerezo A, Muñiz O, et al. Attainment of LDL-cholesterol treatment goals in patients with familial hypercholesterolemia: 5-year SAFEHEART registry follow-up. J Am Coll Cardiol. 2016;67:1278–85.
23. Oliva J, Lopez-Bastida J, Moreno SG, Mata P, Alonso R. Cost-effectiveness analysis of a genetic screening program in the close relatives of Spanish patients with familial hypercholesterolemia. Rev Esp Cardiol. 2009;62:57–65.
24. Moráis-López A, Lama More RA, Dalmau Serra J. Hipercolesterolemia. Abordaje terapéutico. An Pediatr (Barc). 2009;70:488–96.
25. Mata P, Alonso R, Ruiz A, Gonzalez-Juanatey JR, Badimón L, Díaz-Díaz JL, et al. Diagnóstico y tratamiento de la hipercolesterolemia familiar en España: documento de consenso. Aten Primaria. 2015;47:56–65.
26. Argimón J. La aportación reducida de fármacos hipolipemiantes en España Modelo de aplicación y sus resultados. Granada: Sociedad Española de Arteriosclerosis; 2016.

27. Ascaso JF, Mata P, Arbona C, Civeira F, Valdivieso P, Masana L. Hipercolesterolemia familiar homocigota: adaptación a España del documento de posición del grupo de consenso sobre hipercolesterolemia familiar de la Sociedad Europea de Arteriosclerosis. Documento de Consenso de la Sociedad Española de Arteriosclerosis (SEA) y la Fundación Hipercolesterolemia Familiar (FHF). Clin Invest Arterioscler. 2015;27:80–96.
28. Besseling J, Reitsma JB, Gaudet D, Brisson D, Kastelein JJ, Hovingh GK, et al. Selection of individuals for genetic testing for familial hypercholesterolaemia: development and external validation of a prediction model for the presence of a mutation causing familial hypercholesterolaemia. Eur Heart J. 2016 [in press].
29. Braamskamps M, Kuster D, Avis H, Wijburg F, Kastelein J, Wiegman A, et al. Patients with familial hypercholesterolemia who initiated statin treatment in childhood are at lower risk for CHD then their affected parents. Circulation. 2013;128. A17837.
30. Fung MD, Canning KL, Mirdamadi P, Ardern CI, Kuk JL. Lifestyle and weight predictors of a healthy overweight profile over a 20-year follow-up. Obesity (Silver Spring). 2015;23:1320–5.
31. Burrows R, Correa-Burrows P, Reyes M, Blanco E, Albala C, Gahagan S. High cardiometabolic risk in healthy Chilean adolescents: associations with anthropometric, biological and lifestyle factors. Public Health Nutr. 2016;19:486–93.
32. Sánchez-Bayle M, Soriano-Guillén L. Influence of dietary intervention on growth in children with hypercholesterolaemia. Acta Paediatr. 2003;92:1043–6.
33. Guardamagna O, Abello F, Baracco V, Federici G, Bertucci P, Mozzi A, et al. Primary hyperlipidemias in children: effect of plant sterol supplementation on plasma lipids and markers of cholesterol synthesis and absorption. Acta Diabetol. 2011;48:127–33.
34. Gylling H, Plat J, Turley S, Ginsberg HN, Ellegård L, Jessup W, et al. Plant sterols and plant stanol in the management of dyslipidaemia and prevention of cardiovascular disease. Atherosclerosis. 2014;232:345–60.
35. Negele L, Schneider B, Ristl R, Stulnig TM, Willfort-Ehringer A, Helk O, et al. Effect of a low-fat diet enriched either with rapeseed oil or sunflower oil on plasma lipoproteins in children and adolescents with familial hypercholesterolaemia. Results of a pilot study. Eur J Clin Nutr. 2015;69:337–43.
36. McCrindle BW, Helden E, Conner WT. Garlic extract therapy in children with hypercholesterolemia. Arch Pediatr Adolesc Med. 1998;152:1089–94.
37. Davidson MH, Dugan LD, Burns JH, Sugimoto D, Story K, Drennan K. A psyllium-enriched cereal for the treatment of hypercholesterolemia in children: a controlled, blind, crossover study. Am J Clin Nutr. 1996;63:96–102.
38. Morgan RE. Does consumption of high-fructose corn syrup beverages cause obesity in children? Pediatr Obes. 2013;8:249–54.
39. Pérez-Morales E, Bacardi-Gascón M, Jiménez-Cruz A. Sugar-sweetened beverage intake before 6 years of age and weight or BMI status among older children; systematic review of prospective studies. Nutr Hosp. 2013;28:47–51.
40. Lava SA, Bianchetti MG, Simonetti GD. Salt intake in children and its consequences on blood pressure. Pediatr Nephrol. 2015;30:1389–96.
41. LeBlanc AG, Janssen I. Dose-response relationship between physical activity and dyslipidemia in youth. Can J Cardiol. 2010;26:201–5.
42. Fernandes RA, Christofaro DG, Casonatto J, Codogno JS, Rodrigues EQ, Cardoso ML, et al. Prevalence of dyslipidemia in individuals physically active during childhood, adolescence and adult age. Arq Bras Cardiol. 2011;97:317–23.
43. Luepker RV, Perry CL, McKinlay SM, Nader PR, Parcel GS, Stone EJ, et al., CATCH Collaborative Group. Outcomes of a field trial to improve children's dietary patterns and physical activity: the Child and Adolescent Trial for Cardiovascular Health (CATCH). JAMA. 1996;275:768–76.
44. Braamskamp MJ, Stefanutti C, Langslet G, Drogari E, Wiegman A, Hounslow N, et al. Efficacy and safety of pitavastatin in children and adolescents at high future cardiovascular risk. J Pediatr. 2015;167:338–43.
45. Kusters DM, Avis HJ, de Groot E, Wijburg FA, Kastelein JJ, Wiegman A, et al. Ten-years follow-up after initiation of statin therapy in children with familial hypercholesterolemia. JAMA. 2014;312:1055–77.
46. Luirink IK, Hutten BA, Wiegman A. Optimizing treatment of familial hypercholesterolemia in children and adolescents. Curr Cardiol Rep. 2015;17:629.
47. Kusters DM, Caceres M, Coll M, Cuffie C, Gagné C, Jaconbson MS, et al. Efficacy and safety of ezetimibe monotherapy in children with heterozygous familial or nonfamilial hypercholesterolemia. J Pediatr. 2015;166:1377–84.
48. Araujo MB, Botto PM, Mazza CS. [Use of ezetimibe in the treatment of familial hypercholesterolemia in children and adolescents]. An Pediatr (Barc). 2012;77:37–42 [in Spanish].
49. Pintó X, Zúñiga M. Tratamiento de la hipercolesterolemia y prevención de las enfermedades cardiovasculares mediante la inhibición de la reabsorción de ácidos biliares con resincolestiramina. Clin Invest Arterioscler. 2011;23:9–16.
50. Davidson M. The efficacy of colesevelam HCl in the treatment of heterozygous familial hypercholesterolemia in pediatric and adult patients. Clin Ther. 2013;35:1247–52.
51. Davidson M. A systematic review of bile acid sequestrant therapy in children with familial hypercholesterolemia. J Clin Lipidol. 2011;5:76–81.
52. Go GW, Mani A. Low-density lipoprotein receptor (LDLR) family orchestrates cholesterol homeostasis. Yale J Biol Med. 2012;85:19–28.
53. Raal FJ, Stein EA, Dufour R, Turner T, Civeira F, Burgess L, et al. PCSK9 inhibition with evolocumab (AMG 145) in heterozygous familial hypercholesterolaemia (RUTHERFORD-2): a randomised, double-blind, placebo-controlled trial. Lancet. 2015;385:331–40.
54. Robinson JG, Farnier M, Krempf M, Bergeron J, Luc G, Averna M, et al. Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. N Engl J Med. 2015;372:1489–99.
55. Raal FJ, Honarpour N, Blom DJ, Hovingh GK, Xu F, Scott R, et al. Inhibition of PCSK9 with evolocumab in homozygous familial hypercholesterolaemia (TESLA Part B): a randomised, double-blind, placebo-controlled trial. Lancet. 2015;385:341–50.
56. Bruckert E, Blaha V, Stein EA, Raal FJ, Kurtz CE, Honarpour N, et al. Trial assessing long-term use of PCSK9 inhibition in patients with genetic LDL disorders (TAUSSIG): efficacy and safety in patients with homozygous familial hypercholesterolemia receiving lipid apheresis. Circulation. 2014;130. A17016.
57. Cuchel M, Meagher EA, du Toit Theron H, Blom DJ, Marais AD, Hegele RA, et al., Phase 3 HoFH Lomitapide Study investigators. Efficacy and safety of a microsomal triglyceride transfer protein inhibitor in patients with homozygous familial hypercholesterolaemia: a single-arm, open-label, phase 3 study. Lancet. 2013;381:40–6.
58. Alonso R, Mata P, Alonso Y, Gregorio M, de Andrés R. [Homozygous familial hypercholesterolemia. First case in Spain treated with lomitapide, an inhibitor of the synthesis of lipoproteins with apolipoprotein B]. Med Clin (Barc). 2015;145:229–30 [in Spanish].
59. Duell PB, Santos RD, Kirwan BA, Witztum JL, Tsimikas S, Kastelein JJ. Long-term mipomersen treatment is associated with a reduction in cardiovascular events in patients with familial hypercholesterolemia. J Clin Lipidol. 2016;10:1011–21.
60. Raal FJ, Santos RD, Blom DJ, Marais AD, Charng MJ, Cromwell WC, et al. Mipomersen, an apolipoprotein B synthesis inhibitor, for lowering of LDL cholesterol concentrations in patients with homozygous familial hypercholesterolaemia: a randomised, double-blind, placebo-controlled trial. Lancet. 2010;375:998–1006.

2.2. To assess the usefulness of the lipid profile, the concentrations of apoproteins and the biomarkers of LDLR function to improve the detection of FH and the vascular prognosis, according to subclinical data of arteriosclerosis, in children with FH.

ARTICLE 3

*Plana N, **Rodríguez-Borjabad C,** Ibarretxe D, Ferré R, Caselles A, Masana L i grup del projecte DECOPIN. Lipid and lipoprotein parameters for detection of familial hypercholesterolemia in childhood. The DECOPIN Project. Clin Invest Arterioscl.2018;30:170-178.*

ARTICLE 4

*Girona J, **Rodríguez-Borjabad C**, Ibarretxe D, Heras M, Amigo N, Masana L, Plana N. Plasma soluble IDOL, LDLR and PCSK9 levels as biomarkers of familial hypercholesterolemia in children. J Clin Lipidol. 2018;12:211-218.*

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ORIGINAL ARTICLE

Lipid and lipoprotein parameters for detection of familial hypercholesterolemia in childhood. The DECOPIN Project☆

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KEYWORDS
Familial hypercholesterolaemia;
Children;
Apo-B/Apo-A1 ratio

Abstract
Background: Familial hypercholesterolaemia (FH) in children is under-detected and is difficult to diagnose in clinical practice. The aim of this study was to evaluate clinical, biochemical and vascular imaging variables in order to detect children and adolescents with FH.
Methods: A total of 222 children aged 4–18 years old were recruited to participate in a project for the early detection of FH (The DECOPIN Project). They were distributed into 3 groups: FH, if genetic study or clinical criteria were positive ($n=91$); polygenic hypercholesterolaemia (PH) if LDL-cholesterol >135 mg/dl without FH criteria ($n=23$), and control group (CG) if LDL-c <135 mg/dl ($n=108$). Data were collected from family history, anthropometric data, and clinical variables. The usual biochemical parameters, including a complete lipid profile were analysed. The carotid intima-media thickness (cIMT) and thickness of Achilles tendons were determined using ultrasound in all participants.

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◊ The names of the members of the DECOPIN group are given in Appendix 1.

Results: A total of 91 children had a diagnosis of FH, 23 with PH, and 108 with CG. Children with FH had higher concentrations of total cholesterol, LDL-C, ApoB/ApoA1 ratio, and cholesterol-year score, than the other groups. HDL-C was lower in the FH group than in the CG. Thickness of the Achilles tendon and cIMT did not show any differences between groups, although a greater cIMT trend was observed in the FH group. ApoB/ApoA1 ratio >0.82 was the parameter with the highest sensitivity and specificity to predict the presence of mutation in children with FH.
Conclusions: Although LDL-C is the main biochemical parameter used to define FH, the ApoB/ApoA1 ratio (>0.82) may be a useful tool to identify children with FH and a positive mutation.

PALABRAS CLAVE
Hipercolesterolemia familiar;
Niños/as;
Índice ApoB/ApoA1

Valor de los parámetros lipídicos y apoproteicos para la detección de hipercolesterolemia familiar en la infancia. Proyecto DECOPIN

Resumen
Introducción: La hipercolesterolemia familiar (HF) infantil está infradiagnosticada y su diagnóstico no es fácil en la práctica clínica. El objetivo fue evaluar qué características clínicas, bioquímicas y de imagen vascular pueden ayudarnos a detectar a niños/as y adolescentes con hipercolesterolemia afectados de HF.
Métodos: Doscientos veintidós niños y adolescentes de entre 4 y 18 años fueron reclutados para participar en un proyecto de detección precoz de HF (proyecto DECOPIN). La HF se diagnosticó por criterios genéticos o clínicos. Se definió hipercolesterolemia poligénica (HP) cuando el c-LDL > 135 mg/dl pero sin criterios clínicos ni genéticos de HF. Participantes con c-LDL < 135 mg/dl se incluyeron en el grupo control (GC). Se recogieron la historia familiar, los datos antropométricos y las variables clínicas. Se analizaron parámetros bioquímicos y lipídicos. Se determinó el grosor íntima-media carotídeo (GIMc) y los tendones de Aquiles por ecografía.
Resultados: Noventa y un niños fueron diagnosticados de HF y 23 de HP, y 108 como GC. El grupo HF presentó mayores concentraciones de CT, c-LDL, índice ApoB/ApoA1 e índice colesterol año. El c-HDL fue menor en grupo HF que en el GC. Si bien el c-LDL fue el parámetro más definitorio de HF, el índice ApoB/ApoA1 > 0,82 fue el que de forma aislada mostró mayor sensibilidad y especificad para predecir la presencia de mutación en el grupo de niños HF. El grosor de los tendones de Aquiles no mostró diferencias entre grupos. El GIMc fue mayor en los niños HF sin diferencias significativas.
Conclusiones: Los niveles de c-LDL son el marcador de HF. Un índice ApoB/ApoA1 > 0,82 puede ser una herramienta útil para decidir el estudio genético en niños con sospecha de HF.

Introduction

Familial hypercholesterolaemia (FH) is the most common monogenic disorder. It has 2 forms: the homozygous (HoFH) and the heterozygous (HeFH) form. HoFH has a prevalence of 1/160,000–300,000 according to data published for the European population.[1] In Spain, recent data estimate a prevalence of 1/450,000.[2] The latest studies in the European population, establish that the prevalence of HeFH ranges between 1/200–250.[1] The prevalence of people under the age of 18 with phenotype HeFH in the Spanish population is 1/217.[3] In a recent meta-analysis of the paediatric population under 19 years of age, a prevalence of HeFH of 1/279 was observed.[4]

FH is an autosomal dominant disorder, which means that it can be transmitted with a probability of 50% from parent to child, being detectable from birth due to the presence of elevated levels of total cholesterol (TC). Children affected by FH have low-density lipoprotein cholesterol (LDL-C) plasma concentrations of up to three times higher than children who are not affected.[5] This disease is mainly caused by mutations in the gene encoding the LDL-C receptor *(LDL-R)*. To a lesser extent, defects in the gene encoding apolipoprotein B *(ApoB)* and the gene encoding proprotein convertase subtilisin/kexin type 9 *(PCSK9)* have been described. Clinically, these are expressed in the same way and only genetic testing can allow us to tell them apart. Nevertheless, in around 5–30% of current cases with the FH phenotype, the gene responsible for this pathology has

not been identified.[6] Young adults (20–39 years old) diagnosed with FH have a risk 100 times greater of presenting with a premature coronary episode than the unaffected population. Arteriosclerosis in FH begins at an early age in life.[7] For this reason, detection and diagnosis are a priority so that recommendations for healthy life habits can be implemented, and where appropriate, pharmacological treatment can be initiated, as early as possible. This will certainly contribute to the decrease of premature cardiovascular disease (PCVD), characteristic of this population. Despite considerable evidence of the benefits of an early diagnosis, the implementation of detection strategies is still an unresolved issue. Only in Slovenia is universal screening carried out in the paediatric population.[8] Expert consensus does not always follow the same line. The ideal age for diagnosis is between 8 and 10 years of age; however, in our country [Spain] there is no established detection policy. The current reality is that, in most cases, detection is conducted through direct cascade screening or opportunistic detection.[9]

Suspected diagnosis in under-18 patients is established in the face of levels of LDL-C > 130 mg/dl (90th percentile, in the Spanish population),[10] along with a history of severe hypercholesterolaemia in a parent or a history of PCVD in first- or second-degree relatives. Since it is not always possible to conduct a genetic test, in clinical practice it would be ideal if we had biomarkers that would allow us to differentiate hypercholesterolaemia of monogenic origin from that of polygenic hypercholesterolaemia.

The aim of this study was to assess which clinical, biochemical and vascular imaging variables can help us to differentiate those children and adolescents with polygenic hypercholesterolaemia (PH) from those affected by FH.

Subjects and methods

Study design

Transversal Study of the Programme for the Detection of Familial Hypercholesterolaemia in the Paediatric Population (DECOPIN project). A total of 222 children and adolescents aged between 4 and 18 years were included. Three detection strategy types were applied: opportunistic, inverse cascade and direct cascade. To implement opportunistic screening, an information campaign was organised for paediatricians, inviting them to participate in the project. The detection criteria were applied by the Primary Healthcare paediatrician in children and adolescents aged 4 to 14 years. Children with suspected FH were re-evaluated by the Paediatric Endocrinology Department and in turn sent to the Unitat de Medicina Vascular i Metabolisme (UVASMET) of the Hospital Universitari Sant Joan (Reus, Tarragona) for genetic diagnosis. The inverse cascade study was applied to children for whom suspected FH was opportunistically detected if the following criteria were met: LDL-C >135 mg/dl in at least 2 tests and a history of PCVD in first- or second-degree relatives or severe hypercholesterolaemia (TC >300 mg/dl) in one parent or in unspecified relatives. When a child met the criteria for suspected FH, we proceeded to the study of their parents. The clinical criteria from the Dutch Lipid Clinic Network (DLCN) was applied to the parents for the diagnosis of FH, and a genetic test was carried out for those with a score of greater than or equal to 8. In the event of a positive result, the genetic test was conducted for the child[1] and their siblings, if they had any, independently of LDL-C values.

Direct cascade was applied to children and adolescents between 4 and 18 years old, the children of patients diagnosed with FH in our department. When the genetic test was positive in the parent, it was also carried out for their children. If the genetic test result was negative in the parent, it was not carried out for the child.

The Hospital Universitari Sant Joan Ethics Committee approved the study. The parents or legal representatives gave their consent in writing. Their consent was also requested for the genetic test when applicable. The study protocol complied with the ethics regulations of the Declaration of Helsinki, 1975.

Patients

Two hundred and twenty-two children and adolescents aged between 4 and 18 years were included in the DECOPIN Project between March 2013 and June 2017.

The study population was divided into 3 groups:

- Children with FH: mutation carriers or children with LDL-C >135 mg/dl and parent with FH diagnosis by DLCN criteria and score $\geq$8, independent of genetic result.
- Children with PH: LDL-C >135 mg/dl but without clinical or genetic diagnosis of FH in their parents.
- Control children (control group [CG]): children with LDL-C <135 mg/dl.

Children with secondary hypercholesterolaemia: hypothyroidism, renal diseases, hepatic diseases or other chronic diseases and those affected by HoFH, were excluded.

Medical history and physical examination

A personal clinical history was carried out, including family history of PCVD and dyslipidaemia in first- and second-degree relatives. Anthropometric data were collected during the physical examination. The BMI_{Score} (BMI in children − 50th percentile of the Orbegozo growth curves/SD 50th percentile of the Orbegozo growth curves[11]) were used to calculate BMI. Dietary habits and physical activity were assessed. Specific signs of dyslipidaemia were sought in all children. Both parents of the children coming from opportunistic screening were examined for the presence of corneal arcus, tendon xanthomas and xanthelasma.

Biochemical analysis and lipid profile

None of the assessed children had been treated with lipid-lowering agents. Following 8 h of fasting, all participants were subject to a blood extraction. The biochemical and lipid parameters and lipoproteins were determined through enzymatic, colorimetric and immunoturbidimetric methods,

which were adapted to a Cobas Modular 700 self-analyser (Roche®, Basel, Switzerland).

LDL-C was calculated using the Friedewald formula. Cholesterol-year score was calculated (LDL-C × years), as well as ApoB/apoA1 ratio. The presence of proteinuria was ruled out.

Genetic test

The presence of mutations was studied using the Liponext genetic test, which selectively detects mutations in *LDLR*, *APOB*, *PCSK9* and large rearrangements. A genetic sequencing tool (SEQPRO LIPO RS® [Progenika Pharma]) was used.

Ultrasound study

This was carried out at UVASMET in the Hospital Universitari Sant Joan. It was performed with a MyLab 60-X Vision Ultrasound (Esaote, Genova, Italy) ultrasound machine. A 7.5–10 MHz linear probe with semi-automatic software was used. The images were obtained by the same operator to reduce observer variation.[12]

Determination of the carotid intima-media thickness

The carotid intima-media thickness (cIMT) was determined by a semi-automatic method with live images and by radiofrequency. Images were obtained from the posterior wall of the common carotid artery to 1 cm proximal to the carotid bifurcation. The final cIMT was the result of the mean of both common carotid cIMTs.

Determination of the Achilles tendons' thickness

For the testing of this variable, the participants were in prone position with their legs at an angle of flexion of 90°, with a high-resolution probe placed perpendicular to the tendon at 2 cm from the proximal calcaneal insertion site. 3 separate measurements were taken at the area of maximum tendon width with 0.5 between them. The width of the Achilles tendon of each leg is the result of the mean of the 3 measurements. Presence of xanthomas was also checked for.

Statistical analysis

The description of the variables is presented using standard statistics, including mean and standard deviation (SD) for the normal variables, and median and interquartile range (IQR) for non-normal variables. The frequencies are presented as percentages. The normal distribution of continuous variables was studied using the Kolmogorov–Smirnov test. For the categorical variables and to compare frequencies between groups, the Chi-square test was used. The *Student's t-test* or *ANOVA test* with *Bonferroni* post hoc test was used to compare and analyse the continuous variables between groups with normal distribution, and for non-normal, the *Mann–Whitney U* test and *Kruskall–Wallis* tests were used. To be able to connect the variables, the *Pearson* or *Spearman* correlations were carried out between continuous variables of normal or non-normal distribution, respectively. The multivariate analysis was carried out with logistic or linear regression, depending on whether the variables were dichotomous or continuous. ROC curves were generated (acronym of the initials: *receiver operating characteristic curve*) and the area under the curve (AUC) was calculated to assess the capability of identifying children with FH with or without mutation. Statistical significance was considered to be $p < 0.05$.

Statistical analysis was carried out with the SPSS 22.0 for Windows statistical analysis package (SPSS, IBM®, Chicago, IL).

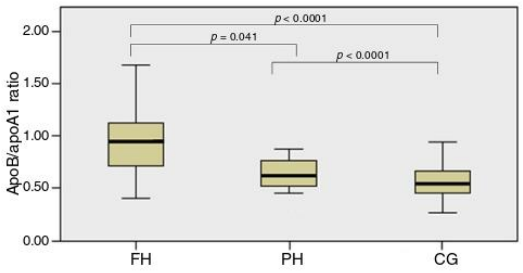

Figure 1 ApoB/apoA1 ratio in the population studied according to diagnosis.

Results

A total of 222 children and adolescents were included through the DECOPIN Project, of whom 91 were diagnosed with FH (53 through direct cascade and 38 by inverse cascade), 23 PH and 108 CG. In Table 1, the baseline characteristics of the population studied are shown distributed according to diagnosis. The clinical variables studied do not demonstrate statistically significant differences, except for age between the FH and CG groups ($p = 0.025$). Statistically significant differences were observed in the lipid profile between the CG with respect to the FH and PH group with TC, LDL-C, ApoB and cholesterol-year score ($p < 0.0001$), which also demonstrated differences between FH and PH ($p = 0.002$). A lower concentration of HDL-C was observed in the FH group in relation to the CG ($p = 0.047$). The Achilles tendon measurement and the cIMT did not demonstrate statistically significant differences between groups. However, in the cIMT there was a superior tendency in the FH group with respect to the other 2 groups. In 66 children with FH, the genetic test was positive (92.5% with mutation in *LDLR*, 3.0% in *APOB* and 4.5% in *PCSK9*). The remainder of the biochemical parameters analysed in blood and urine did not demonstrate significant differences between groups (data not shown).

In Fig. 1, the ApoB/apoA1 ratio is shown between groups which detected statistically significant differences between the FH and PH groups ($p = 0.041$) and between these two groups and the CG ($p < 0.0001$).

In Table 2, data from 81 families with FH is collected, including history of PCVD in first- and second-degree relatives, and lipid deposits in the affected parents, as well as their mean baseline LDL-C and the percentage of those in whom the mutation was detected.

Bivariate correlation was analysed between LDL-C and the ApoB/apoA1 ratio with the anthropometric variables,

Table 1 Data on the population studied distributed according to diagnosis.

	FH ($n=91$)	PH ($n=23$)	CG ($n=108$)	p
Clinical data				
Age (years)	9.00 (6.00–12.00)	11.00 (8.00–12.00)	11.00 (8.00–13.00)	0.025[c]
Males, (n [%])	49 (53.80)	11 (47.80)	60 (55.60)	NS
Weight (kg)	33.50 (23.20–49.50)	38.50 (29.00–51.00)	40.6 (27.35–51.55)	NS
Height (cm)	137.90 ± 25.18	143.30 ± 16.44	144.53 ± 18.66	NS
BMI_{Score}	0.18 ± 0.96	0.17 ± 1.22	0.00 ± 0.95	NS
Waist circumference (cm)	62.54 ± 12.40	66.31 ± 13.66	64.09 ± 12.06	NS
SBP (mmHg)	109.32 ± 12.76	108.85 ± 10.99	111.49 ± 11.23	NS
DBP (mmHg)	64.86 ± 9.40	64.96 ± 9.48	63.59 ± 8.58	NS
Lipid data and genetic test				
Total cholesterol (mg/dl)	262.00 (230.00–302.00)	228.00 (217.00–234.00)	190.00 (168.5–204.5)	<0.0001[b,c]
HDL-C (mg/dl)	62.20 ± 15.32	64.30 ± 16.17	68.08 ± 18.43	0.047[c]
LDL-C (mg/dl)	183.00 (150.00–302.00)	143.00 (139.00–148.00)	107.00 (90.50–123.00)	<0.0001[b,c]
Triglycerides (mg/dl)	64.00 (50.00–87.00)	69.00 (56.00–124.00)	59.00 (46.50–74.00)	NS
ApoA1 (mg/dl)	148.58 ± 26.00	154.24 ± 20.62	158.33 ± 28.42	0.040[c]
ApoB (mg/dl)	129.00 (115.00–154.00)	109.00 (105.00–118.00)	89.00 (74.00–96.00)	<0.0001[b,c]
ApoB/apoA1 ratio	0.95 (0.72–1.12)	0.70 (0.61–0.85)	0.54 (0.45–0.65)	0.041[a] <0.0001[b,c]
Lp(a) (mg/dl)	15.83 (7.08–69.17)	12.09 (7.50–39.42)	12.92 (5.00–40.42)	NS
Cholesterol-year score	1600 (1160–2312)	1551 (1256–1776)	1142 (784–1390)	0.002[b] <0.0001[c]
Ultrasound data				
Achilles tendon (mm)	4.93 ± 0.69	5.25 ± 0.75	5.15 ± 0.75	NS
cIMT (μm)	423.86 ± 63.78	409.17 ± 67.94	413.13 ± 61.43	NS

Data expressed as mean ± SD for the variables that follow a normal distribution, mean (IQR) for the variables that do not follow a normal distribution and n (%) for categorical variables.
The statistical test used was *ANOVA* (normal distribution), *Kruskal–Wallis* (non-normal distribution) or *Chi-square* (for categorical data). A p value of <0.05 was considered to be statistically significant.
ApoA1: apolipoprotein A1; ApoB: apolipoprotein B; BMI_{Score}: body mass index score; CG: control group; cIMT: carotid intima-media thickness; DBP: diastolic blood pressure; FH: familial hypercholesterolaemia; HDL-C: HDL cholesterol; LDL-C: LDL cholesterol; Lp(a): lipoprotein a; PH: polygenic hypercholesterolaemia; SBP: systolic blood pressure.
[a] FH vs PH.
[b] PH vs CG.
[c] FH vs CG.

Table 2 Premature cardiovascular disease in first- and second-degree relatives in FH children from a total of 81 families and physical examination of the parent affected by FH.

	FH relatives, n (%)
Family data	
Premature cardiovascular disease	
First-degree (%)	10 (11.40)
Second-degree (%)	16 (18.20)
Corneal arcus (%)	34 (40.00)
Xanthomas (%)	34 (40.00)
LDL-C (mg/dl)	251.16 ± 64.92
Mutation (%)	75.70

Data expressed as mean ± SD for the variables that follow a normal distribution, and n (%) for categorical variables.

lipid profile and ultrasound study in the studied populations (Table 3).

It is worth noting the statistically significant positive correlations between the ApoB/apoA1 ratio and the complete lipid profile, as well as the cIMT ($p \leq 0.0001$).

In Fig. 2, the ROC curve of the 3 lipid biomarkers that best allow for discrimination of the paediatric population with FH with positive and negative mutation, the best predictor being the ApoB/apoA1 ratio, can be observed. In Table 4, the cut-off point with its sensitivity and specificity can be observed.

Discussion

In the hypercholesterolaemia study in the paediatric age group, the TC test is the most utilised tool between 1 and 9 years of age for detecting FH.[13] In real clinical practice, in the face of all children or adults with hypercholesterolaemia, once secondary causes were discarded, we had to posit the diagnosis of FH. However, since the clinical criteria could not be applied in the paediatric age group and it is not always possible to conduct a genetic test, diagnosing FH is not easy. In this study, we included a total of 222 children, 114 with hypercholesterolaemia detected following the application of direct cascade or opportunistic detection by the Primary Healthcare paediatrician with subsequent application of inverse cascade. Eight children with normal cholesterol were included as a CG originating from

Table 3 Correlations of the anthropometric variables, lipid profile and ultrasound study with LDL-C and ApoB/apoA1 ratio in the population studied ($n = 222$).

	LDL-C		ApoB/apoA1 ratio	
	r	*p*	*r*	*p*
Age	−0.232	<0.0001[a]	−0.115	0.093
Weight	−0.158	0.017	0.043	0.526
Height	−0.197	0.003	−0.084	0.222
BMI_{Score}	0.107	0.112	0.187	0.006[a]
Abdominal girth:	−0.134	0.047	0.025	0.722
Total cholesterol	0.937	<0.0001[a]	0.784	<0.0001[a]
HDL-C	−0.215	0.001[a]	−0.436	<0.0001[a]
LDL-C	–	–	00.867	<0.0001[a]
Triglycerides	0.078	0.246	0.252	<0.0001[a]
ApoA1	−0.242	<0.0001[a]	−0.468	<0.0001[a]
ApoB	0.973	<0.0001[a]	0.907	<0.0001[a]
ApoB/apoA1 ratio	0.818	<0.0001[a]	–	–
Lp(a) (mg/dl)	0.112	0.108	0.122	0.089
Cholesterol-year score	0.341	<0.0001[a]	0.341	<0.0001[a]
Achilles tendon	−0.044	0.515	0.022	0.747
cIMT	0.440	<0.0001[a]	0.593	<0.0001[a]

The statistical test used was the *Pearson* correlation (normal distribution), and *Spearman* (non-normal distribution) or Chi-square (for categorical data).
A p value of <0.05 was considered to be statistically significant.
ApoA1: apolipoprotein A1; ApoB: apolipoprotein B; BMI_{Score}: body mass index score; cIMT: carotid intima-media thickness; HDL-C: HDL cholesterol; LDL-C: LDL cholesterol; Lp(a): lipoprotein a.
[a] The significant p was maintained after adjusting the correlations by age.

Table 4 Cut-off points for the 3 best biomarkers for their discriminative capacity (sensitivity and specificity) to differentiate between the genetically positive and negative FH population.

	AUC	Cut-off point	Sensitivity (%)	Specificity %	*p*
Total cholesterol	0.672	244.50 mg/dl	71.70	60.00	0.013
LDL-C	0.745	170.50 mg/dl	71.70	80.00	0.000
ApoB/apoA1 ratio	0.820	0.80	83.00	68.00	0.000

To analyse these data, the ROC curve was used.
A p value of <0.05 was considered to be statistically significant.
ApoA1: apolipoprotein A1; ApoB: apolipoprotein B; AUC: area under the curve; LDL-C: LDL cholesterol.

the application of direct cascade on having a parent with FH or opportunistic detection. Our objective was to look for lipid or vascular biomarkers that would help us to distinguish those children with a higher probability of being affected by FH.

FH is completely asymptomatic in children and adolescents. For this reason, family history is of great interest where applicable. Similarly, the examination of the parents, in search of stigmas of genetic dyslipidaemia (corneal arch, xanthomas), are elements of great interest for the diagnosis, although it should be taken into account that in young parents, these characteristic clinical signs are not usually present. Another relevant detail is knowledge of the family history of PCVD, taking into account the age of the parents. It is usually more common to find this history in second-degree relatives such as grandparents.[14] In our population, 18.2% of the second-degree relatives had presented with PCVD, while only 10.4% of parents had that history. Klančar et al. reported that only a third of the relatives of children with FH with a genetic diagnosis had a history of PCVD, for which reason they concluded that the detection of FH in the child cannot be based on a family history of PCVD.[7] In the same sense, O'Loughlin et al. reported that the detection of FH in the child, based on a family history of cardiovascular disease, had a low sensitivity and specificity.[15] Current clinical diagnosis is based on LDL-C levels. We know that the phenotype of children with FH diagnosed genetically is very diverse, from presenting with very severe hypercholesterolaemia (LDL-C >190 mg/dl) to normal LDL-C values, albeit with the majority of cases demonstrating figures ranging between 130 and 190 mg/dl. In our study, the LDL-C mean was 183 mg/dl, while in the children with PH it was 143 mg/dl. Variability of the phenotype in children with FH has been objectified in a universal screening study for the detection of FH conducted in 10,095 children between 1 and 2 years of age through genetic analysis. Children

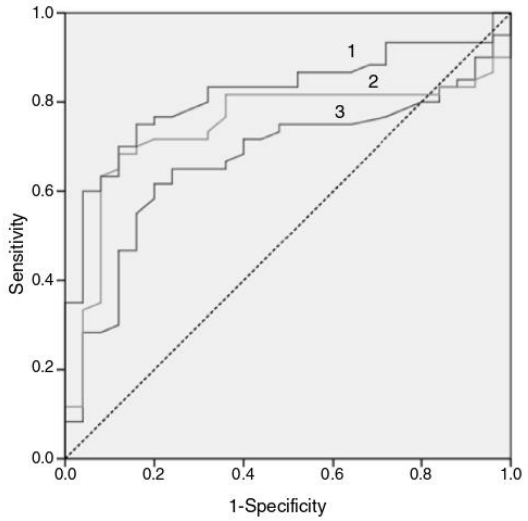

Figure 2 ROC curve of the 3 lipid biomarkers that best distinguish the FH population with or without mutation. 1: ApoB/apoA1 ratio (AUC = 0.820); 2: LDL-C (AUC = 0.745); 3: TC (AUC = 0.672).

with genetically confirmed FH showed mean LDL-C figures of 155 mg/dl, although in some cases the figures were lower than 130 mg/dl. This indicates that detection based solely on phenotype is insufficient.[10] It has been indicated that an ApoB/apoA1 ratio of greater than 0.68 is a more sensitive and specific marker for the detection of FH.[16] This ratio is used to distinguish those patients affected by myocardial infarction in the InterHeart study.[17] More recently, the data from the Pure study[18] have indicated that the lipid parameter associated with cardiovascular risk mediated by an atherogenic diet is also the apoB/apoA1 ratio. The apoB/apoA1 ratio is an estimate of the relationship between the pro- and anti-atherogenic lipoprotein particles. The measurement of these apolipoproteins could have dual significance when assessing cardiovascular risk and increasing detection of FH in children,[19] although it is true that testing of lipoproteins is not commonplace in clinical health practice, a fact that limits its use in Primary Healthcare. However, it is a standard test in the specialised departments. We should highlight the positive correlation and statistical significance between the ApoB/apoA1 ratio and the cIMT observed in the group of participants in our study. Similar data have been described before by other groups, which indicates that a pro-atherogenic lipoprotein profile in children and adolescents predisposes the development of subclinical arteriosclerosis in adults.[19]

In our population of children diagnosed with FH, an apoB/apoA1 ratio of greater than 0.82 showed greater sensitivity and specificity in the detection of carriers of a genetic mutation, compared with LDL-C or CT. This fact is of great interest as it has not been described before.

In our study, the cholesterol-year score is higher in children with hypercholesterolaemia in relation to the CG. The load of accumulated LDL-C in the arterial wall throughout life, along with the presence of other cardiovascular risk factors, will determine the development of arteriosclerosis. In children, one way of measuring the LDL-C load is the cholesterol-year score test (LDL-C per year) without treatment. Torsade et al. observed that children with FH with a greater cholesterol-year score presented with a greater number of plates at the carotid artery level.[20]

In our study, the HDL-C concentration was significantly lower in relation to the CG. Current data indicate that it may be due to less activity in reverse cholesterol transport, as a consequence of a disturbance in the concentration of lipid content of small HDLs.[21]

On the other hand, our group has participated in a study, observing that patients with untreated FH presented with disturbances in the concentrations of enzymes that participate in the remodelling of HDLs; this would cause dysfunctional HDLs, which would bring about a defect in the ability to eliminate cholesterol from macrophages.[22]

The cIMT in children with FH was greater than the group with PH but with no significant difference. These data differ from those described previously by other groups, in those that differences in children have been observed with respect to their unaffected siblings before 8 years of age.[23] Currently, the most recent guidelines on cardiovascular prevention from 2016 do not recommend the cIMT test as a marker of subclinical arteriosclerosis in the general population. However, in children, the cIMT has been demonstrated as having played an acceptable discriminatory role and we believe that it continues to be an appropriate tool in highly selected populations with a high cardiovascular risk, such as children with FH.

In this study, we only measured the Achilles tendons' thickness; neither the deposits of cholesterol nor myofibril network distortion were assessed. We proposed that their determination could help us in the detection and diagnosis of children with FH, as has been described in the adult population.[24] Both by palpation and their measurement by ultrasound, although they seemed to be thicker than expected, there were no significant differences between the 3 populations studied.

As a particular strength of the study, it is worth mentioning that it is the first one conducted in Spain in children for the detection and diagnosis of FH, with the collaboration and involvement of different healthcare areas. The application of opportunistic screening by Primary Healthcare paediatricians and the application of direct and inverse cascade in the specialist department.

As study limitations, the sample size was small, especially for the group of children with PH, but we should also remember that these were children detected in families with FH and not in the general population. Also, the age range studied included the pubertal stage, and it was difficult to assess the hormonal impact on the results obtained.

Conclusions and recommendations

The FH detection strategies via direct and indirect screening allowed for an increase in childhood diagnosis of FH. Concentrations of TC elevated above 245 mg/dl or of LDL-C greater than 170 mg/dl is associated with the presence of FH with a positive genetic mutation. However, the parameter with the best relationship between sensitivity and specificity is an

ApoB/apoA1 ratio of greater than 0.82. Educating the children and their families about following the recommended lifestyle changes and initiating pharmacological treatment in a timely manner will have a clear impact on the prevention of PCVD characteristic of this population.

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Authorship

Dr. Núria Plana: conception and design of the study, clinical monitoring, data collection, monitoring of the collected data, interpretation of the data, draft and final approval of the version presented.

Dr. Cèlia Rodríguez-Borjabad: has contributed to the coordination of the study, obtaining data, analysis and interpretation of the data, critical review of the intellectual content.

Dr. Daiana Ibarretxe: has contributed to obtaining data, analysis and interpretation of the data, critical review of the intellectual content.

Dr. Raimon Ferré: carried out the ultrasound study, critical review of the intellectual content.

Dr. Albert Feliu: conception and design of the study, recruitment of patients, review of inclusion and exclusion criteria, critical review of intellectual content.

Dr. Alejandra Caselles: collaboration with recruitment, critical review of intellectual content.

Dr. Luís Masana: conception and design of the study, interpretation of the data, draft and final approval of the version presented.

Conflicts of interest

For this study, the authors declare that there was no interference in the attaining or interpretation of the results and that they therefore have no conflicts of interest. Some of the authors have received fees for conferences or advice, as detailed below.

Núria Plana has received conference fees from Alexion, Amgem, Ferrer, MSD, Rubio and Sanofi.

Luis Masana has received conference and scientific advice fees from Amgen, MSD, Recordati and Sanofi.

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References

1. Wiegman A, Gidding SS, Watts GF, Chapman MJ, Ginsberg HN, Cuchel M, et al. Familial hypercholesterolæmia in children and adolescents: gaining decades of life by optimizing detection and treatment. Eur Heart J. 2015;36:2425–37.
2. Sánchez-Hernández RM, Civeira F, Stef M, Perez-Calahorra S, Almagro F, Plana N, et al. Homozygous familial hypercholesterolemia in Spain: prevalence and phenotype–genotype relationship. Circulation. 2016;9:504–10.
3. Zamora A, Masana L, Comas-Cufi M, Vila A, Plana N, García-Gil M, et al. Familial hypercholesterolemia in a European mediterranean population. prevalence and clinical data from 2.5 million primary care patients. J Clin Lipidol. 2017;11:1013–22.
4. Akioyamen LE, Genest J, Shan SD, Reel RL, Albaum JM, Chu A, et al. Estimating the prevalence of heterozygous familial hypercholesterolaemia: a systematic review and meta-analysis. BMJ Open. 2017;7:e016461.
5. Ramaswami U, Cooper J, Humphries SE. The UK paediatric familial hypercholesterolaemia register: preliminary data. Arch Dis Child. 2017;102:255–60.
6. Motazacker MM, Pirruccello J, Huigen R, Do R, Gabriel SPJ. Advances ungenetics show the need for extending screening strategies for autosomal dominat hypercholesterolaemia. Eur Heart J. 2012;33:1360–6.
7. McGill H, Mcmahan CA, Gidding SS. Contemporary reviews in cardiovascular medicine preventing heart disease in the 21st century. Implications of the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Study. Circulations. 2008;117:1216–27.
8. Klančar G, Grošelj U, Kovač J, Bratanič N, Bratina N, Podkrajšek KT, et al. Universal screening for familial hypercholesterolemia in children. J Am Coll Cardiol. 2015;66:1250–7.
9. Plana N, Rodríguez-Borjabad C, Ibarretxe D, Masana L. Hipercolesterolemia familiar en la infancia y la adolescencia: una realidad oculta. Clin Investig Arterioscler. 2017;29:129–40.
10. Gómez Gerique J, Gutiérrez Fuentes J, Montoya M, Pores A, Rueda A, Avellaneda A, et al. Perfil lipídico de la población española: estudio DRECE (Dieta y Riesgo de Enfermedad Cardiovascular en España). Med Clin (Barc). 1999;5:730–5.
11. Sobradillo B, Aquirre A, Aresti U, Bilbao A, Fernández-Ramos C, Lizárraga A, et al. Curvas y tablas de crecimiento. Estudio longitudinal de crecimiento y transversal. Bilbao: Fundación Faustino Orbegozo Eizaguirre; 2004.
12. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. J Am Soc Echocardiogr. 2008;21:93–111.
13. Wald DS, Bestwick JP, Wald NJ. Child–parent screening for familial hypercholesterolaemia: screening strategy based on a meta-analysis. BMJ. 2007;335:599.
14. Galema-Boers JMH, Versmissen J, van Lennep HWOR, Williams M, Lennep Van JER. Cascade screening of familial hypercholesterolemia must go on. Atherosclerosis. 2015;242:415–7.
15. O'Loughlin J, Lauzon B, Paradis G, Hanley J, Lévy E, Delvin E, et al. Usefulness of the American Academy of Pediatrics recommendations for identifying youths with hypercholesterolemia. Pediatrics. 2004;113:1723–7.
16. Medeiros AM, Alves AC, Aguiar P, Bourbon M. Cardiovascular risk assessment of dyslipidemic children: analysis of biomarkers to identify monogenic dyslipidemia. J Lipid Res. 2014;55:947–55.
17. Yusuf S, Hawken S, Ôunpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case–control study. Lancet. 2004;36:937–52.
18. Mente A, Dehghan M, Rangarajan S, McQueen M, Dagenais G, Wielgosz A, et al. Association of dietary nutrients with blood lipids and blood pressure in 18 countries: a cross-sectional analysis from the PURE study. Lancet Diabetes Endocrinol. 2017;8587:1–14.
19. Juonala M, Viikari JS, Kähönen M, Solakivi T, Helenius H, Jula A, et al. Childhood levels of serum apolipoproteins B and A-I predict carotid intima-media thickness and brachial endothelial function in adulthood. The cardiovascular risk in young Finns study. J Am Coll Cardiol. 2008;52:293–9.
20. Tonstad S, Joakimsen O, Stensland-Bugge E, Leren TP, Ose L, Russell D, et al. Risk factors related to carotid intima-media thickness and plaque in children with familial hypercholesterolemia and control subjects. Arter Thromb Vasc Biol. 1996;8:984–91.
21. Christensen JJ, Ulven SM, Retterstøl K, Narverud I, Bogsrud MP, Henriksen T, et al. Comprehensive lipid and metabolite profiling of children with and without familial hypercholesterolemia: a cross-sectional study. Atherosclerosis. 2017;266:48–57.
22. Cedó L, Plana N, Metso J, Lee-Rueckert M, Sánchez-Quesada JL, Kovanen PT, et al. Defective HDL remodeling and functionality in familial hypercholesterolemia. J Am Coll Cardiol. 2018;71:466–8.
23. Kusters DM, Wiegman A, Kastelein JJP, Hutten BA. Carotid intima-media thickness in children with hypercholesterolemia. Cir Res. 2014;114:307–11.
24. Junyent M, Gilabert R, Zambón D, Núñez I, Vela M, Civeira F, et al. The use of achilles tendon sonography to distinguish familial hypercholesterolemia from other genetic dyslipidemias. Arter Thromb Vasc Biol. 2005;25:2203–8.

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Plasma inducible degrader of the LDLR, soluble low-density lipoprotein receptor, and proprotein convertase subtilisin/kexin type 9 levels as potential biomarkers of familial hypercholesterolemia in children

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2D-1H-NMR;
Lp(a);
Triglyceride

BACKGROUND: Familial hypercholesterolemia (FH) in children is under-detected. Plasma biomarkers associated with low-density lipoprotein receptor (LDLR) function could help identifying FH children.

OBJECTIVES: We aim to assess the clinical value of inducible degrader of the LDLR (IDOL), soluble LDLR (sLDLR), and proprotein convertase subtilisin/kexin type 9 (PCSK9) plasma concentrations in children with FH compared with control children (CCh).

METHODS: This was a cross-sectional study performed in a Lipid Unit from a University hospital. The participants were 177 children distributed into FH (n = 77) and CCh (n = 100). Main outcomes were changes in IDOL, sLDLR, and PCSK9 plasma concentrations between children groups; secondary outcomes were the association between IDOL, sLDLR, and PCSK9 and lipid profile determined by 2-dimensional nuclear magnetic resonance.

RESULTS: The IDOL levels were higher in FH compared with CCh ($P = .007$). The PCSK9 levels were elevated in FH ($P < .001$). The sLDLR levels had no significant differences between groups. IDOL was significantly positively associated to total and LDL cholesterol and ApoB100 but not to LDL particle number. However, a robust correlation with Lp(a) ($P = .001$) was observed. PCSK9 had the strongest correlation with LDL-associated parameters including particle number. sLDLR

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was associated with triglyceride levels ($P < .001$) and triglyceride-rich particles and inversely to LDL size.

CONCLUSIONS: The IDOL and PCSK9 plasma levels are significantly higher in FH children. Interestingly, sLDLR was associated with atherogenic dyslipidemia components. IDOL concentrations show a robust association with Lp(a) levels. To study the role of plasma biomarkers associated with LDLR expression in FH is warranted.

Background

Familial hypercholesterolemia (FH) affects 1 of 250 children. FH is under-detected, particularly in pediatric age.[1,2] Gene defects in LDL receptor (*LDLR*), apolipoprotein B (*ApoB*), LDLR adaptor protein 1, and proprotein convertase subtilisin/kexin type 9 (*PCSK9*) are clinically expressed as an FH phenotype.[3] The LDLR is the cornerstone molecule regulating the LDL cholesterol (LDL-C) plasma concentrations.[4] LDLR gene mutations, leading to a null or defective receptor, result in a substantial increase in the plasma LDL-C levels and higher cardiovascular risk.[5] On the other hand, drugs that induce LDLR upregulation decrease the LDL-C concentration, reducing cardiovascular event rates. The clinical significance of LDLR warrants interest in the mechanisms controlling its function and expression. Several proteins are involved in LDLR physiology. Its ligand, ApoB, mediates LDL capture. LDLR adaptor protein 1 anchors it to the cell membrane, contributing to LDLR activity. PCSK9 has emerged as a central LDLR functional regulator, and its expression parallels that of LDLR according to intracellular cholesterol content.[6] After being secreted into the bloodstream, PCSK9 binds to LDLR that is internalized with it. PCSK9 precludes LDLR molecule recycling, reducing the expression of LDLR at the hepatocyte membrane. Inhibition of PCSK9 by monoclonal antibodies leads to an increased number of receptors at the cell surface, resulting in a marked LDL-C reduction.[7] In the last few years, the inducible degrader of LDLR (IDOL), a new molecule involved in post-transcriptional LDLR expression regulation, has been identified.[8] IDOL is a sterol-regulated E3 ubiquitin ligase that labels LDLR with ubiquitin, driving it to degradation by proteasome cell mechanisms. Therefore, IDOL activity decreases the level of LDLR, similarly to PCSK9, albeit through a different mechanism. However, interdependent regulation mechanisms have been observed.[9] Furthermore, overexpression of both molecules is associated with nephrotic syndrome and hypercholesterolemia. Similar to PCSK9, IDOL also regulates the very low-density lipoprotein (VLDL) and apoE receptors.[10] Its expression shall be governed by the liver X receptor transcription factor.[11] Interestingly, genome-wide association studies have identified IDOL as a locus associated with circulating LDL-C concentrations.[12–14] Similar to PCSK9, IDOL gene loss-of-function mutations have been associated with low LDL-C levels in humans.[15] Because of all these factors, IDOL has attracted increasing interest as a putative target for hypercholesterolemia treatment.[16] Although IDOL function appears to be intracellular, a plasma-circulating fraction can be detected. Neither its function nor its association with the intracellular portion is known. The mechanisms involved in plasma secretion are equally unknown. However, because of IDOL tight relationship with LDLR expression, we hypothesize that it could be an LDLR functional biomarker. Also, it is known that soluble LDLR (sLDLR) is formed by cleavage of the extracellular domain and can also be detected in the serum of healthy subjects as a biomarker of diseases associated with triglycerides metabolism.[17,18]

To explore a possible role of these 3 circulating proteins as hypercholesterolemia biomarkers, we determined the IDOL, PCSK9, and sLDLR plasma levels in a cohort of FH children.

Subjects and methods

Study design and patients

This study was a cross-sectional study. One hundred seventy-seven children and adolescents, aged 4 to 18 year, who were participating in the "Early Familial Hypercholesterolemia Detection Program in Children" (DECOPIN) to investigate high cholesterol levels or because they were FH family members, were recruited. Children were distributed into 2 groups: (1) familial hypercholesterolemia (FH; n = 77), if they had a positive genetic study or LDL-C > 150 mg/dL if 1 of the parents had FH and (2) control children (CCh; n = 100) if they did not meet the previous criteria. At inclusion, no patient was receiving lipid-lowering therapy. The exclusion criteria were renal, hepatic, or thyroid chronic disease; type I diabetes mellitus; hypercalciuria; eating disorders; autoimmune disease; homozygous FH; and other chronic diseases. An exhaustive medical history, including the familial cardiovascular and dyslipidaemia history, complete physical examination, and anthropometry data, was collected. To calculate the body mass index (BMI) in children, we used the "BMI score" $(BMI_{children} - BMI_{50th\ percentile\ of\ Orbegozo's\ growth\ curves})/SD_{50th\ percentile\ of\ Orbegozo's\ growth\ curves}$. Also, a lifestyle evaluation, including nutritional and physical activity data, was recorded. The Hospital Ethical Committee approved this study, and patients or tutors, depending on

their age, provided written consent in all cases to participate in the study.

Blood sample collection and storage

A blood sample was obtained after overnight fasting. Plasma and serum aliquots were prepared and stored at −80°C in the BioBanc of our center until further use.

Standard biochemical analyses and IDOL, PCSK9, and sLDLR measurements

Biochemical parameters, lipids, and apolipoproteins were measured using colorimetric, enzymatic, and immunoturbidimetric assays that were adapted to a Modular Cobas 700 autoanalyzer (Roche, Basel, Switzerland). The LDL-C levels were calculated with the use of the Friedewald equation: LDL-C = total cholesterol − (high-density lipoprotein cholesterol + [triglycerides/5]). The serum IDOL levels were assessed using an enzyme-linked immunosorbent assay kit (Cusabio, MD). The sensitivity of the assay was 3.9 pg/mL. The circulating PCSK9 and sLDLR levels were measured using commercial enzyme-linked immunosorbent assay kits (R&D Systems, MN). The sensitivities of the assays were 0.096 and 4.72 ng/mL, respectively.

Two-dimensional nuclear magnetic resonance lipid profile evaluation

The Liposcale test was used in all samples. This advanced lipoprotein test is based on 2-dimensional diffusion-ordered ^{1}H nuclear magnetic resonance (NMR) spectroscopy. This method adds diffusion coefficients to classical NMR determinations to provide a direct measure of the mean particle sizes and concentration.[19]

Statistical analysis

The results are expressed as the mean ± standard deviation for normally distributed data, median (second quartile, Q1–fourth quartile, Q3) for data that are not normally distributed and frequencies for categorical data. Kolmogorov–Smirnov tests were used to ensure data were normally distributed. IDOL, PCSK9, and sLDLR were log-transformed. A t-test was used to determine significant differences when data were normally distributed. Mann–Whitney U tests were used to detect significant differences when the data were not normally distributed. Correlations were evaluated using Spearman's test. Multivariate regression models were constructed with the addition of covariates: age, gender, BMI score, and LDL-C for IDOL and PCSK9. The Lp(a) plasma levels were categorized into quartiles, and Kruskal–Wallis test was used to detect significant differences between quartiles. Receiver operating characteristic (ROC) curves were generated, and the area under the ROC curve (AUC) was calculated to evaluate the IDOL and PCSK9 capacity to identify FH children. Statistical analyses were performed using SPSS software (IBM SPSS Statistics, version 22.0). All statistical tests were 2-tailed, and $P < .05$ was considered significant.

Results

Subject characteristics

The clinical, biochemical, and genetic characteristics of the 177 children (FH = 77; CCh = 100) are shown in Table 1. The median age of the study participants was 10 years. Age, cholesterol, LDL-C, non-high-density lipoprotein cholesterol (HDL-C) and ApoB100 showed significant differences between the groups. Seventy-three percent of FH children were genetically diagnosed. As expected, LDL (total and subclasses) particle number, as assessed by 2-dimensional nuclear magnetic resonance (2D-^{1}H-NMR), was significantly higher in FH children compared with CCh (data not shown).

Distribution of the IDOL, sLDLR, and PCSK9 plasma levels

The distribution of the IDOL, sLDLR, and PCSK9 plasma levels in the population (n = 177) is shown in Figure 1. All the parameters showed a dispersed distribution (96.58 [58.44–196.14] pg/mL, 33.97 [25.23–45.52] ng/mL, and 261.30 [216.52–312.16] ng/mL, respectively).

IDOL, PCSK9, and sLDLR circulating levels according to the groups

The IDOL levels were significantly increased in FH children. They were 116.77 (77.55–238.12) pg/mL for FH and 93.18 (43.92–158.77) pg/mL for CCh ($P = .007$). The PCSK9 levels were also different between the groups, which were 281.14 (246.64–329.60) ng/mL for FH and 240.55 (199.61–287.74) ng/mL for CCh ($P < .001$; Fig. 2A and B). The sLDLR levels showed the same trend as IDOL and PCSK9 without reaching statistical significance: 37.75 (26.41–47.52) ng/mL for FH and 31.56 (24.98–44.13) ng/mL ($P = .149$; Fig. 2C).

The IDOL differences between groups persisted after adjusting for age, gender, BMI score, and LDL-C ($P = .045$), while they were lost for PCSK9 ($P = .226$).

Associations of IDOL, PCSK9, and sLDLR with lipid parameters

The IDOL, PCSK9, and sLDLR univariate associations with lipid parameters in the entire population are shown in Table 2. IDOL and PCSK9 were weakly positively

Table 1 Clinical, biochemical, and genetic characteristics of the study subjects (n = 177)

	FH (n = 77)	CCh (n = 100)	*P*
Demographic characteristics and cardiovascular risk factor measures			
Age (y)*	8 (6–11)	11 (8–13)	.004
Gender (% men)	54.50	57.00	.744
BMI score†	0.17 ± 0.92	0.04 ± 0.95	.351
Waist circumference (cm)*	62 (52–69)	63 (56–72)	.130
SBP (mm Hg)†	109.54 ± 12.42	112.68 ± 10.67	.731
DBP (mm Hg)*	64.00 (59.00–70.50)	64.00 (59.00–71.00)	.130
Glucose (mg/dL)*	81.00 (77.00–87.00)	82.50 (78.50–88.00)	.377
Lipids and apolipoproteins			
Cholesterol (mg/dL)*	261.0 (230.0–302.0)	192.5 (171.0–210.0)	<.001
LDL-C (mg/dL)*	183.0 (154.0–224.0)	115.0 (97.0–127.0)	<.001
HDL-C (mg/dL)*	59.00 (51.00–69.00)	63.00 (53.00–76.00)	.060
Non-HDL-C (mg/dL)*	199.00 (170.0–235.0)	128.0 (107.5–141.0)	<.001
Triglycerides (mg/dL)*	65.00 (50.00–88.00)	57.00 (46.00–76.50)	.186
ApoB100 (mg/dL)*	130.5 (115.5–156.5)	92.50 (79.00–103.5)	<.001
ApoA1 (mg/dL)*	146.0 (131.0–162.5)	155.0 (135.0–172.0)	.058
Lp(a) (mg/dL)*	19.58 (7.08–72.4)	13.75 (5.83–43.00)	.195
Genetic diagnosis (%)‡	72.70	–	–

BMI, body mass index; CCh, control children; DBP, diastolic blood pressure; FH, familial hypercholesterolemia; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure.

The data are presented as the mean ± standard deviation for normally distributed data, median (Q1–Q3) for non-normally distributed data or percentage for categorical variables.

*Mann-Whitney U test or χ^2.

†*P* values were obtained by Student t-test.

‡Genetic diagnosis only applies to FH.

associated (r = 0.150, *P* = .047). The IDOL concentrations were positively correlated with the total cholesterol, LDL-C, non-HDL-C, and ApoB100 although the correlation was weaker than for PCSK9 (Table 2). sLDLR was not associated with either IDOL or PCSK9. sLDLR was positively associated with non-HDL-C, ApoB100, but was also associated with triglycerides.

PCSK9 but not IDOL had a significant positive correlation with the LDL particle number, as assessed by 2D-^{1}H-NMR. Interesting, the sLDLR levels positively correlated with the VLDL total and subclass particle numbers (Table 2).

The IDOL concentrations were correlated with Lp(a) (r = 0.242; *P* = .001). Partial correlations showed that the IDOL–Lp(a) correlation was maintained after adjusting for age, gender, BMI score, LDL-C, and group (r = 0.220; *P* = .004). The IDOL level stratified by quartiles of the Lp(a) concentration in the entire cohort is shown in Figure 3. A higher Lp(a) quartile corresponded to higher IDOL levels (*P* = .016). The relationship persisted after adjusting for age, gender, BMI score, LDL-C, and group (*P* = .038).

IDOL determinants and FH discriminant power

After multivariate regression analysis and adjusting for age, gender, BMI score, LDL-C, Lp(a), and group, the IDOL level determinants were age (*P* = .004), group (*P* = .039) and Lp(a) (*P* = .004; R^2 for the model = 0.139, *P* < .001). As expected, the main PCSK9 level determinant was LDL-C (*P* < .020; R^2 for the model = 0.154, *P* < .001).

To determine whether the IDOL and PCSK9 levels could be used to discriminate between the FH and CCh groups, we performed ROC analysis (Supplementary data, Fig. 1S). The IDOL levels were a significant predictor of FH (AUC = 0.618; *P* = .007). Similar to IDOL, PCSK9 levels were a significant predictor of FH (AUC = 0.679; *P* < .001). However, the LDL-C levels were the best diagnostic parameter (AUC = 0.959; *P* < .001), and this diagnostic capacity was not modified by the addition of either IDOL or PCSK9 (AUC = 0.961; *P* < .001 and AUC = 0.960; *P* < .001). These data were similar when only genetically diagnosed FH children were considered (AUC = 0.957; *P* < .001 for LDL-C, AUC = 0.685; *P* = .001 for PCSK9 and AUC = 0.614; *P* = .019 for IDOL).

Discussion

We report data on the IDOL, sLDLR, and PCSK9 plasma concentrations in children with FH and controls. Although in the last years the total PCSK9 plasma levels have been exhaustively studied in different metabolic situations, there is only one publication on sLDLR concentrations in healthy subjects[17] and this is the first time that the IDOL plasma concentrations have been

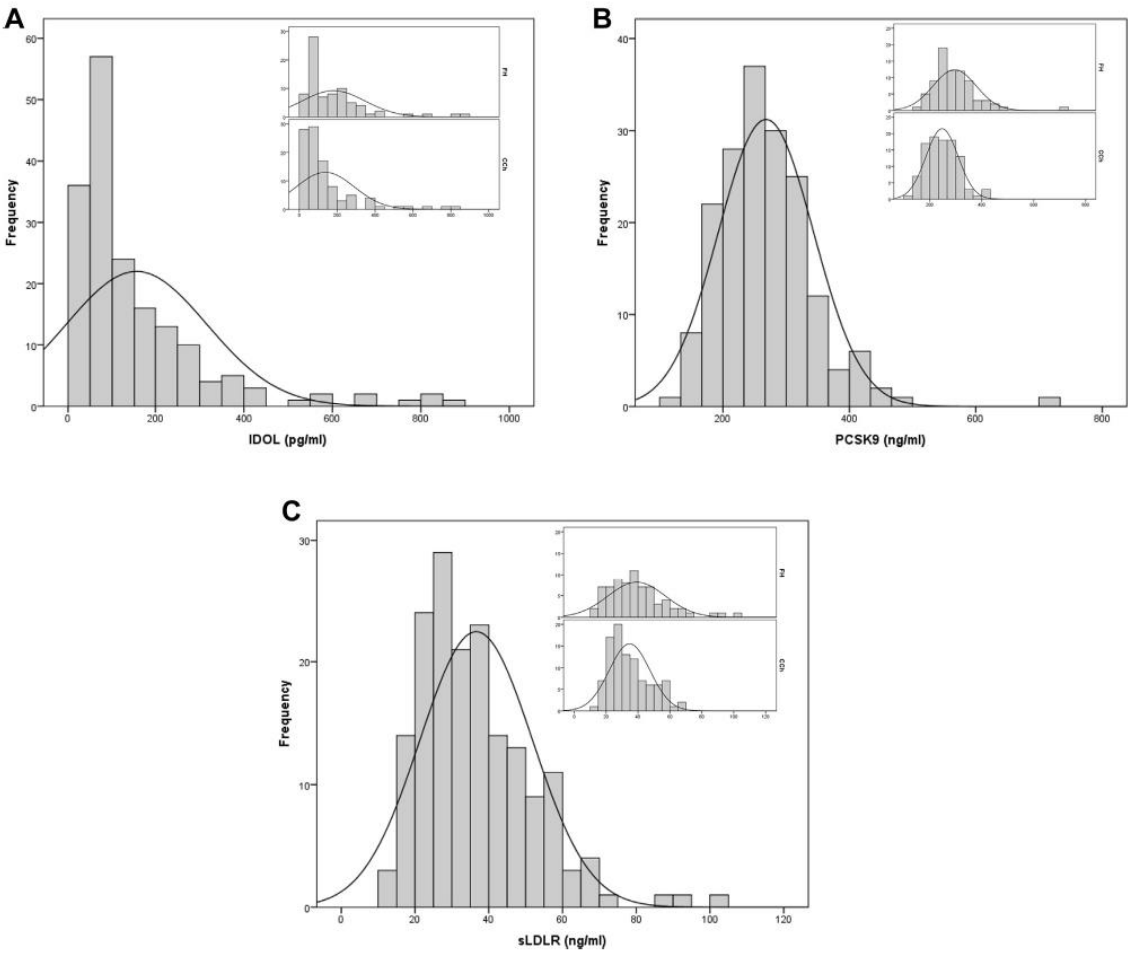

Figure 1 Distribution of IDOL (A), PCSK9 (B), and sLDLR (C) and levels in the whole group (n = 177) and in each studied group: FH (n = 77), CCh (n = 100). The data are presented as frequency distributions. FH, familial hypercholesterolemia; CCh, control children.

reported. A wide concentration range of circulating IDOL levels, including patients with undetectable plasma values, was observed. Both IDOL and sLDLR had a non-normal distribution, which was shifted to the left, whereas the PCSK9 levels followed a near normal shaped curve. The IDOL plasma levels were higher in FH children compared with CCh. On the other hand, the sLDLR concentrations did not show significant differences between groups. However, a surprising trend of higher levels in FH children and a positive correlation with LDL-associated lipid parameters was observed. This fact could be considered a paradox because FH is characterized by lower LDLR activity. One explanation could be that although FH children produce fewer functioning receptors, a compensatory synthesis rate is expected because of lower intrahepatic cholesterol concentrations. Alternatively, higher LDLR liberation from the liver membranes to plasma, dependent on a cell surface metalloproteinase[20] by unknown regulating mechanisms, could also play a role. The correlation between sLDLR and triglycerides is in agreement with Shimohiro et al.[17] who also showed a significantly and positively correlation of sLDLR with triglycerides and LDL-C levels in healthy Japanese individuals; hence, the role of plasma sLDLR concentration as lipid metabolism biomarker remains unknown and the implications of our observations must be further elucidated.

Because genetic testing is not widely available, FH is usually diagnosed based on clinical observations. Therefore, FH plasma biomarkers would be welcome. However, although IDOL concentrations had significant diagnostic power in ROC analyses, circulating levels of IDOL and PCSK9 did not add FH diagnostic discrimination to the LDL-C levels; this was also true when we only considered genetically identified FH children. Whether IDOL and PCSK9 or sLDLR could be helpful to differentiate FH from polygenic hypercholesterolemia would require a larger population study based on FH genetic identification.

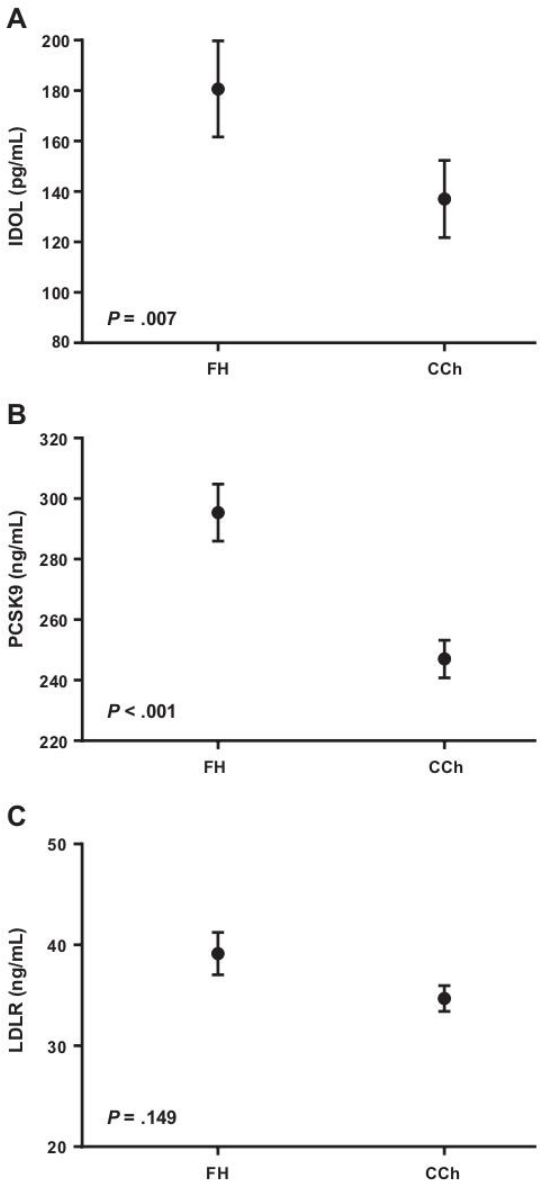

Figure 2 Levels of IDOL (A), PCSK9 (B), and sLDLR (C) according to FH (n = 77) and CCh (n = 100) groups. The data are presented as the mean ± standard error of the mean. *P* values were obtained using Mann–Whitney U test. FH, familial hypercholesterolemia; CCh, control children.

PCSK9 and sLDLR were significantly correlated with LDL subclasses particle number according to 2D-^{1}H-NMR data. The PCSK9 and LDL subclass particle number association was previously reported by our group and others.[21–23] Of interest, sLDLR was positively associated with all VLDL subclasses, which is in accordance with the triglyceride correlation. LDLR has a significant role in VLDL-derived lipoprotein metabolism; however, as previously mentioned, the meaning of this association requires further exploration.

On the other hand, the weak correlation between IDOL and LDL is surprising. IDOL gene variants associated with lower IDOL activity have been associated with lower LDL-C levels, whereas the N342S polymorphism associated with higher LDLR degradation leads to higher LDL-C concentrations although the polymorphism was not associated with increased cardiovascular risk.[24,25] The IDOL plasma concentration was not assessed in any of these prior studies. Interestingly, we found a significant association between the IDOL levels and Lp(a) that we cannot currently explain. It is unknown whether this reflects LDL as the substrate for Lp(a) formation, and further studies are needed to address this robust serendipitous observation. The lack of similarity between IDOL and PCSK9 as FH markers could be because of differences in their modes of action. While PCSK9 acts from plasma, IDOL mainly acts intracellularly; therefore, it is more independent of environmental influences.

The role of circulating IDOL and its secretion mechanisms are unknown. We do not know the relationship between circulating IDOL and its intracellular concentration and function, which is a significant limitation for many intracellular proteins. However, the correlation between the plasma IDOL and LDL-C levels suggests that higher cellular IDOL expression or activity, leading to lower LDLR and higher LDL-C levels, may be paralleled by higher circulating values.

Our study has several limitations. First, the sample size is small, but it has to be considered that the study was performed in children, limiting access to blood sampling. These data cannot be extrapolated to adults. The standard or average IDOL and sLDLR plasma levels are not known, except for the values observed in the control group. Therefore, we cannot perform comparisons with other populations. The age ranges merged into the pubertal period which is associated with changes in LDL, and the influence of hormone changes on IDOL, PCSK9, and sLDLR are not known.

In summary, we report the IDOL and sLDLR plasma levels in familial hypercholesterolemic children for the first time, compared with PCSK9 concentrations. The IDOL concentrations were significantly elevated in FH children. The putative role of circulating IDOL in the diagnosis of FH needs further research.

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Table 2 Associations of the IDOL, PCSK9, and sLDLR levels with the lipids, apolipoproteins, and lipoprotein particle number and size of the whole group (n = 177)

	IDOL		PCSK9		sLDLR	
	R	*P*	r	*P*	r	*P*
Biomarkers and standard lipoprotein profile						
IDOL	–	–	0.150	.047	−0.041	.596
PCSK9	0.150	.047	–	–	0.023	.764
sLDLR	−0.041	.596	0.023	.764	–	–
Cholesterol	0.200	.008	0.398	<.001	0.139	.069
Triglycerides	−0.027	.717	0.095	.210	0.298	<.001
LDL-C	0.168	.025	0.419	<.001	0.127	.098
HDL-C	0.082	.277	−0.074	.329	−0.037	.631
ApoB100	0.190	.013	0.410	<.001	0.194	.013
ApoA1	0.056	.469	−0.046	.553	0.065	.408
Lp(a)	0.242	.001	−0.012	.873	−0.059	.440
Lipoprotein particle number						
Total VLDL	−0.057	.450	−0.013	.860	0.246	.001
Large VLDL	−0.060	.429	−0.009	.901	0.273	<.001
Medium VLDL	−0.083	.273	−0.027	.723	0.254	.001
Small VLDL	−0.047	.531	−0.008	.911	0.242	.001
Total LDL	0.128	.091	0.416	<.001	0.282	<.001
Large LDL	0.143	.057	0.407	<.001	0.119	.120
Medium LDL	0.122	.106	0.404	<.001	0.248	.001
Small LDL	0.118	.116	0.397	<.001	0.361	<.001
Total HDL	0.109	.147	−0.093	.219	−0.038	.622
Large HDL	−0.057	.448	0.021	.777	0.345	<.001
Medium HDL	0.134	.076	−0.006	.932	−0.150	.049
Small HDL	0.085	.259	−0.136	.072	0.021	.787
Lipoprotein particle size						
VLDL	−0.042	.578	−0.061	.417	0.111	.148
LDL	0.055	.468	0.070	.352	−0.243	.001
HDL	0.093	.217	0.105	.165	−0.101	.189

BMI, body mass index; CCh, control children; DBP, diastolic blood pressure; FH, familial hypercholesterolemia; HDL-C, high-density lipoprotein cholesterol; IDOL, inducible degrader of the LDLR; LDL-C, low-density lipoprotein cholesterol; PCSK9, proprotein convertase subtilisin/kexin type 9; SBP, systolic blood pressure; sLDLR, soluble low-density lipoprotein receptor; VLDL, very low-density lipoprotein.
P values were obtained by Spearman's test.

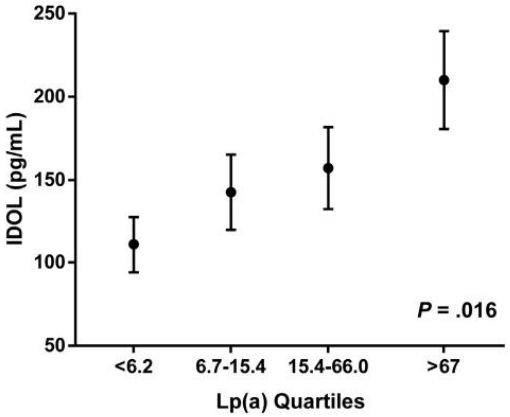

Figure 3 IDOL levels across Lp(a) quartiles in the whole group (n = 177). The data are presented as the mean ± standard error of the mean (mg/dL). *P* values were obtained using the Kruskal–Wallis test.

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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jacl.2017.10.003.

References

1. Wiegman A, Gidding SS, Watts GF, et al. Familial hypercholesterolaemia in children and adolescents: gaining decades of life by optimizing detection and treatment. *Eur Heart J*. 2015;36(36):2425–2437.
2. Plana N, Rodríguez-Borjabad C, Ibarretxe D, Masana L. Hipercolesterolemia familiar en la infancia y la adolescencia: una realidad oculta. *Clin Investig Arterioscler*. 2017;29(3):129–140.
3. Wald DS, Bestwick JP, Morris JK, Whyte K, Jenkins L, Wald NJ. Child–parent familial hypercholesterolemia screening in primary care. *N Engl J Med*. 2016;375(17):1628–1637.
4. Wijers M, Kuivenhoven JA, van de Sluis B. The life cycle of the low-density lipoprotein receptor. *Curr Opin Lipidol*. 2015;26(2):82–87.
5. Dedoussis GVZ, Schmidt H, Genschel J. LDL-receptor mutations in Europe. *Hum Mutat*. 2004;24(6):443–459.
6. Horton JD, Cohen JC, Hobbs HH. PCSK9: a convertase that coordinates LDL catabolism. *J Lipid Res*. 2009;50 Suppl:S172–S177.
7. Bergeron N, Phan BAP, Ding Y, Fong A, Krauss RM. Proprotein convertase subtilisin/kexin type 9 inhibition: a new therapeutic mechanism for reducing cardiovascular disease risk. *Circulation*. 2015;132(17):1648–1666.
8. Zelcer N, Hong C, Boyadjian R, Tontonoz P. LXR regulates cholesterol uptake through idol-dependent ubiquitination of the LDL receptor. *Science*. 2009;325(5936):100–104.
9. Ibrahim S, Somanathan S, Billheimer J, Wilson JM, Rader DJ. Stable liver-specific expression of human IDOL in humanized mice raises plasma cholesterol. *Cardiovasc Res*. 2016;110(1):23–29.
10. Liu S, Vaziri ND. Role of PCSK9 and IDOL in the pathogenesis of acquired LDL receptor deficiency and hypercholesterolemia in nephrotic syndrome. *Nephrol Dial Transplant*. 2014;29(3):538–543.
11. Nelson JK, Cook ECL, Loregger A, et al. Deubiquitylase inhibition reveals liver X receptor-independent transcriptional regulation of the E3 ubiquitin ligase IDOL and lipoprotein uptake. *J Biol Chem*. 2016;291(9):4813–4825.
12. Teslovich TM, Musunuru K, Smith AV, et al. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature*. 2010;466(7307):707–713.
13. Willer CJ, Schmidt EM, Sengupta S, et al. Discovery and refinement of loci associated with lipid levels. *Nat Genet*. 2013;45(11):1274–1283.
14. Waterworth DM, Ricketts SL, Song K, et al. Genetic variants influencing circulating lipid levels and risk of coronary artery disease. *Arterioscler Thromb Vasc Biol*. 2010;30(11):2264–2276.
15. Sorrentino V, Fouchier SW, Motazacker MM, et al. Identification of a loss-of-function inducible degrader of the low-density lipoprotein receptor variant in individuals with low circulating low-density lipoprotein. *Eur Heart J*. 2013;34(17):1292–1297.
16. Zhang C, Tian Y, Zhang M, Tuo Q, Chen J, Liao D. IDOL, inducible degrader of low-density lipoprotein receptor, serves as a potential therapeutic target for dyslipidemia. *Med Hypotheses*. 2016;86:138–142.
17. Shimohiro H, Taniguchi S-I, Koda M, Sakai C, Yamada S. Association between serum soluble low-density lipoprotein receptor levels and metabolic factors in healthy Japanese individuals. *J Clin Lab Anal*. 2015;29(1):52–56.
18. Rebeck GW, LaDu MJ, Estus S, Bu G, Weeber EJ. The generation and function of soluble apoE receptors in the CNS. *Mol Neurodegener*. 2006;1(1):15.
19. Mallol R, Amigó N, Rodríguez MA, et al. Liposcale: a novel advanced lipoprotein test based on 2D diffusion-ordered ^{1}H NMR spectroscopy. *J Lipid Res*. 2015;56(3):737–746.
20. Begg MJ, Sturrock ED, van der Westhuyzen DR. Soluble LDL-R are formed by cell surface cleavage in response to phorbol esters. *Eur J Biochem*. 2004;271(3):524–533.
21. Guardiola M, Plana N, Ibarretxe D, et al. Circulating PCSK9 levels are positively correlated with NMR-assessed atherogenic dyslipidaemia in patients with high cardiovascular risk. *Clin Sci (Lond)*. 2015;128(12):877–882.
22. Ibarretxe D, Girona J, Plana N, et al. Circulating PCSK9 in patients with type 2 diabetes and related metabolic disorders. *Clin Investig Arterioscler*. 2016;28(2):71–78.
23. Girona J, Ibarretxe D, Plana N, et al. Circulating PCSK9 levels and CETP plasma activity are independently associated in patients with metabolic diseases. *Cardiovasc Diabetol*. 2016;15(1):107.
24. Weissglas-Volkov D, Calkin AC, Tusie-Luna T, et al. The N342S MYLIP polymorphism is associated with high total cholesterol and increased LDL receptor degradation in humans. *J Clin Invest*. 2011;121(8):3062–3071.
25. Dhyani A, Tibolla G, Baragetti A, et al. IDOL N342S variant, atherosclerosis progression and cardiovascular disorders in the Italian general population. *PLoS One*. 2015;10(4):e0122414.

2.3. To investigate the alterations, beyond the standard analysis, in the quantity and quality of the lipoprotein profile evaluated by NMR.

ARTICLE 5

***Rodríguez-Borjabad** C, Ibarretxe D, Girona J, Amigo N, Feliu A, Masana L, Plana N. Lipoprotein profile assessed by 2D-1H-NMR in children with familial and non-familial hypercholesterolemia. Atherosclerosis. 2018;270:117-122.*

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Lipoprotein profile assessed by 2D-1H-NMR and subclinical atherosclerosis in children with familial hypercholesterolaemia

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ABSTRACT

Background and aims: Familial hypercholesterolaemia (FH) is underdiagnosed in children. In addition to lipid concentrations, lipoprotein particle quantity and quality could influence cardiovascular risk. We aimed to perform a comprehensive plasma lipid study, including lipoprotein particle number and size assessment by two-dimensional nuclear magnetic resonance (2D-1H-NMR), in children with FH compared to non-affected children and to evaluate the clinical value of these factors as subclinical atherosclerosis biomarkers.
Methods: One hundred eighty-three children participating in the broad "Hypercholesterolemia Early Detection Programme" (Decopin Project) were recruited. They were categorized as FH, if they had either a positive genetic test or clinical certainty, or as control children (CCh). Medical history, anthropometry and clinical variables were recorded. Standard biochemical measurements were performed. The lipoprotein profile was studied by 2D-1H-NMR. Carotid intima-media thickness (cIMT) was assessed by sonography in 177 children.
Results: FH children had a significant 36% increase in LDL particles. The small LDL fraction was increased by 33% compared to CCh. The relative relationship between large, medium and small LDL and the mean LDL particle size was similar between FH children and CCh. The total and small LDL particle numbers were directly associated with and contributed to the determination of the mean cIMT according to bivariate and multivariate analyses in FH children.
Conclusions: The higher cholesterol levels of FH children are due to an overall increased number of all LDL particle subclasses, including a notable 33% increase in small LDL. Total and small LDL particle number shows a good correlation with cIMT in FH children.

1. Introduction

One out of 250 children has familial hypercholesterolaemia (FH), but this clinical diagnosis is largely undetected and untreated [1]. FH is an autosomal-dominant genetic disease that is present in all racial and ethnic groups and has long been recognized as a cause of premature atherosclerotic coronary heart disease [1,2]. Elevated low-density lipoprotein cholesterol (LDL-C) is observed at an early age. In most cases (93%), this disease is caused by mutations in the low-density lipoprotein receptor (*LDLR*) gene [3], but it can also be

Abbreviations: 2D-1H-NMR, two-dimensional nuclear magnetic resonance; CG, Control group; cIMT, carotid intima-media thickness; CCh, control children; DLCN, Dutch Lipid Clinic Network scale; HeFH, heterozygous familial hypercholesterolaemia; HoFH, homozygous familial hypercholesterolaemia; IQR, interquartile range; PCSK9, proprotein convertase subtilisin-like kexin type 9; VLDL, very low-density lipoprotein.

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0021-9150/

caused by mutations in the apolipoprotein B100 (*APOB*) (5%) [4] and proprotein convertase subtilisin-like kexin type 9 (*PCSK9*) (1%) genes [5].

Two types of this disease are known, including the heterozygous form (HeFH), which is the most common inherited disorder with a prevalence of 1/217 children [6], and the homozygous form (HoFH), which has a prevalence of 1/450,000 individuals according to the latest data published for the Spanish population [7].

In Europe, the estimated number of affected individuals is approximately 4.5 million, with children and adolescents accounting for 20–25% of this population [8]. Clinical criteria for FH diagnosis, such as the Dutch Lipid Clinic Network scale (DLCN), cannot be applied to individuals under 18 years of age. Therefore, diagnoses in children should preferably be established by genetic testing. If the genetic test is not available, the diagnosis can be determined by the presence of an increased LDL-C concentration in the context of a family history of FH [9].

In addition to elevated LDL-C levels, the quality of LDL particles is also an important risk determinant, given that smaller LDL particles are associated with a higher cardiovascular risk [10].

NMR methods assess lipoprotein size and then, lipoproteins are arbitrary distributed in different subgroups according particle size. Physiologically the small LDL subclass accounts for 30–50% of total LDL particle number. Some pathological states like hypertriglyceridemia lead to increased number of smaller LDL.

Smaller LDL particles are denser, more atherogenic and more easily oxidized [11]; because they tend to have a lower affinity for the LDL receptor, a harmful increase in the proportion of this LDL subclass in FH children could be anticipated. Although an increase of smaller LDL particles in FH children has been reported [12], information regarding LDL subclass proportion, quantity and quality beyond cholesterol concentrations alone is scarce.

Clear evidence indicates that high levels of LDL-C in a child accelerate the formation and development of atheromatous lesions during the first years of life, as assessed by carotid intima-media thickness (cIMT) and imaging techniques [13]. Different studies have shown faster cIMT progression in children with FH from 7 years of age [8].

The purpose of the present study was to compare lipoprotein subclass particle number and sizes beyond lipid concentrations alone between FH children and control children (CCh) to attain a more comprehensive metabolic characterization of FH children and assess the putative role of this lipid profile as cardiovascular risk marker according to its association to subclinical atherosclerosis.

2. Materials and methods

2.1. Study design and patients

This is a cross-sectional study. One hundred eighty-three children and adolescents aged 4–18 years were enrolled in this study between March 2013 and June 2016. They were participating in the "Early Familial Hypercholesterolemia Detection Project" (DECOPIN Project), which focuses on the implementation of opportunistic, direct and reverse cascade FH screening. Children were classified as FH ($n = 82$) if they had a positive genetic test, or LDL-C >150 mg/dL and one of the parents had a DLCN score >8 in the case of no available or negative genetic test result. The CCh group included children attending our unit because of FH suspicion but not fulfilling FH criteria, and non-affected siblings of FH children studied because of family screening ($n = 101$). At the time of inclusion, no patients were receiving lipid-lowering therapy. The exclusion criteria were chronic renal, hepatic or thyroid disease and type 1 diabetes mellitus, hypercalciuria, eating disorders, autoimmune disease, homozygous FH and other chronic diseases.

An exhaustive medical history, including family cardiovascular and dyslipidaemia history, a complete physical examination and anthropometry data was recorded. To calculate body mass index (BMI) in children, we used the BMI score, which was calculated by the following equation: [(BMI children − BMI 50th percentile of Orbegozo's growth curves)/standard deviation (SD) 50th percentile of Orbegozo's growth curves] [14].

The Hospital's Ethics Committee approved the study protocol. All participants or participants' tutors provided written consent, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

2.2. Blood sample collection and storage

Blood samples were obtained after overnight fasting. Plasma and serum aliquots were prepared and stored at −80 °C in the BioBanc of our centre until further use.

2.3. Standard biochemical analysis

Serum cholesterol and triglyceride levels were measured using enzymatic colorimetric tests (CHOD-POD for cholesterol and GPO-POD for triglycerides), and apolipoprotein A1 (ApoA1) and apolipoprotein B (ApoB) were measured using immunoturbidimetric assays. HDL cholesterol was measured using a direct enzymatic colorimetric method that is dependent on detergents that solubilise only the HDL. The lipid profile was analysed according to Spintrol "H" CAL GC-MS reference methods. Spintrol "H" Normal was used as a quality control. All reagents were from Spinreact SA (Spain) and were performed in a Cobas Mira Plus autoanalyser (Roche Diagnostics, Spain). LDL-C levels were calculated by the Friedewald equation: LDL cholesterol = total cholesterol − (high-density lipoprotein (HDL) cholesterol + [triglycerides ÷ 5]).

2.4. 2D-1H-NMR lipid profile evaluation

The 2D-1H-NMR Liposcale is a new generation 1H-NMR test developed with the collaboration of our group (15). A 500-µl aliquot of plasma was shipped on dry ice to Biosfer Teslab (Reus, Spain) for lipoprotein analysis. The particle sizes and numbers of nine subtypes of lipoproteins [large, medium and small very low-density lipoprotein (VLDL), LDL and high-density lipoprotein (HDL)] were determined as previously reported. Briefly, particle concentrations and diffusion coefficients were obtained from the measured amplitudes and attenuation of their spectroscopically distinct lipid methyl group NMR signals using the 2D diffusion-ordered NMR spectroscopy pulse. By this method, the hydrodynamic characteristics of the molecules can be measured as is the case of the diffusion coefficient associated with each subclass of lipoprotein. From the diffusion coefficients, the sizes of different subclasses of lipoproteins are directly calculated through the Stokes-Einstein equation. The direct measurement of the size, as in this method, is of particular importance since it is used to calculate the number of lipoprotein particles. The methyl signal was surface-fitted with 9 Lorentzian functions associated with each lipoprotein subtype. The area of each Lorentzian function reflected the lipid concentration of each lipoprotein subtype, and the size of each subtype was calculated from the diffusion coefficient. The particle number of each lipoprotein subtype was calculated by dividing the lipid volume by the particle volume of a given class. Lipid volumes were determined using common conversion factors to convert concentration units into volume units. The variation coefficients for particle number were between 2% and 4%. The variation coefficients for particle size were lower than 0.3% [15].

2.5. Carotid intima-media thickness

The cIMT of the right and left common carotid arteries was determined in 177 children using a MyLab 60-X Vision sonographer (Esaote, Genova, Italy). A 7,5–10 MHz linear transducer and semi-automatic radio frequency software were used (QIMT©, Esaote). The images were obtained and measured by a single operator to reduce observer variability. We averaged the measurements of the left and right carotid arteries to obtain the mean cIMT [16,17].

2.6. Statistical analysis

The results are expressed as the mean ± SD for normally distributed data, as the median (interquartile range (IQR)) for data that were not normally distributed and as frequencies for categorical data. Kolmogorov-Smirnov tests were used to ensure that the data had a normal distribution. *t*-tests were used to determine significant differences when the data were normally distributed. Mann-Whitney tests were used to detect significant differences when the data were not normally distributed. Correlations were evaluated using Pearson's test for normally distributed data and Spearman's test for data that were not normally distributed. A multiple linear regression analysis using the enter method was used to test the associations of cIMT values with the different LDL measurements (apoB, LDL-C, LDL particle number, small LDL particle number) adjusted for gender, age, SBP and TG.

All analyses were performed using the statistical package SPSS 22.0 for Windows (SPSS, IBM®, Chicago, IL). A two-tailed *p*-value <0.05 was considered statistically significant in all analyses.

3. Results

3.1. Baseline characteristics

Anthropometry, clinical and standard biochemical data from the 183 children according to the diagnostic group are shown in Table 1. Of the 82 children with FH, 61 have a causal genetic mutation, representing a 74.4% of the sample. The genetically negative FH were younger and predominantly girls precluding any further comparison. The median age of the study participants was approximately 10 years, with significant differences ($p = 0.004$) between groups. No differences in gender distribution were observed. Apart from the lipid profile, differences were not observed in anthropometry or standard biochemical values. As expected, significant differences in total cholesterol, LDL-C, ApoB and ApoB/ApoA ratio values were observed between the FH and CCh groups ($p \leq 0.001$). HDL-C and ApoA1 concentrations also showed significant differences ($p = 0.019$ and $p = 0.034$, respectively).

3.2. 2D-1H-NMR lipid profile evaluation

FH children had significantly higher concentrations of total, large, medium and small LDL subclasses ($p \leq 0.001$) (Table 2). The number of small LDL particles was 33% higher in FH children than that in CCh, representing approximately half of the total particle numbers. No significant differences were observed in the percentage of small LDL particles between groups. FH children showed a statistically significant trend in larger HDL ($p = 0.022$) and smaller VLDL particles than CCh ($p = 0.043$) (Table 2).

3.3. Correlations between cIMT and 2D-1H-NMR lipid profile parameters

cIMT was obtained in 177 children (76 FH children and 101 CCh). No differences in the mean cIMT were observed between the groups (Table 1). The bivariate correlations between cIMT and 2D-1H-NMR lipid profile parameters in both groups, adjusted for age, are shown in Table 3. ApoB and LDL-C, but not total cholesterol, were associated with cIMT. However, the correlations between

Table 1
Characteristics of the FH and control children groups.

	FH (n = 82)	CCh (n = 101)	*p* value
Clinical data			
Age (years)	9.28 ± 3.69	10.80 ± 3.42	0.004
Diagnosis age (years)	8.32 ± 3.63		
Male (%)	53.80	52.60	0.631
BMI score	0.19 ± 0.91	0.04 ± 0.97	0.283
Waist circumference (cm)	62.34 ± 12.16	65.38 ± 11.34	0.087
SBP (mmHg)	109.53 ± 12.89	113.07 ± 10.87	0.061
DBP (mmHg)	64.69 ± 9.15	64.70 ± 8.77	0.993
Biochemical data			
Total cholesterol (mg/dL)	266.46 ± 47.97	190.53 ± 28.26	<0.0001
HDL-C (mg/dL)	61.40 ± 14.70	67.51 ± 19.25	0.019
LDL-C (mg/dL)	185.50(154.00–220.00)	112.00(96.00–125.00)	<0.0001
Triglycerides (mg/dL)	64.50(50.00–88.00)	58.00(48.00–75.00)	0.205
ApoA1 (mg/dL)	148.44 ± 26.27	157.58 ± 29.34	0.034
ApoB (mg/dL)	132.00(116.00–156.00)	90.50(78.00–99.00)	<0.0001
ApoB/ApoA ratio	0.95(0.72–1.13)	0.56(0.46–0.68)	<0.0001
Lp(a) (mg/dL)	18.79(7.08–71.26)	13.33(5.83–40.42)	0.187
Glucose (mg/dL)	82.05 ± 8.91	82.40 ± 8.13	0.783
Creatinine (mg/dL)	0.54 ± 0.16	0.57 ± 0.15	0.205
AST (U/L)	26.00(22.00–31.00)	26.00(22.00–29.00)	0.501
ALT (U/L)	17.50(13.00–21.00)	17.00(14.00–20.00)	0.597
GGT (U/L)	11.00(9.00–13.00)	12.00(10.00–13.00)	0.296
TSH (μU/mL)	2.71 ± 1.06	2.48 ± 1.13	0.928
Vitamin D (ng/mL)	28.54 ± 9.52	31.22 ± 8.87	0.116
cIMT (μm)	424.97 ± 68.31	420.97 ± 62.48	0.685

FH, familial hypercholesterolaemia; CCh, Control children.
Data are presented as mean ± SD for normally distributed data and as median and interquartile range (IQR) for non-normally distributed data. The percentage is used for categorical variables. The Mann-Whitney test was used for data with a non-normal distribution. *t*-tests were used for data with a normal distribution, and X^2 tests were used for categorical variables.

Table 2
Lipoprotein subclass analysis assessed by 2D-1H-NMR.

	FH (n = 82)	CCh (n = 101)	*p* value
Lipoprotein particle number			
VLDL (nM)	17.15(13.00–25.92)	18.16(14.27–23.69)	0.455
Large VLDL (nM)	0.61(0.43–0.92)	0.63(0.46–0.87)	0.566
Medium VLDL (nM)	3.04(2.22–4.22)	3.20(2.41–4.33)	0.207
Small VLDL (nM)	13.69(10.26–20.77)	14.32(11.24–19.09)	0.533
LDL (nM)	917.26 ± 187.69	676.25 ± 128.51	<0.0001
Large LDL (nM)	137.10 ± 30.92	102.09 ± 22.46	<0.0001
Medium LDL (nM)	344.52(295.63–409.12)	255.03(225.45–285.11)	<0.0001
Small LDL (nM)	419.89(352.25–486.42)	315.05(277.13–341.07)	<0.0001
HDL (μM)	27.84 ± 4.20	29.06 ± 4.93	0.078
Large HDL (μM)	0.13(0.10–0.18)	0.14(0.09–0.18)	0.918
Medium HDL (μM)	9.02 ± 1.67	9.23 ± 1.99	0.461
Small HDL (μM)	18.64 ± 2.84	19.69 ± 3.20	0.022
Lipoprotein size (diameter, nm)			
VLDL	42.65 ± 0.25	42.73 ± 0.3	0.043
LDL	21.30 ± 0.14	21.30 ± 0.16	0.388
HDL	8.23(8.22–8.25)	8.22(8.21–8.24)	0.004

FH, familial hypercholesterolaemia; CCh, Control children.
Data are presented as mean ± SD for normally distributed data and as median (IQR) for non-normally distributed data. The Mann-Whitney test was used to compare data with a non-normal distribution, and *t*-tests were used for normally distributed data.

Table 3
Correlations between 2D-1H-NMR lipoprotein profile data and the mean carotid intima media thickness (cIMT) sorted by group.

	FH (n = 76)		CCh (n = 101)	
	r	*p*	r	*p*
Total cholesterol	0.221	0.063	−0.096	0.352
HDL-C	−0.163	0.174	−0.276	0.006
LDL-C	0.241	0.043	0.057	0.578
Triglycerides	0.129	0.285	0.208	0.041
ApoA1	−0.080	0.506	−0.276	0.018
ApoB	0.237	0.046	0.097	0.343
ApoB/ApoA ratio	0.187	0.118	0.116	0.258
Lipoprotein particle number				
VLDL	0.057	0.626	0.248	0.013
Large VLDL	0.042	0.719	0.210	0.036
Medium VLDL	0.068	0.564	0.237	0.018
Small VLDL	0.056	0.636	0.250	0.012
LDL	0.249	0.032	0.057	0.570
Large LDL	0.123	0.292	−0.049	0.632
Medium LDL	0.218	0.061	0.053	0.602
Small LDL	0.299	0.009	0.093	0.358
HDL	−0.061	0.606	−0.097	0.337
Large HDL	−0.057	0.628	0.094	0.355
Medium HDL	−0.023	0.844	−0.098	0.333
Small HDL	−0.071	0.546	−0.092	0.363
Lipoprotein size				
VLDL	0.027	0.818	−0.038	0.708
LDL	−0.148	0.206	−0.143	0.156
HDL	0.009	0.936	−0.016	0.876

FH, familial hypercholesterolaemia; CCh, Control children.
p values were obtained by Pearson's test adjusted by age.

total and small LDL particle number with cIMT in the FH group were more strongly associated (r = 0.249, $p = 0.032$ and r = 0.299, $p = 0.009$, respectively). Moreover, in the multivariate analysis with cIMT as dependent variable, the models including total, and particularly small LDL particle number, significantly contribute to determine cIMT values contrary to those including standard LDL-C or ApoB (Table 4). In contrast, in the CCh group, the VLDL particle parameters were significantly correlated with cIMT rather than LDL (total VLDL r = 0.248, $p = 0.013$; Large VLDL r = 0.210 $p = 0.036$; Medium VLDL r = 0.237, $p = 0.018$; Small VLDL r = 0.250, $p = 0.012$).

Interestingly, in the entire population (FH + CCh), the correlations between cIMT and total and small LDL particle numbers were significant in older children (>14 y/o) (r = 0.407 $p = 0.015$ and r = 0.507 $p = 0.002$, respectively) (Supplementary Fig. 1).

Table 4
Impact of LDL parameters on cIMT according to the multiple regression analysis in FH children.

	Beta	*p value*	R^2
Model 1			
ApoB	0.165	0.136	0.316
Model 2			
LDL-C	0.189	0.078	0.285
Model 3			
LDL particle number	0.230	0.039	0.297
Model 4			
Small LDL particle number	0.282	0.013	0.316

The linear regression analysis results are displayed as beta coefficients with *p* and R^2 values for each model. cIMT is the dependent variable. The additional variables included in the models were: gender, age, SBP and TG.

4. Discussion

In the clinical setting, hypercholesterolaemia is usually determined by high total and calculated LDL-C concentrations. These concentrations are very high in FH subjects compared to normal individuals. LDL transports approximately 80% of our blood cholesterol. This particle has a variable size, ranging from approximately 20 to 30 nm in diameter [18]. It has been shown that small LDLs are more atherogenic than large LDL [10,19]. Individuals with a higher proportion of small LDLs need more particles to transport the same amount of cholesterol. Furthermore, small LDLs tend to have a greater affinity for subendothelial components and consequently exhibit increased retention times in the artery wall and are more prone to oxidation, leading to higher susceptibility to atherosclerosis [11,20,21]. Therefore, cholesterol concentrations are only partially informative in terms of cardiovascular risk associated with LDL. LDL particle number and size have been shown to be better predictors of risk than standard LDL-C concentrations [22,23]. In this study, we analysed particle numbers and sizes of the full lipoprotein profile by 2D-1H-NMR in children with FH. We aimed to study whether their lipid profile could be associated with an increased atherosclerosis risk beyond that determined by cholesterol concentrations alone. The 2D-1H-NMR method used was developed with the collaboration of our group by improving upon previous 1H-NMR methods by incorporating diffusion as a second dimension in the calculations [13], providing a direct particle size measurement. In our study, we showed that FH children

had 35% more LDL particles than the CCh group. Importantly, a 33% increase was also observed in the small, more-atherogenic LDL particles. These data highlight the importance of LDL particles in the pathophysiology of increased cardiovascular risk in FH patients. In our study, the metabolic alterations were only quantitative. Although it has been suggested that small LDL have a different affinity for LDL receptors, our data show that the proportions of large, medium and small LDL particles were similar in FH and CCh groups, suggesting that LDL receptor defects impact similarly to all LDL subclasses. This finding is consistent with the results from the Van der Graaf group, however, they observed an increased proportion of smaller VLDLs in FH children [12]. The reasons for this difference could be the different age ranges between cohorts and the distinct basal lipid concentrations. Their patients were approximately three years older. With increasing age, environmental factors play an important role that must be considered. Our study group was quite young and relatively unexposed to environmental influences. In fact, the CCh group, which was older, tended to have a higher number of VLDLs and smaller HDLs, reflecting the impact of environmental factors.

The overall mean cIMT was similar between groups although a trend to higher cIMT in FH children was observed, despite they were younger and had lower SBP. The sample size and short evolution time because of children age, precluded detecting significant differences at this stage. However, the association between cIMT and LDL particle number, in particular for smaller LDLs, was significant in the FH group and stronger than LDL-C and apoB. This fact was corroborated by multivariate analyses in which total and small LDL particle numbers were found to be independent predictors of subclinical atherosclerosis in FH children, while neither LDL-C nor apoB contributed significantly. These data suggest that despite no differences in cIMT were observed between groups, FH children have a lipid profile leading to accelerate vascular wall thickening over time. Interestingly, when we distribute the entire population by age group, the correlation between LDL particle number and cIMT was only observed in the greater than 14 y/o subgroup, suggesting that the impact of LDL on vessels is influenced by exposure time (Supplementary Fig. 1). On the other hand, the correlation between LDL particles and cIMT was not observed in CCh, probably due to lower LDL particle number determining an even slower cIMT progression.

A limitation of our study is the relatively small sample size, although it was the largest FH children cohort to undergo NMR lipoprotein profile and cIMT assessments. Moreover, the age ranges of these groups coincide with puberty and we do not know the impact of hormone changes on these lipid parameters.

In conclusion, we showed the complete lipoprotein profile as assessed by 2D-1H-NMR in FH and CCh groups. The main observation was that the higher cholesterol levels in FH children are driven by quantitative rather than qualitative differences. LDL particle number showed a strong association with cIMT, particularly in FH patients and older children, reinforcing the putative role of LDL particle number in the cardiovascular risk evaluation of FH children.

Conflict of interest

Daiana Ibarretxe: lecture from Sanofi, MSD and Esteve. Núria Plana: lectures from Amgen, MSD, Ferrer and Alexion. Luís Masana: lecture and advisory fees from Amgen, Sanofi and MSD. Núria Amigó: shareholder of Biosfer Teslab. The other authors have indicated they have no potential conflicts of interest to disclose.

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Author contributions

Cèlia Rodríguez-Borjabad: contributed to the collection, analysis and interpretation of the data and drafted the initial manuscript.

Daiana Ibarretxe: conceptualized and designed the study, contributed to the collection, analysis and interpretation of the data and critically reviewed the manuscript.

Josefa Girona: contributed to the data analysis and critically reviewed the manuscript.

Raimon Ferre: performed carotid sonography and critically reviewed the manuscript.

Albert Feliu: conceptualized and designed the study, contributed to the recruitment of patients and to initial review of eligibility according to the inclusion criteria and critically reviewed the manuscript.

Núria Amigó: carried out a study of lipoproteins by NMR.

Luís Masana: conceptualized and designed the study, drafted the initial manuscript, supervised the data collection and approved the final manuscript as submitted.

Núria Plana: conceptualized and designed the study, contributed to the data collection, supervised the data collection, drafted the initial manuscript and approved the final manuscript as submitted.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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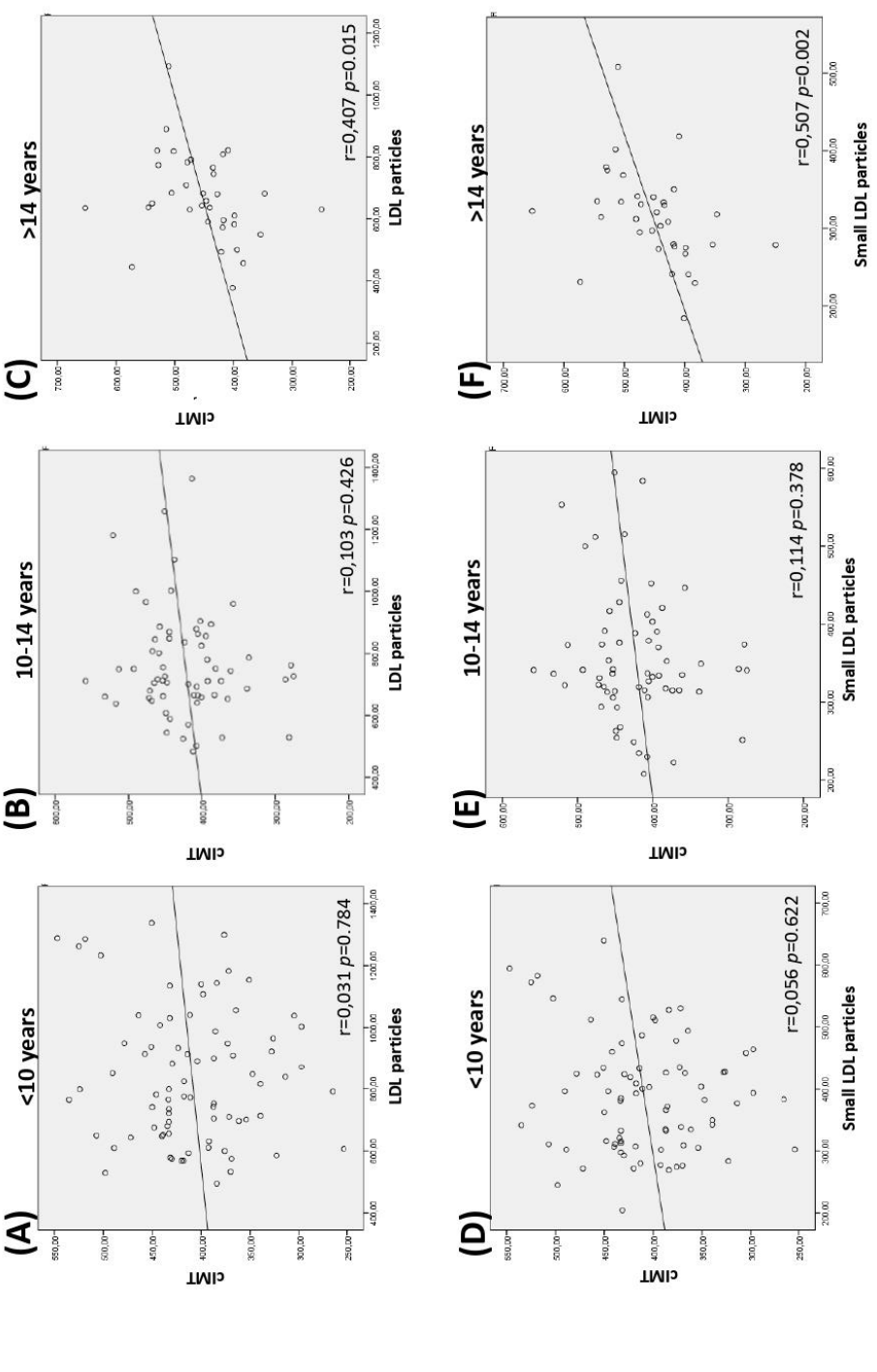
Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.atherosclerosis.2018.01.040.

References

[1] B.G. Nordestgaard, M.J. Chapman, S.E. Humphries, H.N. Ginsberg, L. Masana, O.S. Descamps, et al., Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society, Eur. Heart J. 34 (2013) 3478–3490.
[2] S.S. Gidding, M.A. Champagne, S.D. de Ferranti, J. Defesche, M.K. Ito, J.W. Knowles, et al., The agenda for familial hypercholesterolemia: a scientific statement from the American heart association, Circulation 132 (2015) 2167–2192.
[3] J.L. Goldstein, H.H. Hobbs, M.S. Brown, Familial hypercholesterolaemia, in: C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Vale (Eds.), The Metabolic and Molecular Basis of Inherited Disease, vol. III, McGraw Hill, New York, 2001, pp. 2863–2914.
[4] A.J. Whitfield, P.H. Barrett, F.M. van Bockxmeer, J.R. Burnett, Lipid disorders and mutations in the ApoB gene, Clin. Chem. 50 (2004) 1725–1732.
[5] J.D. Horton, J.C. Cohen, H.H. Hobbs, PCSK9: a convertase that coordinates LDL catabolism, J. Lipid Res. 50 (2009). Suppl:S172-7.
[6] A. Zamora, M. Masana, M. Comas-Cufí, A. Vila, N. Plana, M. García-Gil, Familial hypercholesterolemia in a European Mediterranean population. Prevalence and clinical data from 2.5 million primary Care patients, J Clin Lipidol 11 (2017) 1013–1022.
[7] R.M. Sánchez-Hernández, F. Civeira, M. Stef, S. Perez-Calahorra, F. Almagro, N. Plana, et al., Homozygous familial hypercholesterolemia in Spain: prevalence and phenotype-genotype relationship, Circ Cardiovasc Genet 9 (2016) 504–505.
[8] A. Wiegman, S.S. Gidding, G.F. Watts, M.J. Chapman, H.N. Ginsberg, M. Cuchel, et al., Familial hypercholesterolaemia in children and adolescents: gaining decades of life by optimizing detection and treatment, Eur. Heart J. 36 (2015) 2425–2437.
[9] U. Ramaswami, J. Cooper, S.E. Humphries, FH paediatric register steering group. The UK paediatric familial hypercholesterolaemia register: preliminary data, Arch. Dis. Child. 102 (2017) 255–260.
[10] G.J. Blake, J.D. Otvos, N. Rifai, P.M. Ridker, Low-density lipoprotein particle concentration and size as determined by nuclear magnetic resonance spectroscopy as predictors of cardiovascular disease in women, Circulation 106 (2002) 1930–1937.
[11] D.L. Tribble, L.G. Holl, P.D. Wood, R.M. Krauss, Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size, Atherosclerosis 93 (1992) 189–199.
[12] A. Van der Graaf, J. Rodenburg, M.N. Vissers, B.A. Hutten, A. Wiegman, M.D. Trip, et al., Atherogenic lipoprotein particle size and concentrations and the effect of pravastatin in children with familial hypercholesterolemia, J. Pediatr. 152 (2008) 873–878.
[13] D.M. Kusters, A. Wiegman, J.J.P. Kastelein, B.A. Hutten, Carotid intima-media thickness in children with hypercholesterolemia, Circ. Res. 114 (2014) 307–311.
[14] Curvas y Tablas de crecimiento (estudios longitudinal y transversal), Instituto de Investigación sobre crecimiento y desarrollo, Fundación Faustino Orbegozo Eizaguirre, María Diaz de Haro, 10 bis. 48013. Bilbao.
[15] R. Mallol, N. Amigó, M.A. Rodríguez, M. Heras, M. Vinaixa, N. Plana, et al., Liposcale: a novel advanced lipoprotein test based on 2D diffusion-ordered 1H NMR spectroscopy, J. Lipid Res. 56 (2015) 737–746.
[16] J.H. Stein, C.E. Korcarz, R.T. Hurst, E. Lonn, C.B. Kendall, E.R. Mohler, et al., Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine, J. Am. Soc. Echocardiogr. 21 (2008) 93–111.
[17] M. Junyent, R. Gilabert, I. Núñez, E. Corbella, M. Vela, D. Zambón, et al., Carotid ultrasound in the assessment of preclinical atherosclerosis. Distribution of intima-media thickness values and plaque frequency in a Spanish community cohort, Med. Clin. 125 (2005) 770–774.
[18] R.M. Krauss, D.J. Burke, Identification of multiple subclasses of plasma low density lipoproteins in normal humans, J. Lipid Res. 23 (1982) 97–104.
[19] M.A. Austin, J.L. Breslow, C.H. Hennekens, J.E. Buring, W.C. Willett, R.M. Krauss, Low- density lipoprotein subclass patterns and risk of myocardial infarction, JAMA 260 (1988) 1917–1921.
[20] H. Campos, K.S. Arnold, M.E. Balestra, T.L. Innerarity, R.M. Krauss, Differences in receptor binding of LDL subfractions, Arterioscler. Thromb. Vasc. Biol. 16 (1996) 794–801.
[21] J. de Graaf, H.L. Hak-Lemmers, M.P. Hectors, P.N. Demacker, J.C. Hendriks, A.F. Stalenhoef, Enhanced susceptibility to in vitro oxidation of the dense low density lipoprotein subfraction in healthy subjects, Arterioscler. Thromb. 11 (1991) 298–306.
[22] S. Mora, J.E. Buring, P.M. Ridker, Discordance of low-density lipoprotein (LDL) cholesterol with alternative LDL-related measures and future coronary events, Circulation 129 (2014) 553–561.
[23] D.M. Tehrani, Y. Zhao, M.J. Blaha, S. Mora, R.H. Mackey, E.D. Michos, et al., Discordance of low-density lipoprotein and high-density lipoprotein cholesterol particle versus cholesterol concentration for the prediction of cardiovascular disease in patients with metabolic syndrome and Diabetes Mellitus (from the multi-ethnic Study of atherosclerosis [MESA]), Am. J. Cardiol. 117 (2016) 1921–1927.

Supplementary Figure



Associations between cIMT and LDL particles (A, B, C) and small LDL particles (D, E, F). The FH and CCh groups were pooled and then divided by age groups: <10 years (n=82/57%FH), 10-14 years (n=64/32%FH) and >14 years (n=37/38% FH).
P values were obtained by Pearson's test.

Objective 3

3. To describe the impact of diet on children with FH and design techniques to implement lifestyle changes in these children.

3.1. To analyse the clinical and biochemical performance of an educational program focused on improving the lifestyle (diet, PA, smoking) of children with FH.

ARTICLE 6

***Rodríguez-Borjabad C**, Malo AI, Ibarretxe D, Girona J, Heras M, Ferré R, Feliu A, Salvadó M, Varela A, Amigó N, Masana L, Plana N on behalf of DECOPIN Group. Efficacy of therapeutic lifestyle changes on lipid profiles assessed by NMR in children with familial hypercholesterolemia. Clin Invest Arterioscl. 2020;32:49-58.*

ARTICLE 7

***Rodríguez-Borjabad C**, Andreychuk N , Ibarretxe D, Girona J, Guerrero C, Feliu F, Plana N, Masana L, on behalf of the DECOPIN Group.Effectiveness of an infantile educational program on healthy lifestyle addressed to children with genetic and non-genetic driven hypercholesterolemia. Learning while playing! (in preparation).*

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ORIGINAL ARTICLE

Efficacy of therapeutic lifestyle changes on lipid profiles assessed by NMR in children with familial and non-familial hypercholesterolemia

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KEYWORDS
Familial hypercholesterolaemia;
Type IIa hyperlipidaemia;
Lipoprotein profile;
NMR;
Children;
Healthy lifestyle

Abstract
Background and aims: The first line of therapy in children with hypercholesterolaemia is therapeutic lifestyle changes (TLSC). The efficacy of lifestyle intervention in children with familial hypercholesterolaemia (FH), where LDL-C levels are genetically driven, deserves a focused study.
Aims: To evaluate the impact of a lifestyle education program, focused on food patterns and physical activity, on lipid profiles assessed by nuclear magnetic resonance (NMR) in children with FH vs. non-FH.
Methods: Phase 1 was a cross-sectional study of baseline characteristics, and phase 2 was a prospective TLSC intervention study. In total, the study included 238 children (4 to 18 years old; 47% girls) attending the lipid unit of our hospital due to high cholesterol levels. Eighty-five were diagnosed with FH (72% genetic positive), and 153 were diagnosed with non-Familial

Abbreviations: 2D-1H-NMR, two-dimensional nuclear magnetic resonance; Apo A, apolipoprotein; Apo B, apolipoprotein B100; BMI, body mass index; FH, familial hypercholesterolemia; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein (a); LDLR, low-density lipoprotein receptor; MUFA, monounsaturated fatty acid; PA, physical activity; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; SD, standard deviation; TLSC, therapeutic lifestyle changes; VLDL, very low-density lipoprotein.

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hypercholesterolaemia. A quantitative food frequency questionnaire (FFQ) including 137 items was used. Physical activity (PA) was assessed by the Minnesota questionnaire. The lipid profile was assessed using the 2D-1H-NMR (Liposcale test). A total of 127 children (81 in the FH group) participated in the prospective phase and were re-assessed after 1 year of the TLSC intervention, consisting of education on lifestyle changes delivered by a specialized nutritionist.
Results: The FH and non-FH groups were similar in anthropometry and clinical data, except that those in the FH were slightly younger than those in the non-FH group. Both the FH and non-FH groups showed a similar diet composition characterized by a high absolute calorie intake and a high percentage of fat, mainly saturated fat. The PA was below the recommended level in both groups. After one year of TLSC, the percentage of total and saturated fats was reduced, and the amount of fiber increased significantly in both groups. The percentage of protein increased slightly. The number of children engaged in at least 1 hour/day of PA increased by 56% in the FH group and by 53% in the non-FH group, and both these increases were significant. The total and small-LDL particle numbers were reduced in both groups, although the absolute change was greater in the FH group than in the non-FH group.
Conclusions: Educational strategies to implement TLSC in children lead to empowerment, increased adherence, and overall metabolic improvement in children with high blood cholesterol, including those with FH.

PALABRAS CLAVE
Hipercolesterolemia familiar;
Hiperlipidemia tipo IIa;
Perfil lipoproteico;
RMN;
Niños;
Estilo de vida saludable

Eficacia de los cambios terapéuticos en el estilo de vida en el perfil lipídico evaluado por RMN en niños con hipercolesterolemia familiar y no familiar

Resumen
Antecedentes y objetivos: La primera línea de terapia en niños con hipercolesterolemia son los cambios terapéuticos en el estilo de vida (TLSC). La eficacia de la intervención en el estilo de vida en niños con hipercolesterolemia familiar (HF), en los que los niveles de LDL-C son generados genéticamente, merece un estudio específico.
Objetivos: Evaluar el impacto de un programa de educación sobre el estilo de vida, centrado en los patrones alimentarios y la actividad física, sobre el perfil lipídico evaluado por resonancia magnética nuclear (RMN) en niños con HF versus no HF.
Métodos: La fase 1 fue un estudio transversal de las características basales, y la fase 2 fue un estudio prospectivo de intervención mediante TLSC. En total, el estudio incluyó a 238 niños (de 4 a 18 años; 47% niñas) que asistieron a la unidad de lípidos de nuestro hospital debido a los altos niveles de colesterol. Ochenta y cinco fueron diagnosticados con HF (72% genéticamente positivos), y 153 fueron diagnosticados de no HF. Se utilizó un cuestionario cuantitativo de frecuencia de alimentos (FFQ) que incluye 137 ítems. La actividad física (AF) se evaluó mediante el cuestionario de Minnesota. El perfil lipídico se evaluó mediante la prueba 2D-1H-NMR (Liposcale Test). Un total de 127 niños (81 en el grupo HF) participaron en la fase prospectiva y fueron reevaluados después de 1 año de la intervención mediante TLSC, que consistió en educación sobre cambios en el estilo de vida impartida por una nutricionista especializada.
Resultados: Los grupos HF y no HF fueron similares en los datos antropométricos y clínicos, excepto que los HF eran ligeramente más jóvenes que los no HF. Los participantes de ambos grupos mostraron una composición de dieta similar caracterizada por un alto consumo de calorías totales y un alto porcentaje de grasas, principalmente grasas saturadas. La AF estuvo por debajo del nivel recomendado en ambos grupos. Después de un año de TLSC, se redujo el porcentaje de grasas totales y saturadas, y la cantidad de fibra aumentó significativamente en ambos grupos. El porcentaje de proteína aumentó ligeramente. El número de niños involucrados en al menos 1 hora/día de AF aumentó en un 56% en el grupo de HF y en un 53% en el grupo sin HF, y ambos aumentos fueron significativos. Los números de partículas LDL totales y pequeñas se redujeron en ambos grupos, aunque el cambio absoluto fue mayor en el grupo HF que en el grupo no HF.
Conclusiones: Las estrategias educativas para implementar TLSC en niños conducen al empoderamiento, al aumento de la adherencia y a la mejora metabólica general en niños con colesterol alto en sangre, incluidos aquellos con HF.

Introduction

Familial hypercholesterolemia (FH) is an autosomal-dominant disorder of lipid metabolism characterized by an increase in low-density lipoprotein cholesterol (LDL-C).[1] The elevation of LDL is observed at an early age and results in early atherosclerotic lesions.[2] This disease has been described as a cause of premature atherosclerotic coronary heart disease, and for this reason, it is very important to start treating children.[3]

In Catalonia, one out of 217 children has the FH phenotype.[4] This disease is very prevalent worldwide and is underdiagnosed.[2] In recent years, several studies have been published to improve the detection of FH.[5–7] The need for improved detection arises from the need to start treatment earlier to improve the prognosis.[1,8–10] In addition, early detection strategies are efficient.[11–13]

The first line of therapy in hypercholesterolemic children is therapeutic lifestyle changes (TLSC), and only FH children with very high LDL-C will require medication[1,8,14,15] to achieve the LDL-C goal of 130 mg/dL.[2,8,16] Total cholesterol and LDL-C levels can be modulated by dietary intake.[17–18] Studies have shown that dietary adjustments can reduce plasma cholesterol by 20–30%[17] and LDL atherogenicity.[19] The efficacy of lifestyle interventions in children with FH, where LDL-C levels are genetically driven, deserves focused study. Some authors have observed that the reduction in LDL-C in patients with FH is lower than in patients without FH.[19–21] The effect of diet on the lipid profile of these children is little known.

The atherogenicity of LDL particles is not measured only by the concentration of cholesterol. The LDL particle number is considered an even better risk marker. Interestingly, small LDL particles are more atherogenic because they are more easily oxidizable, they can pass through the membrane, and they have less affinity for the receptor.[22–23] Small LDL is viewed as an important cardiovascular risk factor.[24–25] FH children exhibit a significant absolute increase in the LDL particle number, which includes the most atherogenic small LDL particles.[26–27] These characteristics can be evaluated using metabolomics techniques based on nuclear magnetic resonance (NMR). Studies showing the effect of diet on lipoprotein particle number and size in FH children are scarce.

The purpose of the present study was to characterize the total lipid profile of FH and non-FH hypercholesterolemic children, to appraise the diet and PA of FH and non-FH hypercholesterolemic children and to prospectively evaluate the impact of a lifestyle intervention on food patterns, PA and lipid profiles of FH and non-FH hypercholesterolemic children.

Methods

Study design and patients

The first phase of this study was a cross-sectional study, and the second phase was a prospective post-TLSC study. From March 2013 to May 2019, 274 children and adolescents aged 4–18 years attending the lipid unit of our University Hospital because of high cholesterol were selected to participate in the ''Early Familial Hypercholesterolemia Detection Project'' (DECOPIN Project).[7] Thirty-six children were not included because of a lack of full clinical information or secondary causes of hypercholesterolemia; therefore, 238 children were included in the cross-sectional phase of the study. Children were classified as FH ($n = 85$) if they had a positive genetic test or LDL-C > 160 mg/dL and one of the parents had a Dutch Lipid Clinic Network score >8, in the case of no available genetic test result. Children who did not meet the FH criteria were included in the non-FH ($n = 153$) group. None of the children were on lipid-lowering therapy at baseline. The exclusion criteria were chronic renal, hepatic or thyroid disease and type 1 diabetes mellitus, hypercalciuria, eating disorders, autoimmune disease, homozygous FH and other chronic diseases.

All FH children were asked to take part in the prospective phase and all of them accepted. although only 81 have completed the 1 year follow-up and were included in the final analysis. Forty-six non-FH children were also included. In this case, were asked to take part in the prospective phase those children with higher cholesterol (>135 mg/dL) and FH siblings willing to participate. After one-year follow-up the main changes in diet and physical activity were reevaluated. Standard lipid profile was also determined. We also communicate the results of a complete 2D-1H-NMR lipid profile that was available at baseline and after one year of follow-up in 97 children (64 FH and 33 non-FH) participating in the prospective phase of the study.

Anthropometry, clinical history and biochemical parameters

Anthropometry, demography and clinical data were recorded at basal time (and after the 1-year follow-up in the prospective study). Body mass index (BMI) score was calculated according to the following formula: [(BMI children–BMI 50th percentile of Orbegozo's growth curves)/standard deviation (SD) 50th percentile of Orbegozo's growth curves].[28]

Standard biochemical analyses were performed at similar times. Blood samples were obtained after overnight fasting. Serum cholesterol and triglyceride levels were evaluated using an enzymatic colorimetric test (CHOD-POD and GPO-POD, respectively), high density lipoprotein cholesterol (HDL-C) was evaluated using a direct enzymatic colorimetric method, and apolipoprotein levels were measured by immunoturbidimetric assays. LDL-C levels were calculated by the Friedewald equation. A portion of the blood sample was stored at −80 °C in the BioBanc of our Research Institute.

Full lipoprotein profile (Liposcale Test®)

The Liposcale Test® (actualized 2018 version) was used to assess the full lipoprotein profile. As previously reported, this method is based on 2D-1H-NMR.[29] This method determines the lipid concentration and particle number for the large, medium and small subclasses of the main lipoprotein classes (VLDL, LDL and HDL) and their size-associated diffusion coefficients. In the actualized version, the LDL lipoprotein class has been linearly calibrated to the LDL

particle number according to the standard FDA-approved NMR based on the lipoprotein methodology developed by Otvos and colleagues[30] to obtain the best agreement of the absolute LDL particle numbers between the two techniques.[31] The variation coefficients for the particle number were between 2% and 4%. The variation coefficients for particle size were lower than 0.3%.

Diet and physical activity assessment and implementation of TLSC

The diet data were collected with a quantitative food frequency questionnaire (FFQ) that included 137 items[32] plus alcohol, as validated in the PREDIMED study. The diet data were collected from children and families by a registered nutritionist. The TLSC intervention consisted of nutritionist visits at which children and their families were taught to eat healthy, using simple and healthy culinary techniques and meal plans. They were also motivated to perform physical exercise and avoid alcohol and tobacco consumption. At the end of the clinical visits, a personalized lifestyle recommendation report, consisting in a tailored advice to approach diet to international guidelines according personal deviations, was provided to each participant.

Physical activity (PA) was assessed by the Minnesota leisure-time physical activity questionnaire. According to international guidelines, we quantified the number of hours of physical activity per week.

The Hospital's Ethics Committee approved the study protocol, and this conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Before participation in the study, informed consent was signed by one of the parents.

Table 1 Clinical, biochemical and lipoprotein subclass analysis assessed by 2D-1H-NMR of the study subjects at basal time.

	Non FH ($n=153$)	FH ($n=85$)	p
Demographic characteristics			
*Age (y)**	11 (8–13)	9 (6–12)	0.003
Gender (% girls)	47.70	47.10	0.547
BMI score†	0.11 ± 1.05	0.22 ± 0.90	0.425
Waist circumference (cm)†	65 ± 12	62 ± 12	0.117
Lipids and apolipoproteins			
*Cholesterol (mg/dL)**	201.08 (177.11–222.55)	266.05 (233.95–298.15)	<0.0001
*LDL-C (mg/dL)**	132.28 (111.14–149.06)	189.95 (157.81–227.07)	0.025
*HDL-C (mg/dL)**	52.78 (46.02–64.00)	53.36 (48.34–59.94)	<0.001
*Triglycerides (mg/dL)**	62.00 (50.48–84.14)	68.20 (54.03–96.54)	0.320
*ApoB100 (mg/dL)**	94 (79–104)	133 (115–156)	0.009
ApoA1 (mg/dL)†	157 ± 3	147 ± 25	<0.0001
*Ratio ApoB100/ApoA1**	0.58 (0.46–0.75)	0.96 (0.72–1.15)	<0.0001
*Lp(a) (mg/dL)**	31.5 (11.50–113.30)	38.00 (16.00–158.39)	0.249
Lipoprotein particle number			
*Total VLDL (nmol/L)**	24.81 (18.44–35.71)	25.16 (18.75–39.33)	0.632
Large VLDL (nmol/L)*	0.77 (0.53–1.06)	0.75 (0.51–1.02)	0.506
Medium VLDL (nmol/L)†	3.16 ± 1.84	3.51 ± 1.54	0.131
Small VLDL (nmol/L)*	21.06 (16.11–30.60)	21.43 (15.67–33.57)	0.825
*Total LDL (nmol/L)**	1294.02 (1117.99–1423.46)	1804.19 (1485.75–2078.17)	<0.0001
Large LDL (nmol/L)*	184.52 (157.39–204.20)	259.85 (220.38–303.38)	<0.0001
Medium LDL (nmol/L)*	390.06 (296.90–455.78)	613.47 (466.07–768.33)	<0.0001
Small LDL (nmol/L)*	716.08 (626.57–771.40)	851.81 (770.93–1012.23)	<0.0001
*Total HDL (μmol/L)**	26.39 (23.8131.12)	26.99 (23.95–30.52)	0.639
Large HDL (μmol/L)*	0.23 (0.21–0.26)	0.28 (0.25–0.31)	<0.0001
Medium HDL (μmol/L)†	8.92 ± 1.78	9.59 ± 1.42	0.003
Small HDL (μmol/L)†	18.48 ± 4.03	17.84 ± 3.64	0.224
Lipoprotein size (diameter. nm)			
VLDL-Z (nm)†	42.17 ± 0.38	42.26 ± 0.34	0.071
LDL-Z (nm)†	21.06 ± 0.29	21.33 ± 0.30	<0.0001
HDL-Z (nm)†	8.25 ± 0.06	8.30 ± 0.07	<0.0001

FH: familial hypercholesterolemia; BMI: body mass index; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; ApoB100: apolipoprotein B100; ApoA1: apolipoprotein A1; Lp(a): lipoprotein (a); Z: size.
The data are presented as the mean ± standard deviation for normally distributed data. median (Q1–Q3) for non-normally distributed data or percentage for categorical variables.
* Mann–Whitney U test or χ^2.
† p Values were obtained by Student t-test.

Table 2 Nutritional information of the daily intake of children evaluated from a food frequency questionnaire (FFQ) at basal time ($N = 238$).

	Non FH ($n = 153$)	FH ($n = 85$)	p
Total intake (kcal/day)*	2767.44 (2337.06–3292.49)	2861.94 (2483.00–3260.18)	0.326
Carbohydrate (%)†	47.45 ± 4.83	48.17 ± 5.44	0.296
Protein (%)†	17.24 ± 3.05	16.60 ± 1.82	0.080
Fat (%)†	35.26 ± 4.16	35.23 ± 4.91	0.931
MUFA (%)†	14.86 ± 2.39	14.83 ± 2.61	0.924
PUFA (%)†	5.17 ± 1.21	5.03 ± 1.25	0.421
SFA (%)†	10.72 ± 2.2429	11.02 ± 2.40	0.343
Fiber (g/day)*	20.48 (16.91–25.81)	20.21 (16.0726.38)	0.779
Cholesterol (g/day)†	0.36 ± 0.11	0.36 ± 0.12	0.546

FH: familial hypercholesterolemia; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid. The data are presented as the mean ± standard deviation for normally distributed data. median (Q1–Q3) for non-normally distributed data or percentage for categorical variables.
* Mann–Whitney U test or χ^2.
† p Values were obtained by Student t-test.

Statistical analysis

The results are expressed as the mean ± SD for normally distributed data, as the median (interquartile range (IQR)) for data that were not normally distributed and as frequencies for categorical data. Kolmogorov–Smirnov tests were used to ensure that the data had a normal distribution. T-tests were used to determine significant differences when the data were normally distributed. Mann–Whitney tests were used to detect significant differences when the data were not normally distributed. The paired T-test were used in normal variables and Wilcoxon signed-rank test in non-parametric variables to know the difference after 1 year of TLSC.

All analyses were performed using the statistical package SPSS 25.0 for Windows (SPSS, IBM®, Chicago, IL). A p-value <0.05 was considered statistically significant in all analyses.

Results

Clinical, biochemical and lipoprotein subclass analysis at basal time

Anthropometry, clinical and lipid profile data from the 238 children are shown in Table 1 according to the diagnostic group. Of these, 85 children had FH (72% positive mutation). The FH children included in the cross-sectional phase were younger than the non-FH children ($p = 0.003$). In the prospective phase, there were no age differences between the groups. As expected, significant differences in total cholesterol ($p < 0.0001$), LDL-C ($p = 0.025$), apolipoprotein B (ApoB) ($p = 0.009$) and apolipoprotein A (ApoA) and its ratio ($p < 0.0001$) were observed. HDL-C was lower in the non-FH group than in the FH group ($p < 0.0001$). No differences in Lp(a) were detected.

FH children had significantly higher absolute concentrations of total, large, medium and small LDL subclasses ($p < 0.0001$) than the non-FH children. The number of small LDL particles was approximately 20% higher in FH children than in non-FH children. The mean LDL size was slightly larger in FH children than in non-FH children because of a higher proportion of medium-sized LDL at baseline.

Diet composition and intake of macronutrients at basal time

The intake of macronutrients is shown in Table 2. At basal time, we did not find differences in diet composition between groups. Both groups ingested a high amount of calories (median values for FH = 2767 kcal and non-FH = 2861 kcal). The consumption of protein, saturated fatty acid (SFA) and cholesterol was well above the general recommendations. The calorie intake delivered by SFA was 11%, which is much higher than the recommended 7–8%. The amount of fiber was approximately 20 g/day, which was closer to the lower recommended limits.

Composition of diet and physical activity after one year of the TLSC intervention

One hundred twenty-seven children participated in the prospective phase (FH $n = 81$ and non-FH $n = 46$). After one year of TLSC, both diet composition and physical activity changed significantly in a positive way (Figs. 1 and 2).

The percentages of calories due to total fat (FH $p < 0.0001$ and non-FH $p = 0.002$) and saturated fat (FH $p < 0.0001$ and non-FH $p = 0.020$) were significantly reduced. The amount of fiber increased significantly by approximately 10% in both groups (FH $p < 0.0001$ and non-FH $p = 0.003$).

Interestingly, in Fig. 2, we show the percentage of children who performed 1 h/day of PA before and after the intervention. There were significant increases of 4,6-fold in the FH group and of 4,8-fold in the non-FH group ($p < 0.0001$ in both cases).

Lipid profile after one year of the TLSC intervention

The standard lipid profile, prospectively determined in 127 children, showed a decrease in total and LDL cholesterol

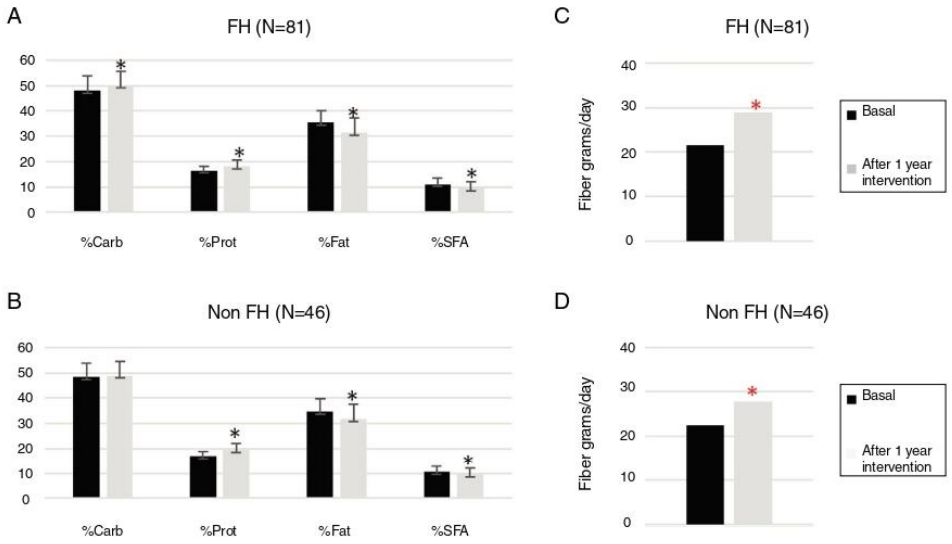

Figure 1 Changes in the daily intake evaluated from a food frequency questionnaire (FFQ) at basal time and after intervention.
FH: familial hypercholesterolemia; Carb: carbohydrate; Prot, protein; SFA, saturated fatty acid.
p Values were obtained by paired sample *T*-test.
*The difference after 1 year of TLSC is statistically significant ($p < 0.05$).

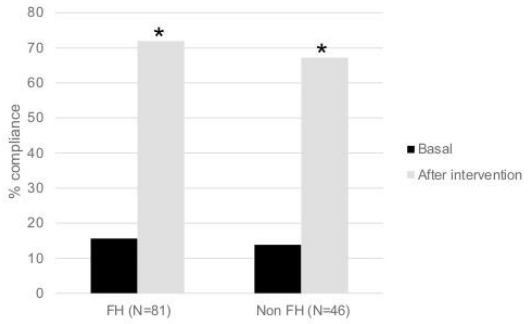

Figure 2 Percentage of compliance of 1 hour a day of physical exercise.
FH: familial hypercholesterolemia.
p Values were obtained by paired sample *T*-test.
*The difference after 1 year of TLSC is statistically significant ($p < 0.05$).

in both hypercholesterolemic groups, although the LDL-C reduction was only statistically significant in FH children (FH $p < 0.0001$ and non-FH $p = 0.172$) (Table 3). The magnitude of change was larger in the FH group than in the non-FH group (Table 3). There were no significant changes in HDL-C and triglycerides.

The lipid profile assessed by 2D-1H-NMR determined prospectively in 97 children, Table 4, showed a significant decrease in total LDL particles in both groups (FH and non-FH $p = 0.002$), while maintaining the same proportion of particles among subclasses. The absolute number of small LDL particles was significantly reduced in both cases (FH $p = 0.002$ and non-FH $p = 0.003$). The mean LDL size was not changed in any group. VLDL and HDL total particle number was also reduced in both groups although the difference was only significant in the FH group, probably reflecting the sample size differences.

Table 3 Lipid profile after 1-year intervention ($N = 127$).

	Non FH ($n = 46$)			FH ($n = 81$)		
	Baseline	After 1 year intervention	*p*-Value	Baseline	After 1 year intervention	*p*-Value
TC (mg/dL)	214 (192–229)	202 (189–224)	0.033	265 (232–302)	229 (206–281)	<0.0001
LDL-C (mg/dL)	132 (124–145)	127 (115–140)	0.172	186 (150–220)	154 (132–200)	<0.0001
HDL-C (mg/dL)	62 (53–76)	59 (52–71)	0.073	59 (51–70)	59 (51–67)	0.069
TG (mg/dL)	61 (54–81)	75 (63–94)	0.184	64 (50–88)	61 (51–80)	0.0893

FH: familial hypercholesterolemia; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: blood triglycerides.
p Values were obtained by paired sample T-test for normal variables and Wilcoxon signed-rank test for non-parametric variables.

Table 4 Changes in lipoprotein subclass analysis assessed by 2D-1H-NMR after one year of intervention in lifestyle changes ($N = 97$).

	Non FH ($n = 33$)		FH ($n = 64$)	
	Differences (media ± SD)	*p*-Value	Differences (media ± SD)	*p*-Value
Lipoprotein particle number				
Total VLDL (nmol/L)	2.16 ± 15.54	0.430	−4.43 ± 13.73	0.012
Large VLDL (nmol/L)	0.39 ± 0.32	0.481	−0.11 ± 0.31	0.009
Medium VLDL (nmol/L)	0.50 ± 2.15	0.189	−0.67 ± 1.20	<0.0001
Small VLDL (nmol/L)	1.62 ± 13.64	0.500	−3.65 ± 12.54	0.023
Total LDL (nmol/L)	−102.01 ± 176.72	0.002	−146.87 ± 355.57	0.002
Large LDL (nmol/L)	−19.66 ± 29.81	0.001	−28.83 ± 52.15	<0.0001
Medium LDL (nmol/L)	−33.64 ± 100.06	0.062	−52.87 ± 186.52	0.027
Small LDL (nmol/L)	−48.70 ± 87.51	0.003	−65.18 ± 159.65	0.002
Total HDL (μmol/L)	−0.66 ± 2.97	0.207	−1.20 ± 3.60	0.010
Large HDL (μmol/L)	−0.01 ± 0.02	0.054	−0.02 ± 0.04	<0.0001
Medium HDL (μmol/L)	−0.60 ± 1.20	0.001	−0.60 ± 1.20	<0.0001
Small HDL (μmol/L)	−0.56 ± 0.91	0.845	−0.57 ± 2.73	0.100
Lipoprotein size (diameter, nm)				
VLDL	0.01 ± 0.40	0.905	−0.03 ± 0.37	0.527
LDL	−0.04 ± 0.29	0.418	−0.50 ± 0.25	0.107
HDL	−0.02 ± 0.05	0.034	−0.01 ± 0.05	0.035

FH: familial hypercholesterolemia; VLDL: very low-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; SD: standard deviation.
p Values were obtained by paired sample *T*-test for normal variables and Wilcoxon signed-rank test is a non-parametric variables.

Discussion

In this study, we report that TLSC implemented in children with genetically driven hypercholesterolemia produced a global benefit on diet and PA, leading to lipoprotein profile improvement and therefore cardiovascular risk reduction.[33–34]

Lifestyle changes are the first option in the treatment of FH children. We have observed that healthy lifestyle recommendations lead to a beneficial change in both diet composition and the amount of PA.[1,2,8,35] In our study, the diet of both FH and non-FH children improved. The percentages of fats and SFA were reduced in both groups. SFA intake is associated with higher total cholesterol and LDL-C,[8] and controlling cholesterol and SFA intake has no negative impact on the growth and development of children.[36] The percentage of protein increased slightly in both groups, but the type of food carrying them changed positively (data not shown). The fiber content of the children's diet increased significantly in the two groups. An increase in dietary fiber has been associated to a lipid profile improvement.[17,20]

PA was also improved. Regular and sustained physical activity protects against cardiovascular disease with a dose-response relationship. It is recommended that children perform 60 min of moderate to vigorous PA every day.[37]

Another aspect to be highlighted is the importance of early TLSC. Establishing lifestyle changes at early ages is more effective, better assimilated and better maintained over time than those established later in life.[1,8] Early prevention of atherosclerotic disease should begin in childhood with lifestyle education.[34]

Interestingly, TLSC were associated with positive modifications of the lipid profile, not just on standard parameters but also on total and small LDL particle numbers.[27,34] Notably, these changes were more significant in FH children, probably because the differences in the sample size and that they started from higher basal values, but a greater empowerment and adherence, because children and their families were more aware of the disease could also play a role. Interesting, VLDL and HDL were also reduced probably reflecting the impact on global fat burden. The reduction on HDL particles, without a HDL-C lowering, could reflect cholesterol redistribution among bigger particles.

Despite the genetic origin of hypercholesterolemia, TLSC have a significant impact on lipid parameters. According to the Cholesterol Treatment Trialist Collaboration meta-analyses, based on statin randomized control trials, each 1 mmol/L of LDL-C reduction is associated with a 22% relative cardiovascular risk reduction.[38] Data from observational studies and mainly from Mendelian randomized studies show a greater benefit when the LDL-C reduction starts earlier.[39] Accordingly, our results suggest a higher impact on cardiovascular risk in these groups of children over time. Moreover, TLSC are associated not only with LDL-C reduction but also with total and, interestingly, small LDL particles, which should induce an incremental overall benefit.

The main limitations of this study are, first, the limited sample size. However, this is one of the larger cohorts reported prospectively. The sample sizes of the crossectional study, the global prospective and the 2D-1H-NMR prospective study are different, according data availability.

However, the demographic data of groups was not significantly different.

In our cohort, we have adolescents, and in this period of development, LDL-C levels are changing, which could jeopardize the real effect of TLSC.[40] Finally, diet assessments are always inaccurate, even though we used validated questionnaires for the Spanish population, and the data acquisition was performed by the same nutritionist.

In conclusion, educational strategies to implement TLSC in children lead to empowerment, increased adherence and overall metabolic improvement in hypercholesterolemic children, including those with FH. Early intervention should result in an important impact on future cardiovascular prognosis.

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Authors' contributions

Cèlia Rodríguez-Borjabad: Rodríguez-Borjabad contributed to the collection, analysis and interpretation of the data and drafted the initial manuscript.

Ana Irene Malo: Dr. Malo contributed to the collection and critically reviewed the manuscript.

Daiana Ibarretxe: Dr. Ibarretxe conceptualized and designed the study, contributed to the collection, analysis and interpretation of the data and critically reviewed the manuscript.

Josefa Girona: Dr. Girona contributed to the data analysis and critically reviewed the manuscript.

Merche Heras: Mrs contributed to analysis of blood samples

Raimon Ferre: Dr. Ferré performed carotid sonography and critically reviewed the manuscript.

Albert Feliu: Dr. Feliu conceptualized and designed the study, contributed to the recruitment of patients and to the initial review of eligibility according to the inclusion criteria and critically reviewed the manuscript.

Maria Salvadó: Dr. Salvadó contributed to the recruitment of patients.

Anna Varela: Mrs. Varela was the nurse in charge of collecting the blood samples.

Núria Amigó: Dr. Amigó carried out a study of lipoproteins by NMR.

Luís Masana: Dr. Masana conceptualized and designed the study, drafted the initial manuscript, supervised the data collection and approved the final manuscript as submitted. Núria Amigó: contributed to the NMR analysis and critically reviewed the manuscript.

Núria Plana: Dr. Plana conceptualized and designed the study, contributed to the data collection, supervised the data collection, drafted the initial manuscript and approved the final manuscript as submitted.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Conflicts of interest

Daiana Ibarretxe: lecture stipends from Sanofi, MSD and Esteve. Núria Plana: lecture stipends from Amgen, MSD, Ferrer and Alexion. Luís Masana: lecture stipends and advisory fees from Amgen, Sanofi and MSD. Núria Amigo: shareholder of Biosfer Teslab. None of these conflicts of interest are related to the contents of the present manuscript. The other authors have indicated that they have no potential conflicts of interest to disclose.

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Appendix A. DECOPIN GROUP: Investigators who have participated in patient recruitment

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References

1. Wiegman A, Gidding SS, Watts GF, Chapman MJ, Ginsberg HN, Cuchel M, et al. Familial hypercholesterolaemia in children and adolescents: gaining decades of life by optimizing detection and treatment. Eur Heart J. 2015;36:2425–37.
2. Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. Eur Heart J. 2013;34:3478–90.
3. Gidding SS, Champagne MA, de Ferranti SD, Defesche J, Ito MK, Knowles JW, et al. The agenda for familial hypercholesterolemia: a scientific statement from the American Heart Association. Circulation. 2015;132:2167–92.
4. Zamora A, Masana L, Comas-Cufi M, Vila A, Plana N, Garcia-Gil M, et al. Familial hypercholesterolemia in a European Mediterranean population-prevalence and clinical data from 2.5 million primary care patients. J Clin Lipidol. 2017;11:1013–22.
5. Kusters DM, de Beaufort C, Widhalm K, Guardamagna O, Bratina N, Ose L, et al. Paediatric screening for hypercholesterolaemia in Europe. Arch Dis Child. 2012;97:272–6.
6. Groselj U, Kovac J, Sustar U, Mlinaric M, Fras Z, Podkrajsek KT, et al. Universal screening for familial hypercholesterolemia in children: the Slovenian model and literature review. Atherosclerosis. 2018;277:383–91.
7. Ibarretxe D, Rodríguez-Borjabad C, Feliu A, Bilbao JÁ, Masana L, Plana N. Detecting familial hypercholesterolemia earlier in life by actively searching for affected children: The DECOPIN project. Atherosclerosis. 2018;278:210–6.
8. Descamps OS, Tenoutasse S, Stephenne X, Gies I, Beauloye V, Lebrethon MC, et al. Management of familial hypercholesterolemia in children and young adults: consensus paper developed by a panel of lipidologists, cardiologists, paediatricians, nutritionists, gastroenterologists, general practitioners and a patient organization. Atherosclerosis. 2011;218:272–80.
9. Lughetti L, Predieri B, Balli F, Calandra S. Rational approach to the treatment for heterozygous familial hypercholesterolemia in childhood and adolescence: a review. J Endocrinol Invest. 2007;30:700–19.
10. Plana N, Rodríguez-Borjabad C, Ibarretxe D, Masana L. Hipercolesterolemia familiar en la infancia y la adolescencia: una realidad oculta. Clin Investig Arterioscler. 2017;29:129–40.
11. Kerr M, Pears R, Miedzybrodzka Z, Haralambos K, Cather M, Watson M, et al. Cost effectiveness of cascade testing for familial hypercholesterolaemia, based on data from familial hypercholesterolaemia services in the UK. Eur Heart J. 2017;38:1832–9.
12. Pears R, Griffin M, Futema M, Humphries SE. Improving the cost-effectiveness equation of cascade testing for familial hypercholesterolaemia. Curr Opin Lipidol. 2015;26:162–8.
13. Lázaro P, Pérez de Isla L, Watts GF, Alonso R, Norman R, Muñiz O, et al. Cost-effectiveness of a cascade screening program for the early detection of familial hypercholesterolemia. J Clin Lipidol. 2017;11:260–71.
14. Daniels SR, Gidding SS, de Ferranti SD. National Lipid Association Expert Panel on Familial Hypercholesterolemia. Pediatric aspects of familial hypercholesterolemias: recommendations from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. J Clin Lipidol. 2011;5:S30–7.
15. Harada-Shiba M, Arai H, Ishigaki Y, Ishibashi S, Okamura T, Ogura M, et al. Guidelines for diagnosis and treatment of familial hypercholesterolemia 2017. J Atheroscler Thromb. 2018;25:751–70.
16. Plana N, Rodríguez-Borjabad C, Ibarretxe D, Ferré R, Feliu A, Caselles A, et al. Valor de los parámetros lipídicos y apoproteicos para la detección de hipercolesterolemia familiar en la infancia. Proyecto DECOPIN. Clin Investig Arterioscler. 2018;30:170–8.
17. Threapleton DE, Greenwood DC, Evans CE, Cleghorn CL, Nykjaer C, Woodhead C, et al. Dietary fibre intake and risk of cardiovascular disease: systematic review and meta-analysis. BMJ. 2013;347:f6879.
18. Chiavaroli L, Nishi SK, Khan TA, Braunstein CR, Glenn AJ, Mejia SB, et al. Portfolio dietary pattern and cardiovascular disease: a systematic review and meta-analysis of controlled trials. Prog Cardiovasc Dis. 2018;61:43–53.
19. Hernáez Á, Castañer O, Goday A, Ros E, Pintó X, Estruch R, et al. The Mediterranean Diet decreases LDL atherogenicity in high cardiovascular risk individuals: a randomized controlled trial. Mol Nutr Food Res. 2017:61.
20. Negele L, Schneider B, Ristl R, Stulnig TM, Willfort-Ehringer A, Helk O, et al. Effect of a low-fat diet enriched either with rapeseed oil or sunflower oil on plasma lipoproteins in children and adolescents with familial hypercholesterolaemia. Results of a pilot study. Eur J Clin Nutr. 2015;69:337–43.
21. Mateo-Gallego R, Perez-Calahorra S, Cofán M, Baila-Rueda L, Cenarro A, Ros E, et al. Serum lipid responses to weight loss differ between overweight adults with familial hypercholesterolemia and those with familial combined hyperlipidemia. J Nutr. 2014;144:1219–26.
22. Tribble DL, Holl LG, Wood PD, Krauss RM. Variations in oxidative susceptibility among six low-density lipoprotein subfractions of differing density and particle size. Atherosclerosis. 1992;93:189–99.
23. de Graaf J, Hak-Lemmers HL, Hectors MP, Demacker PN, Hendriks JC, Stalenhoef AF. Enhanced susceptibility to in vitro oxidation of the dense low-density lipoprotein subfraction in healthy subjects. Arterioscler Thromb. 1991;11:298–306.
24. Krauss RM. Lipoprotein subfractions and cardiovascular disease risk. Curr Opin Lipidol. 2010;21:305–11.
25. Blake GJ, Otvos JD, Rifai N, Ridker PM. Low-density lipoprotein particle concentration and size as determined by nuclear magnetic resonance spectroscopy as predictors of cardiovascular disease in women. Circulation. 2002;106:1930–7.
26. Christensen JJ, Ulven SM, Retterstøl K, Narverud I, Bogsrud MP, Henriksen T, et al. Comprehensive lipid and metabolite profiling

of children with and without familial hypercholesterolemia: a cross-sectional study. Atherosclerosis. 2017;266:48–57.
27. Rodríguez-Borjabad C, Ibarretxe D, Girona J, Ferré R, Feliu A, Amigó N, et al. Lipoprotein profile assessed by 2D-1H-NMR and subclinical atherosclerosis in children with familial hypercholesterolaemia. Atherosclerosis. 2018;270:117–22.
28. Curvas y Tablas de crecimiento (estudios longitudinal y transversal), Instituto de Investigacion sobre crecimiento y desarrollo, Fundación Faustino Orbegozo Eizaguirre, María Diaz de Haro, 10 bis. 48013. Bilbao.
29. Mallol R, Amigo N, Rodríguez MA, Heras M, Vinaixa M, Plana N, et al. Liposcale: a novel advanced lipoprotein test based on 2D diffusion-ordered 1H NMR spectroscopy. J Lipid Res. 2015;56:737–46.
30. Jeyarajah EJ, Cromwell WC, Otvos JD. Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. Clin Lab Med. 2006;26:847–70.
31. Watson PF, Petrie A. Method agreement analysis: a review of correct methodology. Theriogenology. 2010;73:1167–79.
32. Martin-Moreno JM, Boyle P, Gorgojo L, Maisonneuve P, Fernandez-Rodriguez JC, Salvini S, et al. Development and validation of a food frequency questionnaire in Spain. Int J Epidemiol. 1993;22:512–9.
33. Cicero AFG, Fogacci F, Giovannini M, Bove M, Debellis G, Borghi C. Effect of quantitative and qualitative diet prescription on children behavior after diagnosis of heterozygous familial hypercholesterolemia. Int J Cardiol. 2019;293:193–6.
34. Torvik K, Narverud I, Ottestad I, Svilaas A, Gran JM, Retterstøl K, et al. Dietary counseling is associated with an improved lipid profile in children with familial hypercholesterolemia. Atherosclerosis. 2016;252:21–7.
35. Pérez-Jiménez F, Pascual V, Meco JF, Pérez Martínez P, Delgado Lista J, Domenech M, et al. Document of recommendations of the SEA 2018. Lifestyle in cardiovascular prevention. Clin Investig Arterioscler. 2018;30:280–310.
36. Morenga LT, Montez JM. Health effects of saturated and trans-fatty acid intake in children and adolescents: systematic review and meta-analysis. PLOS ONE. 2017;12:e0186672.
37. Kavey RE, Daniels SR, Lauer RM, Atkins DL, Hayman LL, Taubert K, et al. American Heart Association guidelines for primary prevention of atherosclerotic cardiovascular disease beginning in childhood. Circulation. 2003;107:1562–6.
38. Cholesterol Treatment Trialists' (CTT) CollaborationBaigent C, Blackwell L, Emberson J, Holland LE, Reith C, et al. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet. 2010;376:1670–81.
39. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J. 2017;38:2459–72.
40. Eissa MA, Mihalopoulos NL, Holubkov R, Dai S, Labarthe DR. Changes in fasting lipids during puberty. J Pediatr. 2016;170:199–205.

Effectiveness of an infantile educational program on healthy lifestyle addressed to children with genetic and non-genetic driven hypercholesterolemia. Learning while playing!

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Abbreviations:

ApoA: apolipoprotein A

ApoB: apolipoprotein B

ASCVD: arteriosclerotic cardiovascular diseases

BMI: body mass index

CV: cardiovascular

FH: familial hypercholesterolemia

HDL-C: high-density lipoprotein cholesterol

HRQOL: health-related quality of life

IQR: interquartile range

LC: lifestyle changes

LDL-C: low-density lipoprotein cholesterol

Lp(a): lipoprotein (a)

LDLR: low-density lipoprotein receptor

PA: physical activity

SD: standard deviation

TC: total cholesterol

TLSC: therapeutical lifestyle changes

VLDL: very low-density lipoprotein

Abstract

Background: Metabolic alterations, including hypercholesterolemia are expressed at earlier ages. Detection of multifactorial and familial hypercholesterolemia (FH) during childhood should allow a prompt therapeutic lifestyle change (TLSC) implementation. Dedicated infantile educational programs on healthy habits must be developed.

Aim: Design and appraise the effect of an infantile education method, addressed to implement healthy hablts in FH anf non-FH hypercholesterolemic children.

Methods: Prospective intervention study. We studied 117 children (5 to 12 y/o, 50% girls) with hypercholesterolemia (64 FH and 53 non-FH) . A full "learning while playing" TLSC educational program based on workshops, comics and magazines, and an interactive blog was developed. Forty-eight children (38 with FH) were assigned to follow a six month TLSC intensive program (IP). Its effect on dietary habits physical activity, lipid parameters and quality of life was compared with sixty-nine children (26 with FH) following the standard of care (SoC).

Results: In both groups a change towards healthier lifestyle habits was observed. Children who followed the TLSC-IP had a greater increase in the consumption of fruits and vegetables, legumes and fish servings and reduced more red and processed meats and sweet products.Both groups increased significantly their PA. Lipid parameters were improved by both interventions. The impact on lipids of both interventions was similar. The FH children on TLSC-IP show a trend to higher total, LDL-C and apo B reduction than those on SoC.

Conclusions: A lifestyle education program aimed at younger children with FH and non-FH hypercholesterolemia provides great impact in healthy lifestyles and improves lipid profile even in FH. The implementation of educational programs based on play activities is welcomed by children and families.

Keywords: diet, physical activity, children, familial hypercholesterolemia, lifestyle education program.

INTRODUCTION

Metabolic diseases associated with poor eating and lifestyle habits are observed at an increasingly early age. Hypercholesterolemia (HC) in children is nowadays highly prevalent. Although the interaction between environmental factors and polygenic predisposition is at the basis of high cholesterol levels in most children the highly prevalent monogenic familial hypercholesterolemia (FH) should always be suspected (66,71). FH is an autosomal dominant hereditary disorder of lipid metabolism that causes elevation of low-density lipoprotein cholesterol (LDL-C) (186). One out of 200-250 children are affected (66,71,96). Children affected have high circulating LDL-C levels since birth leading to early arteriosclerotic cardiovascular diseases (ASCVD) (119). Therefore, early diagnosis and treatment is important to extend ASCVD free life expectancy (48). The first line of treatment in both, FH and non-FH hypercholesterolemic children should be therapeutically lifestyle changes (TLSC) (70,119,181). Along with an appropriate information about the disease, children with FH should receive education about healthy diet, physical activity (PA) and avoiding smoking from a healthcare professional with specific expertise (73,186,304) in order to implement a lifelong healthy lifestyle.

The effect of healthy diets on cardiovascular (CV) prevention has been extensively demonstrated (73,195,197–199); however, the impact of lipid lowering diets in FH, where LDL-C levels are determined by a genetic defect, has been discussed (305,306). Appropriated alimentary recommendations can reduce LDL-C levels by 10 to 15% in FH patients (307), depending on adherence and type of genetic defect (279). Apart from its impact on lipid levels some heathy nutrition patterns as the Mediterranean diet has been associated to lower cardiovascular events independently of its effects on lipid concentrations (308). Poor lifestyle habits such as reduced consumption of fruits, vegetables, legumes and whole grain and the increase consumption of processed meat, sugar and salt increases the risk (309,310). In the same way, PA is also inversely related to increased incidence of HC, obesity, diabetes and CV risk (6).

Although, the impact of TLSC on children with HC and specifically on FH has not been systematically studied, seems worthy implementing healthy behaviours in order to decrease ASCVD risk in this population (66).

Childhood is considered a critical period, in which the dietary patterns and lifestyle are learnt and rooted (311). Regarding infantile education, a positive aspect is that children learn quickly incorporating the new knowledge very fast (66,210,211). If an adequate lifestyle education is started before pubertal age, adequate habits will last during the following years (119).

On the other hand, strategies addressed to implement healthy behaviours in children should involve the whole family (312). Results of a meta-analysis revealed that interventions that included family members produced larger effects than interventions focusing only in children (313,314). Despite people with FH are more aware about the importance of a healthy diet and tend to get better results, establishing TLSC is not easy and maintaining them lifetime is an important challenge (168).

A good way to educate children is through play (30,31). When children have fun and play, they are more receptive and acquire new knowledge and skills more quickly (32), while strategies based on prohibition have shown greater stress and poor results. It is important to show that eating a healthy diet can be fun (30). At a very young age, it is difficult to learn concepts through theoretical learning, while "learning by doing" is a great way to instill behavioral skills. Another important aspect is to carry out group activities to promote and enhance the health education process. Group strategies have shown greater benefits because problems are solved among all, more ideas are exposed and there is less anxiety, helping to achieve the main objective that is behavior change, both for the family and for the child. Group support is one of the most valuable forms of support, especially when it comes to motivation (33).

We have designed a play-based healthy habits teaching program for children with HF. In this article we report the implementation of the program, the acceptance by children and parents with and without FH and its effects on lifestyle behavior and lipid profile.

MATERIAL AND METHODS

Study design and patients

This is an open prospective intervention study. A total of 117 children aged 5-12 years attending the Lipid Unit Sant Joan Hospital of Reus were recruited. These children were taking part in the "Early Detection of FH in Children" project (DECOPIN - NCT04370899) which has been previously described (315). In brief, primary care pediatritians were asked to include cholesterol measurement to blood test preformed to children for any cause (oportunistic screening). Those with high LDL-C levels above 135 mg/dl after excluding secondary causes were sent to our specialized lipid clinic. To detect FH, we applied the inverse cascade protocol studying the parents. If one of the parents had definite FH we studied clinicaly and genetically the children.

Children were classified into 2 groups:

-*FH group*: Including 64 children with definite FH diagnosis based on positive genetic testing (81%) or LDL-C >135 mg/dL and one parent with definite FH .

-*Non-FH group*: Including 53 HC children sent to our unit with no complete FH criteria.

During 2016, all 5 to 12 y/o children sent to our Lipid Unit who had HC were proposed to enter an intensive program of lifestyle changes. A total of 48 children (38 FH, and 10 non-FH) accepted to take part in the TLSC-IP. Children with FH and non-FH between 5-12 years old attending the lipid Unit from 2017 were use as standard of care control group (SoC). Sixty-nine children (26 FH and 43 non-FH) were included

Children with psychiatric disorders, mobility problems, or severe chronic diseases were not included. None of them were on lipid lowering drug therapy.

The study was approved by the Ethical Committee of "Institut Investigació Sanitaria Pere Virgili". A parent or tutor of all children signed an informed consent.

Variables

According the DECOPIN protocol we recorded data on anthropometry, diet, PA and standard lipid parameters at the first visit and then yearly. For the study, the baseline data were compared with the first clinical assessment obtained after the TLSC-IP in the intensive intervention group (mean follow-up 1 year). In the SoC group the baseline data were compared with those obtained after the first year follow-up. A quality of life questionnaire (Health-Related Quality of Life) (HRQOL) was performed by the FH children in the TLSC-IP group before and after the intervention.

Nutritional evaluation: it was carried out by semi-quantitative assessment of the consumption of the different food groups through a frequency questionnaire, validated by the Spanish population (316). A semi-quantitative food frequency questionnaire (FFQ) of 137 items was used to characterize the diet. It was validated by personal interview performed by a registered dietitian. The frequencies of consumption were reported on an incremental scale with nine levels (never or almost never, 1–3 times per month, once per week, 2–4 times per week, 5–6 times per week, once per day, 2–3 times per day, 4–6 times per day and more than six times per day). The reported frequencies of food consumption were converted to the number of intakes per day or week according to the recommendations. In addition, nutrients were transformed in food groups (supplementary table 1) to facilitate understanding the global diet patterns instead of macronutrients. The results were reported as rations of food groups to be compared with the international recommendations.

Physical activity: The degree of PA was assessed using the Minnesota leisure time PA Questionnaire validated for the Spanish population (317,318). From this questionnaire, the minutes of weekly exercise performed by the children were determined and was used as the main variable. The percentage of children who performed more than 3h per week of PA was also analysed.

Lipid profile: Standard biochemical analyses were performed in blood samples obtained after overnight fasting. Total Cholesterol (TC) and triglyceride (TG) levels were evaluated using enzymatic colorimetric tests, high-density lipoprotein cholesterol (HDL-C) was evaluated using a direct enzymatic colorimetric method, and

apolipoprotein levels were measured by immunoturbidimetric assays. LDL-C levels were calculated by the Friedewald equation.

Blood tests were performed at the first clinic visit and annually thereafter. For ethical reasons, additional blood tests were not allowed in young children; therefore, to assess lipid changes, we compared the baseline values with those obtained in the first scheduled control after the intervention period in the TLSC-IP group. In the SoC group, the baseline values were compared with those obtained after the first year of follow-up.

Health-related quality of life: We aim to evaluate the impact of an intensive medical intervention on the quality of life of children with a chronic disease (FH). The quality of life of children between 8-12 years was assessed by the KIDSCREEN questionnaire that subjectively assesses the health and welfare of children and adolescents (319). These tests have been developed to be self-administered to children and adolescents (8 to 18 years), both healthy and with chronic health problems. This questionnaire was completed by 24 FH children in the TLSC-IP group at baseline. The last question ""In general, how would you say your health is…" was also performed after the workshops.

Intensive TLSC intervention:

Children aged between 5 and 12 years selected to follow the intensive intervention arm were enrolled accompanied by one their parents. They were distributed in groups of 5 to 8 and 9 to 12 y/o to take part in the workshops. Children and their families followed the intensive intervention for 6 months. The TLSC-IP consisted in 6 educational workshops: *1. Cooking is fun, 2. Catching food, 3. Let's move, 4. Healthy breakfast, 5. The jorney of sense* and *6. A celebration of colors* (workshops explanation in supplementary table 2). They attend 1 per month lasting 1 hour each, whit the presence of children and parents. Moreover , an informative magazine was handedout to participants and dedicated blog was also set-up to reinforce the programme (see below).

Two sets of educational materials and activities were designed by the multidisciplinary team of the Clinical Unit in order to adapt them to the educational level and the two age groups (5-8 and 9-12 y/o) .

The main objective was improving children's diet and optimize the amount of PA according to international standards and recommendations.

Magazine: After each workshop, a fun magazine containing the main concepts of the workshop was handedout to children. The magazine included different games in order to improve awareness and epowerment of the lifestyle and encourage the correct choice of healthy foods (supplementary figure 1) .

Blog: A closed group blog was created to share experiences and photos of the workshops, questions, exchanging recipes or including contests to motivate the participation of families.

Standard of care intervention

Children not selected to take part in the TLSC-IP, follow the SoC of our specialized unit. It consist in a first visit with a dietitian, identical to the TLSC-IP group, where dietary history is recall and information about healthy diet and PA is explained. A follow-up visit to reinforce the recommendations is schedule after one month and annually there after. In the meantime, parents and children could also contact the dietitian at any time through-out the social nets.

Statistical analysis

The descriptive results are expressed as the median (interquartile range (IQR)) for data that were not normally distributed and as frequencies for categorical data. T-tests were used to determine significant differences when the data were normally distributed. Mann-Whitney test were used determine significant differences when the data were not normally distributed. The paired T-test were used in normal variables and Wilcoxon signed-rank test in non-parametric variables to know the difference after 1 year of intervention.

All analyses were performed using the statistical package SPSS 25.0 for Windows (SPSS, IBM®, Chicago, IL). A p-value <0.05 was considered statistically significant in all analyses.

RESULTS

All 48 children taking part in the TLSC-IP followed all the workshops and their activities until the end. The percentage of attendance was 97.6%. Three out of four children also completed the quiz, games and questions included in the reinforcing magazines. The blog had 1706 entries in the intervention period.

In the table 1 we show the baseline demographic and lipid data of participating children sorted by diagnosis and intervention group (SoC or TLSC-IP). There were more FH children in the TLSC-IP group because the recruitment method. Consequently, this group showed a higher LDL-C level. In table 2 we described the baseline diet characteristics presented as the number of rations of each food group separated by intervention group. In the same table we include the current international recommendation for a healthy diet in children. Regardless of group, children were far from guidelines particularly in consumption of meats and sweet products. The figure 1 shows the changes in the intake of rations of the different food groups observed after the intervention sorted by the SoC or TLSC-IP. It can be observed that both interventions strategies moved the comsumption toward a helthier diet, increasing the amount of vegetebles, fruit, legumes and fish and reducing the meat, the processed meat and important, the amount of sweetened drinks and precooked foods. These changes were more pronounced in the TLSC-IP group, achieving statistical significant differences in vegetables (p=0.001), sweet products (p=0.022) and precooked food (p=0.029).

The changes in the amount of PA are shown in figure 2. A significant increase in PA from baseline values to more than >150min/week, was observed in both groups (p<0.0001) with no differences between intervention intensity groups (p=0.334).

Children who performed more than 3h per week of physical activity during their leisure time changed from….% to 70% in both groups at the end of the study.

Changes in the lipid profile are shown in figure 3. Both interventions had a beneficial impact on lipid profile. Significant decrease were observed on total and LDL cholesterol and ApoB, while HDL-C was not changed or minimally reduced in both groups. These effects were magnified by the TLSC-IP. Interesting, in FH children, in which the total and LDL-C concentrations are genetically determined, we observed a significant impact of the intervention, particularly the intensive intervention (TC: p=0.010 and LDL-C: p=0.011).

In parallel, we seek to asses the feeling of well-being of FH children taking part in an intensive intervention program. Interesting they considered themselves as having a good health at baseline and this consideration increase to very good or excellent after the intervention from 67% to 88% (supplementary figure 2). We show the rest of answers in supplementary figure 3.

DISCUSSION

The pandemic of metabolic diseases including obesity, metabolic syndrome, diabetes and dyslipidaemia is expanding worldwide mainly because unhealthy diets and sedentarism. In this study we have evaluated the impact of educational activities addressed to implement healthy lifestyles on children with both genetic driven (FH) and multifactorial HC. Nevertheless, a firmer research base is needed to establish the efficacy and effectiveness of nutrition education and dietary behaviour change games (320). An important aspect of this work is that it focuses in young children in which the information about dietary advice effects is scarce. Another important aspect is that we address the efficacy of such intervention measures to FH children, in which the importance of genetic factors is undubtably very strong. Starting the education on healthy habits during childhood will lead to an early improvement of CV risk in FH patients and non-FH individuals in general. However, working with children is a two edged sword because the messages can be quickly adopted but it is more difficult to deliver them. We have designed a set of tools adapted to younger FH children (from 5 to 12 y/o) addressed to teach about the healthy habits including diet and PA. This

program was based on the concept that children learn while playing, therefore we prepare a group of workshop activities based on kid games. Through these workshops in which we also involved the parents, many of them also affected by FH, we introduce the main concepts of healthy nutrition and physical activity benefits. The inclusion of the family in the intervention is more successful than programs addressed only to children.

We have compared an innovative intervention based on children education with the usual clinical care of FH and non-FH-HC children in an specialized lipid clinic. The first point to be underlined is that the workshop and their supporting activities, as an educational magazine and a dedicate blog, had an enthusiastic reception by both the young children (5 to 12 y/o) and their parents with an almost 100% participation rate.

The baseline diet analysis showed that children were quite far from international guidelines in terms of food groups rations, with an increase of meat and sweet beverages and foods, precooked foods and low amount of vegetables in general. All these aspects were reversed by both interventions although the TLSC-IP had a higher yield. Moreover, the TLSC-IP also increased the empowerment of both parents and children respect to healthy habits. The game was an excellent vehicle for teaching lifestyle changes, as the children had a good time while learning. The children came to the workshops happy and eager to learn. This aspect is very important to maintain adherence.

During intervention the leisure time dedicated to PA also increased significantly. Interesting all these activities were considered amusement and educational activities contributing to the welfare of kids.

Also important, through-out the quality of life questionnaries, children expressed that their health status was very good or excellent despite taking part in a therapeutic activity. Not talking about diseases and basing information on games could contribute to this result.

Adopting a healthy diet itself was a goal of this study. The healthy diet per se is associated to CV protection, even independently of its effect on intermediate factors

as dyslipidaemia. Therefore, following healthy habits is of paramount importance in FH children. However, this lifestyle change was also associated to improvement in lipoprotein levels. All participants reduce total and LDL-C including FH children. Even a trend to better results in FH following the TLSC-IP was observed, with higher reductions in TC, LDL-C and Apo B levels.

Our study has several limitation and also some strengths. The sample size is rather small, however it should be taking into account that is a group of little FH children involved in a long educational intervention. In fact, this one of the larger groups of young FH children involved in a prospective TLSC activity available in the literature. The distribution of groups was not randomized and the results were based on clinicaly scheduled visits. Furthermore, the assessment of the diet, despite being based on validated questionnaires compiled by the same nutritionist, always contains some inaccuracy.

In conclusion, a lifestyle education in FH children must be considered a medical priority. Implementing infantile education programs based on play activities are well received by children and families. This activity has a significant impact on healthy lifestyle modification.

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Declarations of interest

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References

1. Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: Guidance for clinicians to prevent coronary heart disease. Eur Heart J. 2013;34:3478-90a

2. Wiegman A, Gidding SS, Watts GF, Chapman MJ, Ginsberg HN, Cuchel M, et al. Familial hypercholesterolæmia in children and adolescents: Gaining decades of life by optimizing detection and treatment. Eur Heart J. 2015;36:2425–37.

3. Ramaswami U, Humphries SE. Management of familial hypercholesterolaemia in childhood. Curr Opin Pediatr. 2020;32(5):633-640

4. Zamora A, Masana L, Comas-Cufí M, Vila À, Plana N, García-Gil M, et al. Familial hypercholesterolemia in a European Mediterranean population—Prevalence and clinical data from 2.5 million primary care patients. J Clin Lipidol. 2017;11:1013–22.

5. Pederiva C, Capra ME, Viggiano C, Rovelli V, Banderali G, Biasucci G. Early prevention of atherosclerosis: Detection and management of hypercholesterolaemia in children and adolescents.MDPI.2021;11:345.

6. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J. 2020;41:111-188.

7. Harada-Shiba M, Arai H, Ishigaki Y, Ishibashi S, Okamura T, Ogura M, et al. Guidelines for diagnosis and treatment of familial hypercholesterolemia 2017. J Atheroscler Thromb. 2018;25:751–70.

8. Luirink, Ilse K, Hutten, Barbara A, Wiegman A. Optimizing Treatment of Familial Hypercholesterolemia in Children and Adolescents. Curr Cardiol Rep. 2015;17:78.

9. Diamond DM, Alabdulgader AA, de Lorgeril M, Harcombe Z, Kendrick M, Malhotra A, et al. Dietary Recommendations for Familial Hypercholesterolaemia: an Evidence-Free Zone. BMJ Evidence-Based Med.2020; bmjebm-2020-111412.

10. Watts GF, Gidding S, Wierzbicki AS, Toth PP, Alonso R, Brown WV, et al. Integrated guidance on the care of familial hypercholesterolaemia from the International FH Foundation. Int J Cardiol. 2014;171:309–25.

11. Dalen JE, Devries S. Diets to prevent coronary heart disease 1957-2013: What have we learned?. American Journal of Medicine. 2014;11: 364–369.

12. Mente A, De Koning L, Shannon HS, Anand SS. A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. Ann Intern Med. 2014;161:456.

13. McCaulley M. Association of dietary, circulating, and supplement fatty acids with coronary risk. [Internet]. Vol. 161, Annals of internal medicine. American College of Physicians; 2014 [cited 2021 May 28]. p. 456.

14. Casas R, Castro-Barquero S, Estruch R, Sacanella E. Nutrition and cardiovascular health. Int J Mol Sci. 2018;19:3988 .

15. Barkas F, Nomikos T, Liberopoulos E, Panagiotakos D. Diet and Cardiovascular Disease Risk Among Individuals with Familial Hypercholesterolemia: Systematic Review and Meta-Analysis. Nutrients. 2020;12:2436.

16. Roy G, Boucher A, Couture P, Drouin-Chartier J-P. Impact of Diet on Plasma Lipids in Individuals with Heterozygous Familial Hypercholesterolemia: A Systematic Review of Randomized Controlled Nutritional Studies. Nutrients. 2021;13:235.

17. Lambert CT, Sandesara P, Isiadinso I, Gongora MC, Eapen D, Bhatia N, et al. Current treatment of familial hypercholesterolaemia. Eur Cardiol Rev . 2014;9:76–81.

18. Malhotra A, Shafiq N, Arora A, Singh M, Kumar R, Malhotra S. Dietary interventions (plant sterols, stanols, omega-3 fatty acids, soy protein and dietary fibers) for familial hypercholesterolaemia. Cochrane Database Syst Rev. 2014;2014:CD001918.

19. Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J, Ruiz-Gutiérrez V, Covas MI, et al. Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. Ann Intern Med. 2006;145:1–11.

20. Sotos-Prieto M, Bhupathiraju SN, Mattei J, Fung TT, Li Y, Pan A, et al. Association of Changes in Diet Quality with Total and Cause-Specific Mortality. N Engl J Med.2017;377:143-153.

21. Micha R, Peñalvo JL, Cudhea F, Imamura F, Rehm CD, Mozaffarian D. Association between dietary factors and mortality from heart disease, stroke, and type 2 diabetes in the United States. JAMA. 2017;317(9):912-924.

22. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, et al. Global Burden of Cardiovascular Diseases and Risk Factors, 1990–2019: Update From the GBD 2019 Study. J Am Coll Cardiol. 2020;76:2982-3021.

23. Capra ME, Pederiva C, Viggiano C, De Santis R, Banderali G, Biasucci G. Nutritional Approach to Prevention and Treatment of Cardiovascular Disease in Childhood. Nutrients . 2021;13:2359.

24. Molven I, Retterstøl K, Andersen LF, Veierød MB, Narverud I, Ose L, et al. Children and young adults with familial hypercholesterolaemia (FH) have healthier food choices particularly with respect to dietary fat sources compared with non-FH children. J Nutr Sci. 2013; 2: e32.

25. Burrows R, Correa-Burrows P, Reyes M, Blanco E, Albala C, Gahagan S. High cardiometabolic risk in healthy Chilean adolescents: associations with anthropometric, biological and lifestyle factors. Public Health Nutr.2016;19:486-493.

26. Kinnear FJ, Hamilton-Shield JP, Stensel DJ, Bayly G, Searle A, Thackray AE, et al. Nutrition and physical activity intervention for families with familial hypercholesterolaemia: protocol for a pilot randomised controlled feasibility study. Pilot Feasibility Stud. 2020;6:42.

27. Black MM, Walker SP, Fernald LCH, Andersen CT, DiGirolamo AM, Lu C et al. Early childhood development coming of age: science through the life course. Lancet. 2017;389:77–90.

28. Jeong J, Franchett EE, Ramos de Oliveira C V, Rehmani K, Yousafzai AK. Parenting interventions to promote early child development in the first three years of life: A global systematic review and meta-analysis. PLOS Med.2021;18:e1003602.

29. Daniels SR, Gidding SS, de Ferranti SD. Pediatric aspects of familial hypercholesterolemias: recommendations from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. J Clin Lipidol.2011;5:S30-7.

30. Espinosa-Curiel IE, Pozas-Bogarin EE, Lozano-Salas JL, Martínez-Miranda J, Delgado-Pérez EE, Estrada-Zamarron LS. Nutritional Education and Promotion of Healthy Eating Behaviors Among Mexican Children Through Video Games: Design and Pilot Test of FoodRateMaster. JMIR Serious Games.2020;8:e16431.

31. Minossi V, Pellanda LC. The “Happy Heart” educational program for changes in health habits in children and their families: protocol for a randomized clinical trial. BMC Pediatr. 2015;15:19.

32. Mellecker R, Lyons EJ BT. Mellecker R, Lyons EJ, Disentangling fun and enjoyment in exergames using a expanded design, play, experience framework: A narrative review. Games Heal J. 2013;2:142–149.

33. Borek AJ, Abraham C, Greaves CJ, Gillison F, Tarrant M, Morgan-Trimmer S, et al. Identifying change processes in group-based health behaviour-change interventions: development of the mechanisms of action in group-based interventions (MAGI) framework. Health Psychol Rev. 2019;13:1-21.

34. Ibarretxe D, Rodríguez-Borjabad C, Feliu A, Bilbao J, Masana L, Plana N. Detecting familial hypercholesterolemia earlier in life by actively searching for affected children:The DECOPIN project. therosclerosis. 2018;278:210-216.

35. Vázquez C, Alonso R, Garriga M, de Cos A, de la Cruz JJ, Fuentes-Jiménez F, et al. Validation of a food frequency questionnaire in Spanish patients with familial hypercholesterolaemia. Nutr Metab Cardiovasc Dis. 2012;22:836–42.

36. Elosua R, Marrugat J, Molina L, Pons S, Pujol E. Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish men. The MARATHOM Investigators. Am J Epidemiol. 1994;139:1197–209.

37. Elosua R, Garcia M, Aguilar A, Molina L, Covas MI, Marrugat J. Validation of the Minnesota Leisure Time Physical Activity Questionnaire In Spanish Women. Investigators of the MARATDON Group. Med Sci Sports Exerc. 2000;32:1431–7.

38. Ravens-Sieberer U, Gosch A, Rajmil L, Erhart M, Bruil J, Duer W, ET AL. KIDSCREEN-52 quality-of-life measure for children and adolescents. Expert Rev Pharmacoecon Outcomes Res. 2005;5:353–64.

39. BaranowskiTom, RyanCourtney, Hoyos-CespedesAndrés, Shirong L. Nutrition Education and Dietary Behavior Change Games: A Scoping Review. Games Health J. 2019;8:153-176

Table 1: Description of baseline characteristics of the hypercholesterolemic children included in the study.

	SoC			TLSC-IP		
	All (N=69)	FH (N=26)	non-FH (N=43)	All (N=48)	FH (N=38)	non-FH (N=10)
Age (y)	9(7-11)	8,5(7-11)	9(7-11)	7(6-9)	7(6-8)	8(7-9)
Gender (% girls)	42,03	38,46	44,19	53,19	51,35	60
Z-score-BMI	-0,13(-0,56-0,61)	0,18(-0,275-1,06)	-0,26(-0,67-0,36)	0,00(-0,57-1,01)	0,19(-0,57-1,2)	-0,07(-0,2-0,95)
TC (mg/dL)	218(205-241)	241(219-305)	210(192-228)	262(221-302)	269(230-308)	192(191-193)
HDL-C (mg/dL)	64(54-72)	63,5(50-75)	65(56-71)	59,(52-68)	59(55-67)	54,5(51-58)
LDL-C (mg/dL)	138(125-161)	164(138-219)	128(120-144)	183(146-225)	197(160-235)	125(122-128)
TG (mg/dL)	61(48-81)	60,5(48-81)	61(48-81)	65(50-87)	65(50-87)	63,50(56-71)
ApoA (mg/dL)	152,50(137-168)	141(130,5-156)	153(144-171)	146(135-161)	147(135-161)	139,50(135-144)
ApoB (mg/dL)	107(96-122)	121,5(103,5-147,5)	103,5(94-116)	136(113-159)	139(116-169)	102,50(96-109)

Data are presented as the median (25th-75th percentile).

SoC, standard of care; TLSC-IP, therapeutical lifestyle changes-intensive protocol; FH, familial hypercholesterolemia; BMI, body mass index; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoB, apolipoprotein B100; ApoA, apolipoprotein A1.

Table 2: Description of baseline diet expressed as food rations of two groups compared to international recommendations.

		SoC	TLSC
	International recommendations	Rations	Rations
Cereal	4-6 times/week	2,78(1,86-3,71)	3,36(2,56-4,29)
Vegetables	>2 times/week	2,35(1,55-3,7)	1,91(1,14-2,78)
Fruit	≥3 times/week	2,41(1,56-3,77)	2,19(1,33-3,42)
Nuts	3-7 times/week	1(0,56-6)	1,28(0,56-3,28)
Legumes	2-4 times/week	2,28(1,28-3)	2,28(1,28-3,28)
Meat	3-4 times/week	6,56(5,28-7,56)	6,34(4,84-8,78)
Red meat	<2 times/week	2,28(1,28-3,56)	2,28(1,28-3,56)
Red processed meat	≤1 time/week	4(2,28-6,56)	4,84(3-7,06)
Fish	3-4 times/week	2(1-3)	2(2-3)
Eggs	3-4 times/week	3(3-3)	3(1-3)
Total dairy	2-3 times/day	2,43(1,71-3,5)	3(2,28-3,93)
Fat dairy products	Occasional weekly consumption	0,56(0,28-1,28)	1,28(0,56-3)
Dressing	Occasional weekly consumption	1(0-3)	1,28(0-3)
Sweet products	Occasional weekly consumption	7,4(4,84-9,62)	10,34(7,28-14,28)
Precooked food	Occasional weekly consumption	1(0,56-2)	1(0,56-2,56)
Beverages with sugar	Occasional weekly consumption	1(0,28-4)	3(0,28-5,78)
Artificial-sweeteners	Occasional weekly consumption	0(0-0)	0(0-0)

SoC, standard of care; TLSC-IP, therapeutical lifestyle changes-intensive protocol

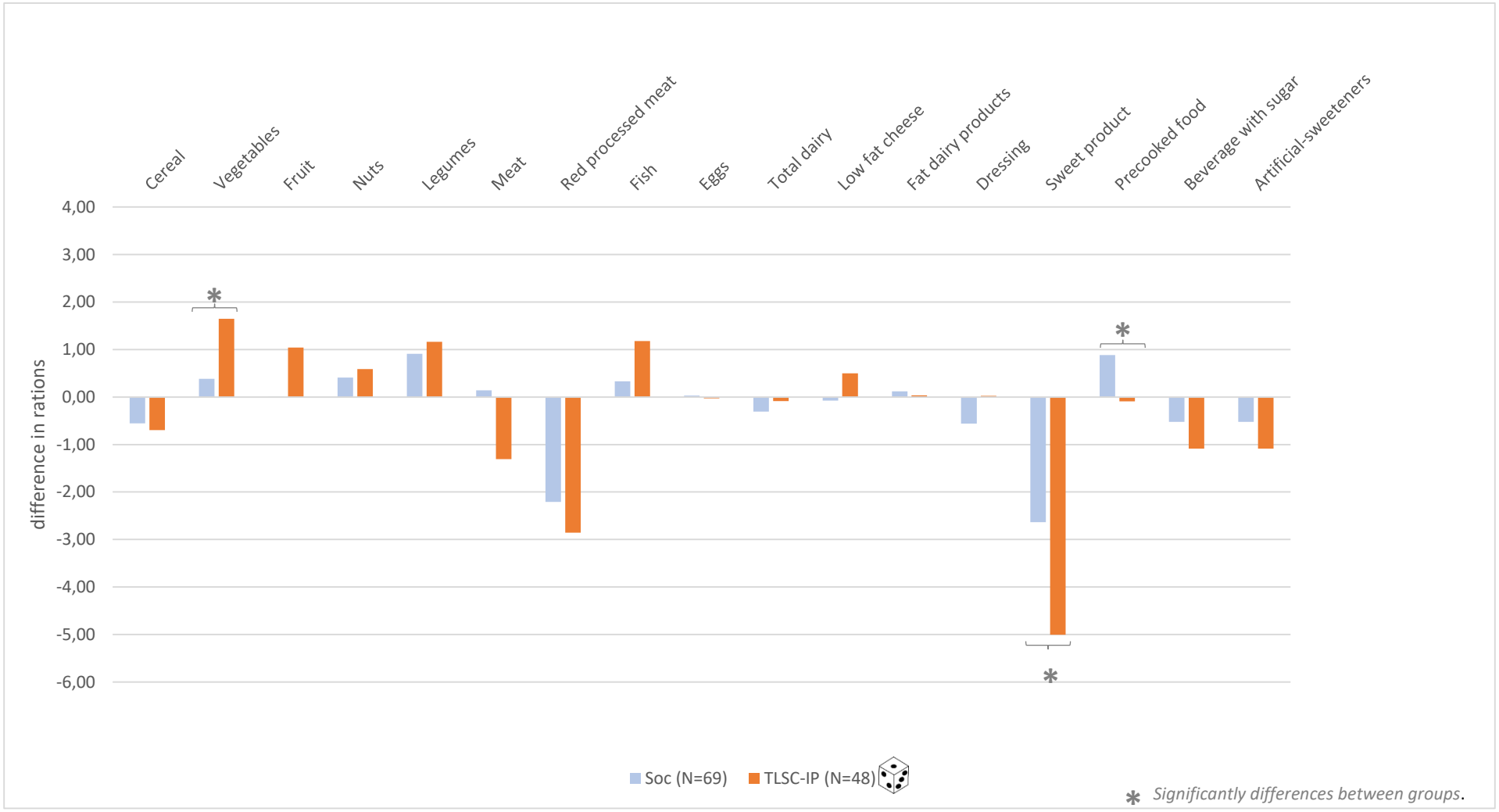


Figure 1. Dietary changes expressed as food rations after the intervention in two groups.

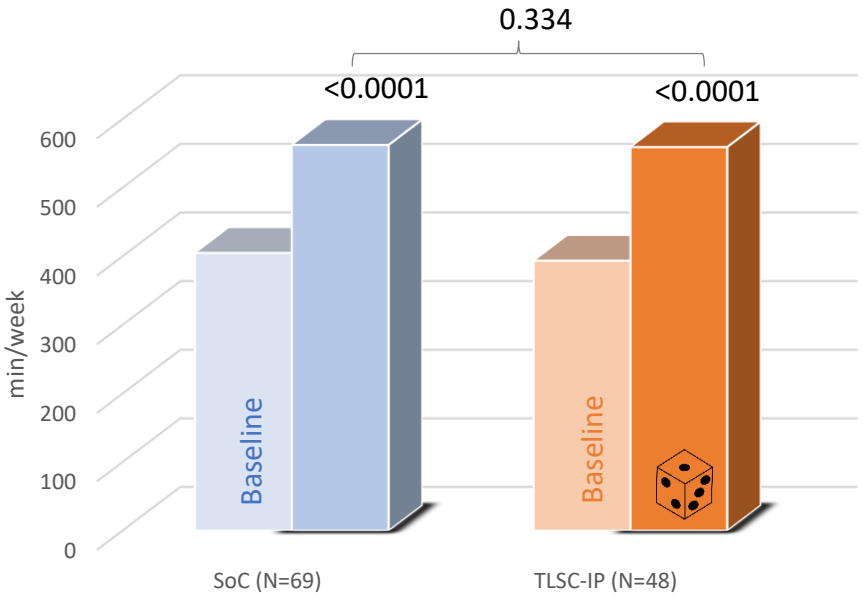


Figure 2: Physical activity (min/week) during leisure time at baseline and after intervention in the two groups.

SoC, standard of care; TLSC-IP, therapeutical lifestyle changes-intensive protocol

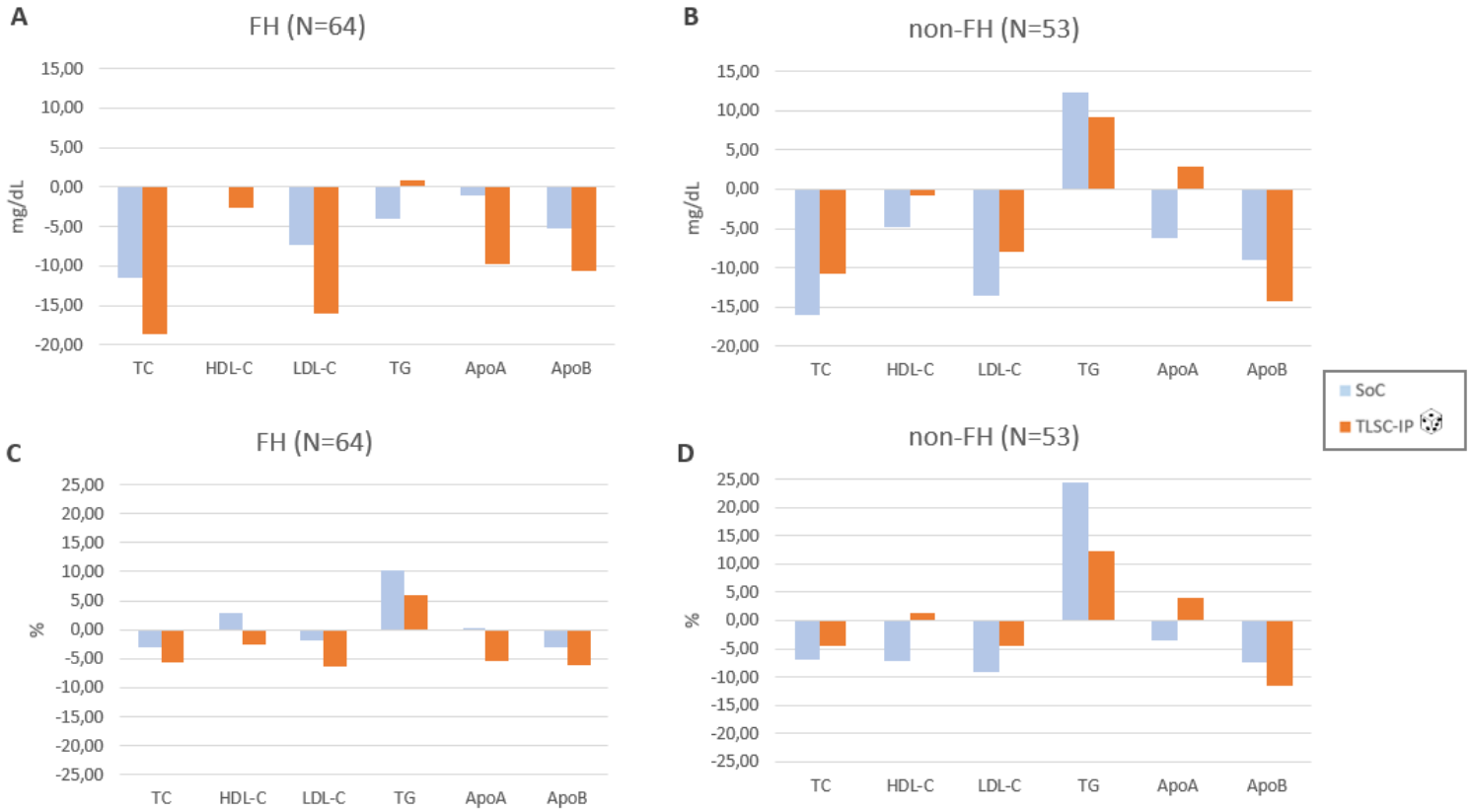


Figure 3: Changes of lipid parameters, in absolute values (A,B) and percentage (C,D) after the intervention in the two groups (SoC and TLSC-IP) divided by diagnosis.

SoC, standard of care; TLSC-IP, therapeutical lifestyle changes-intensive protocol; FH, familial hypercholesterolemia; BMI, body mass index; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoB, apolipoprotein B100; ApoA, apolipoprotein A1.

Supplementary table 1. Description about foods included in each group.

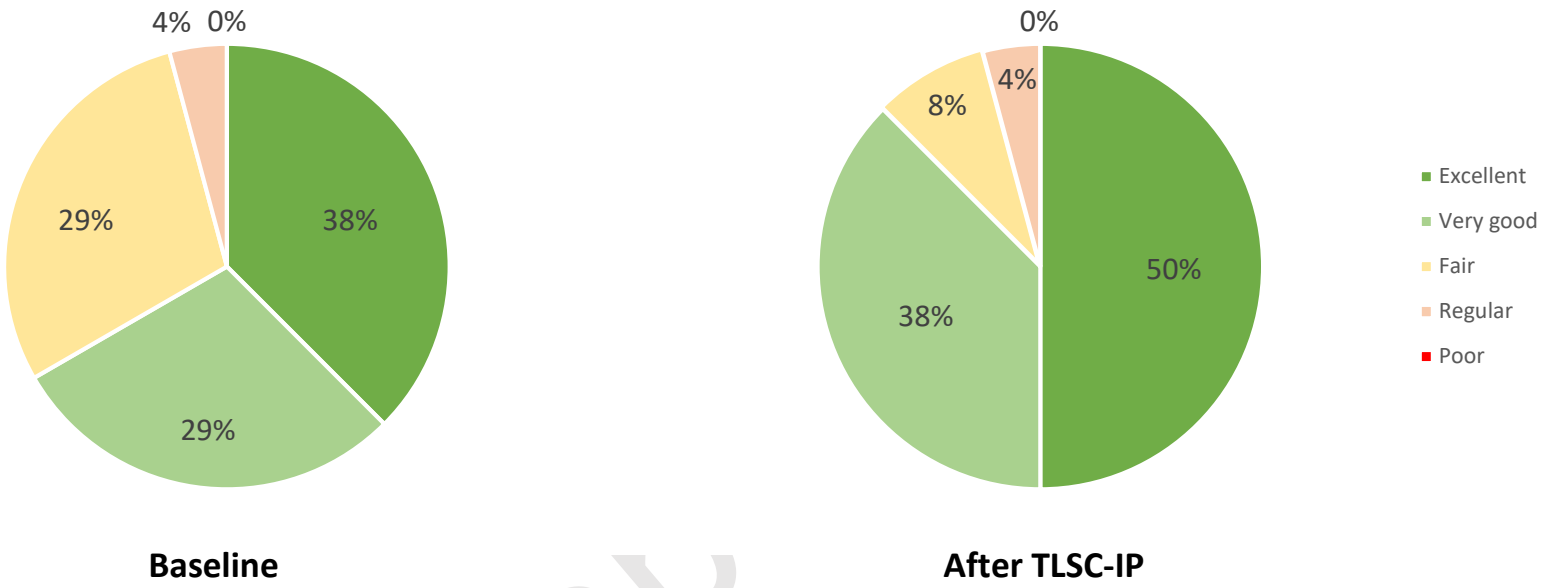
	The group include the following foods:
Cereal	Rice, pasta, breakfast cereal and all kind of bread.
Vegetables	Chard, spinach, cabbage, cauliflower, broccoli, lettuce, endives, escarole, tomato, carrot, pumpkin, green beans, eggplant, zucchini, cucumber, pepper, paprika, asparagus, artichoke, leek, celery, onion, garlic and mushrooms.
Fruits	Orange, tangerine, grapefruit, banana, apple, pear, strawberry, peach, figs, apricot, nectarine, melon, watermelon, grapes, kiwi and berries.
Nuts	Almonds, walnuts, pistachios, hazelnut, pinions, cashews and raisins.
Legumes	Chickpeas, lentils, beans and peas.
Meat	Chicken, turkey, rabbit, pork, veal, cow, beef, sheep, lamb and goat.
Processed red meat	*Salami, serrano ham, liver pate, bacon, hamburger.*
Fish	All the types of fish: fresh fish, smoked fish, canned fish and seafood.
Eggs	Eggs and omelettes.
Total dairy	Milk, yoghurt and cheese.
Low-fat cheese	*Cheeses with less than 30% fat.*
Fat dairy products	Cream, sour cream, ice cream and dairy desserts.
Dressings	Ketchup, mayonnaise and mustard.
Sweet products	Cakes, pastries and cookies.
Precooked food and fast food	Pizza, casserole dishes, spring rolls, etc
Beverages with sugar	Juice and soft drinks with sugar
Artificial-sweeteners	Soft drink with artificial sweeteners.

Supplementary table 2. Information about workshops in the TLSC-IP group.

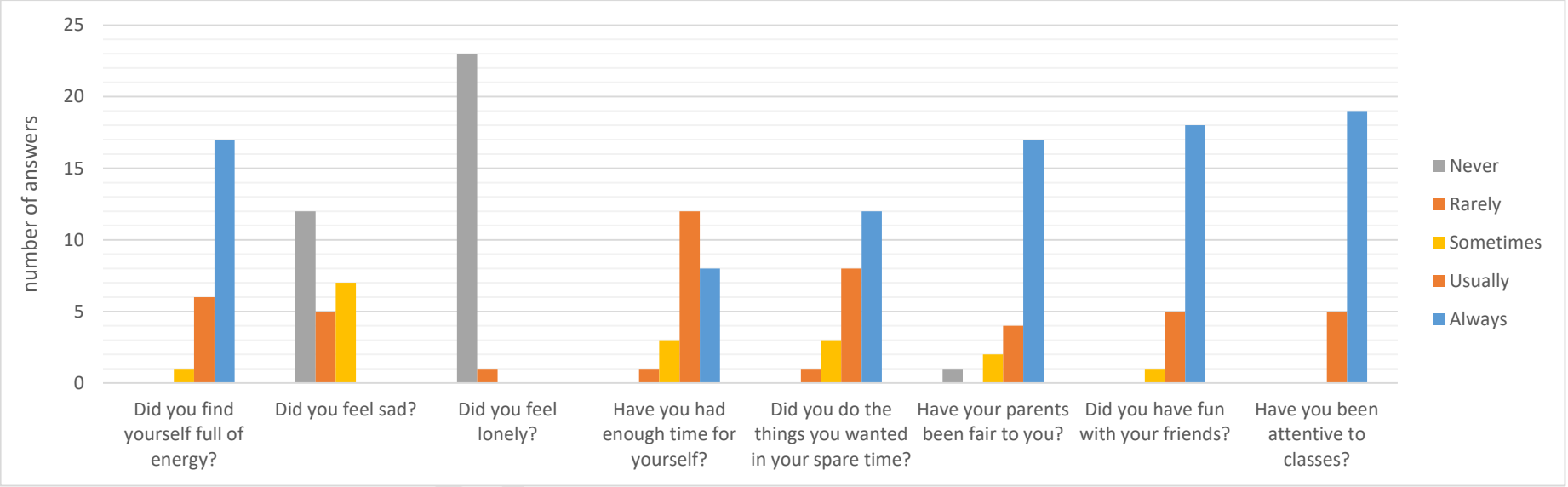
	Name of activity	Description
1	**Cooking is fun**	Practical workshop on healthy habits when cooking. The workshop explained the importance of using healthy cooking techniques (baking, steaming, *papillote*, boiling, etc.), of mixing different food groups and colours and of making more appetizing dishes with foods that you don't like very much. A chef with experience in leading cooking workshops led the event and demonstrated how to prepare a healthy dish (healthy crepe with vegetable spaghetti, almonds and fresh low-fat cheese). There was an emphasis on hygiene (the importance of washing food and washing hands well). While the children cooked, parents were taught to fill in a 3-day diary.
2	**Catching food**	The children played traditional board games adapted to deal with concepts regarding lifestyle changes. Those over 8 years played "Trivial Pursuit" and those under 8 years played "The Game of the Goose." -In the version of Trivial Pursuit, the six different colours represented different food groups: carbohydrates, fruits, vegetables, dairy products, proteins and extras. When the children landed on a square, they had to take a card of that colour and complete a random test (do mime, make something with plasticine, draw, answer a riddle or a true/false question). All of the answers were a food from one of the different food groups. -In the Game of the Goose the children learnt that could move forward round the board if they landed on fruits, fish, legumes and vegetables, but if they landed on foods such as sugary drinks or jelly beans, they had to move back. The squares usually occupied by the Goose had two children playing sports, and each time they landed on one of these squares, the children could throw again. In this way, the children made a positive association between playing sports and progressing in the game (and therefore in life).

3	**Let's move on**	Using traditional games, parents and children increased their PA. In addition to moving, some of the games reinforced a good body posture
4	**Healthy breakfast**	Through playful games, they learned to make a healthy breakfast using the main food groups (dairy, cereals and fruit). We also taught them about phytosterols and about the importance of having breakfast at home and at school.
5	**The journey of the senses**	The children were blindfolded and experienced different foods with their other 4 senses (taste, touch, hearing and smell). The aim of the workshop to encourage children to try new things
6	**A celebration of colours**	A cooking workshop and crafts involving fruits were held. The children had to create succulent dishes from fruit to encourage people to increase their intake. In this activity, we showed that celebrations do not always have to feature cakes and that guests can be offered fruit instead.

Supplementary figure 1: Collage of workshops and drawings used in magazines.



Supplementary figure 2: Percentage of responses from *"In general, how would you say your health is…"* question of Health-related quality of life questionnaire (HRQOL) about 24 FH children who participate in TLSC-IP at basaline and after the intervention.



Supplementary figure 3. Response of the 24 FH children in the TLSC-IP about HRQOL questions at basal time of the workshops.

3.2. To describe the impact of two different diets (Nordic and Mediterranean) on the lipid profile of children with FH

ARTICLE 8

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Dietary intake and lipid levels in Norwegian and Spanish children with familial hypercholesterolemia

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Abstract *Background and aims:* Both the Nordic and Mediterranean diets claim to have a beneficial effect on lipid metabolism and cardiovascular prevention. The objective of this study was to compare diets consumed by children with FH at the time of diagnosis in Norway and Spain and to study their relationship with the lipid profile.
Methods and results: In this cross-sectional study, we appraised the dietary intake in children (4−18 years old) with (n = 114) and without FH (n = 145) from Norway and Spain. We compared Nordic and Mediterranean diet composition differences and determined the association between food groups and lipid profiles.
Results: The Spanish FH group had a higher intake of total fats (mainly monounsaturated fatty acids (MUFAs)), cholesterol and fibre, but a lower intake of polyunsaturated fatty acids (PUFAs) compared to the Norwegian FH group. The Norwegian children consumed more rapeseed oil, low-fat margarine and whole grains and less olive oil, eggs, fatty fish, meat, legumes and nuts. In the Norwegian FH group, fat and MUFAs were directly correlated with total cholesterol, low-density lipoprotein cholesterol and apolipoprotein B and inversely correlated with high-density lipoprotein (HDL-C). In Spanish children with FH, the intake of fats (mainly MUFAs) was directly associated with HDL-C and apolipoprotein A1.
Conclusions: Despite a similar lipid phenotype, diets consumed by children with FH in Norway and Spain have significant differences at time of diagnosis. Nutrition advice should be more adapted to local intake patterns than on specific nutrient composition.

Abbreviations: ApoA1, apolipoprotein A1; ApoB, apolipoprotein B100; BMI, body mass index; CVD, cardiovascular disease; FH, familial hypercholesterolaemia; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SD, standard deviation; SFA, saturated fatty acid; TC, Total cholesterol; VLDL, very-low-density lipoprotein; Z-score, standard deviation scores.

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Introduction

Familial hypercholesterolaemia (FH) is an autosomal-dominant disorder of lipid metabolism characterized by elevated levels of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) [1,2]. The elevation of LDL-C is observed from the first years of life and may result in premature cardiovascular disease (CVD) [2–4]. The prevalence is estimated to be 1/200–500 [4–7], affecting approximately 1.5–3.7 million people (20–25% children) in Europe [2].

According to European recommendations, the LDL-C goal in children with FH should be below 3.5 mmol/L [2]. To improve the prognosis later in life, early diagnosis and treatment are important. The first line of therapy in children with FH is lifestyle changes, which are recommended to be combined with lipid-lowering therapy from 8 to 10 years of age [4]. Dietary components influence lipid metabolism, and healthy changes have been shown to reduce TC levels by 15–30% in the general population, but the association of food patterns to FH clinical expression in children is unknown [8–12]. It is important to know how intake of different macronutrients influence the lipid profile, and which specific food groups have the highest impact on the lipid profile. The new European guidelines on lipid management recommend healthy dietary patterns supported by scientific evidence rather than specific macronutrient composition [13–16], therefore, improving our knowledge on food-based and dietary patterns effects is justified. In summary, many studies have shown associations between macronutrients and the lipid profile in healthy populations; however, few studies have investigated how food groups are associated with the lipid profile in children with FH [16,17].

Nordic [18,19] and Mediterranean diets [20,21] have been suggested to improve cardiovascular health. In the PREDIMED study, the Mediterranean diet was associated with a 30% CVD relative risk reduction [22]. This diet is characterized by a high intake of vegetables and fruits, legumes, olive oil, cereals and nuts, a moderate intake of fish and lean dairy products and a low intake of red meat, and olive oil as a major fat source [23,24]. However, the beneficial effect has not been shown outside of the Mediterranean zone, potentially due to factors such as food availability, food compositions and culinary traditions [25,26]. On the other hand, the Nordic diet has emerged as an alternative beneficial diet in Northern Europe. The Nordic diet is characterized by a high intake of apples, pears, berries, root and cruciferous vegetables, whole grain and rye bread and cereals, fish (particularly fatty fish), low-fat dairy products, rapeseed oil, potatoes and vegetable fats, among others [27,28]. The Nordic diet has been shown to have an effect on cardiovascular health risk markers [29–31].

The aim of the present study was to characterize the diets consumed by children with FH at the time of diagnosis compared to children without FH in Norway and Spain and to evaluate the relationship between a Nordic or Mediterranean diet and the lipid profiles in these children.

Methods

Study design and participants

This was a cross-sectional study. Children with and without FH from Spain and Norway, were included. We obtained information about the dietary intakes and lipid profiles of these children and compared the differences between the Spanish and Norwegian children.

The study participants have been described previously [8,32,33]. Briefly, 114 children with FH and 145 children without FH, aged 4–18 years, were recruited from the lipid clinics at Oslo University Hospital (Oslo, Norway) and the Saint Joan University Hospital (Reus, Spain). Norwegian children with FH were recruited between September and December 2013 with positive genetic FH mutations and untreated (n = 29) [8]. Healthy children included in the Stork-child study (2014–2015) with full diet composition data available were included as controls (n = 23) [32]. The group of Spanish children with FH was recruited from The DECOPIN Project (The Early Familial Hypercholesterolemia Detection Project [33]) from March 2013 to May 2019 (n = 85), including children with a positive genetic test (81%) or LDL-C >4,14 mmol/L and one parent with a DLCN score >8 if unavailable genetic study. The children evaluated for suspected FH who did not meet the FH criteria were included in the non-FH control group (n = 122). The exclusion criteria included the presence of secondary hyperlipidaemia, severe chronic disease and other conditions that could alter nutrition or lipid metabolism.

None of the participants had received lipid-lowering medication before the study visit. Before study participation, one of the parents (or the child, if above 16 years old) signed the informed consent form. The study was approved by the Regional Committee for Medical and Health Research Ethics (East region of Norway) and by the Ethics Committee of the Hospital Research Institute in Reus (Spain). The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

The family history, anthropometry data and all relevant clinical information, including data on clinical and genetic FH diagnoses, were obtained from the clinical records. Z-score is the deviation of an individual's value from the median value of a reference population, divided by the standard deviation of the reference population (or transformed to normal distribution) [34,35].

Routine laboratory assays

The Norwegian group

Non-fasting blood samples were obtained, and standard blood biochemistry and lipid parameters were measured using routine laboratory methods. LDL-C was measured by the direct method.

The Spanish group

Standard biochemical analyses were performed in blood samples obtained after overnight fasting. TC and triglyceride levels were evaluated using enzymatic colorimetric

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tests, high-density lipoprotein cholesterol (HDL-C) was evaluated using a direct enzymatic colorimetric method, and apolipoprotein levels were measured by immunoturbidimetric assays. LDL-C levels were calculated by the Friedewald equation: LDL-C= TC-(HDL-C + [triglycerides/5]).

Dietary intake evaluation

The diet data were collected from children and families by registered nutritionists in both cohorts during the first medical visit, therefore these data represent the baseline nutrition habits in these FH populations before intervention.

Norwegian group

The diet data were collected from 4 pre-coded food diaries. This diary was developed and validated by the Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, and has previously been validated in Norwegian children (9–13 years of age) [36–39]. The PFDs contain a list of 277 food items, drinks and supplements. Household units and a validated photographic booklet were used for the estimation of portion sizes. The participant was asked to report all consumed foods and drinks for four days, including one Saturday or Sunday. The daily intake of energy and nutrients was computed using the AE-14 food database and the "kost beregnings system (KBS)" software (version 7.1, year 2014).

Spanish group

The diet data were collected with a quantitative food frequency questionnaire that includes 137 food items [40] (plus alcohol) and has been validated in the PREDIMED study [22]. The participants were asked to report all foods and drinks consumed over a year. The frequencies of consumption were reported on an incremental scale with nine levels (never or almost never, 1–3 times per month, once per week, 2–4 times per week, 5–6 times per week, once per day, 2–3 times per day, 4–6 times per day and more than six times per day). The reported frequencies of food consumption were converted to the number of intakes per day and multiplied by the weight of the portion size indicated.

Food groups assessment

To study in-depth the associations between different foods and lipid profiles, we defined 55 food groups, 20 main categories and 35 subcategories, according to standard definitions (Ex: milk, vegetables, fruits, meats, fish, etc.). These food groups were defined from the 277 and 137 food items contained in the food frequency questionnaires from Norway and Spain respectively, and that were applied to the study population. This grouping into similar food categories allowed us a comparative analysis of intake between both countries. All of this information is shown in supplementary table 1. This information was available for all children with FH and also for the Spanish children without FH.

Statistical analysis

Continuous data are shown as the mean ± standard deviation (SD) for normally distributed data, as the median (25th-75th percentile) for skewed data, or as frequencies (percent) for categorical data. Kolmogorov–Smirnov tests were used to ensure check for normality. T-tests were used to determine significant differences if the data were normally distributed and Mann–Whitney tests were used to detect significant differences if the data were not normally distributed. Chi-square or Fisher's exact tests were used for categorical variables. All p-values were adjusted for age with ANCOVA tests. Correlations were performed using Pearson's test for normally distributed data and Spearman's test for data that were not normally distributed. A partial correlation was done to adjust for sex and age. We performed a stepwise linear regression with either total cholesterol or LDL-cholesterol as dependent variable and cohort (Norway vs Spain), gender, age, z-score, with the most important diet covariates (percentage of calories provided by macronutrients according Table 2). We have divided the sample into two groups depending on FH status and run the models separately.

All analyses were performed using the SPSS 25.0 statistical package for Windows (SPSS, IBM®, Chicago, IL) and the R statistical programme (R Core Team, 2014). A p-value <0.05 was considered statistically significant in all analyses.

Results

Characteristics of the study sample

The characteristics of the study sample are shown in Table 1. Spanish children with FH were younger (median 9.0 (6.0–12.0) years) than both Spanish children without FH (median 11.0 (8.0–13.0) years; $P = 0.006$) and Norwegian children with FH (median 11.0 (8.0–13.0) years; $P = 0.02$). No differences in the sex distribution or z-score were observed between any of the groups. As expected, both TC and LDL-C were higher in children with FH than in the respective control groups. The mean TC and LDL-C were 5.8 mmol/L and 4.1 mmol/L and 6.8 and 4.8 mmol/L in the FH Norwegian and Spanish cohorts, respectively. Differences in TC and Apolipoprotein B (Apo B) were found between the Norwegian and Spanish children with FH; however, these differences disappeared after adjustment for age. Spanish children without FH had higher TC, LDL-C and HDL-C levels than Norwegian children without FH.

Macronutrient diet composition

The average daily energy percent (E%) delivered by macronutrient intake and the amount of cholesterol and fibre in the FH and non-FH groups is shown in Table 2. The percentage of energy derived from polyunsaturated fatty acids (PUFAs) was higher in the Norwegian FH group than in the Norwegian control children and was the only difference between these groups. There were no differences

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Table 1 Demography and standard lipid profile in Norwegian and Spanish cohorts of children with and without Familial Hypercholesterolemia.

	NORWEGIAN COHORT (n = 52)			SPANISH COHORT (n = 207)			FH Norwegian vs Spanish cohort (n = 114)	Non FH Norwegian vs Spanish cohort (n = 145)
	FH (n = 29)	Non FH (n = 23)	*P*	FH (n = 85)	Non FH (n = 122)	*P*		
Demographic characteristics								
Age (y)	11.0 (8.0–13.0)	10.5 (7.8–12.2)	0.44	9.0 (6.0–12.0)	11.0 (8.0–13.0)	**0.01**	**0.02**	0.68
Gender (% girls)	55.2	52.2	0.52	47.1	46.7	0.54	0.29	0.40
Z-score	0.32 (−0.24–0.91)	0,17 (−0.51–0.53)	0.59	0.27 (−0.42–0.83)	−0.06 (−0.65–0.67)	0.13	0.55	0.41
Biochemical measurements								
TC (mmol/L)	5.8 (5.2–7.1)	3.8 (3.4–4.5)	<**0.001**[a]	6.8 (5.9–7.8)	4.9 (4.3–5.3)	<**0.001**[a]	**0.03**	<**0.001**[a]
LDL-C (mmol/L)	4.1 (3.4–5.2)	2.0 (1.6–2.5)	<**0.001**[a]	4.8 (3.9–5.7)	2.7 (2.3–3.1)	<**0.001**[a]	0.06	<**0.001**[a]
HDL-C (mmol/L)	1.4 (1.3–1.7)	1.5 (1.3–1.8)	0.46	1.5 (1.3–1.7)	1.7 (1.4–1.9)	**0.01**[a]	0.27	**0.02**[a]
Triglycerides (mmol/L)	0.8 (0.6–1.1)	0.7 (0.5–1.0)	0.22	0.7 (0.6–0.9)	0.67 (0.5–0.4)	0.17	0.21	0.72
ApoB100 (g/L)	1.2 (0.9–1.4)	–	–	1.3 (1.1–1.6)	0.9 (0.7–0.9)	<**0.001**[a]	**0.02**	–
ApoA1 (g/L)	1.4 (1.3–1.6)	–	–	1.5 (1.3–1.6)	1.55 (1.4–1.7)	**0.01**[a]	0.81	–
Lp(a) (mg/L)	293 (154–581)	–	–	158 (66.7–660)	129 (45.8–25.0)	0.14	0.14	–

Data are presented as the median (25th–75th percentile) for non-normally distributed data and as percentage for categorical variables. Significant *P* values are in bold. Mann–Whitney U test was used for non-normally distributed variables, Chi square for categorical data and ANCOVA for p values adjusted by age.
FH, familial hypercholesterolemia; Z-score: standard deviation scores; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; ApoB, apolipoprotein B100; ApoA, apolipoprotein A; Lp(a), lipoprotein (a).
- Data not available.
[a] *P* remains significant after adjusting by age.

in macronutrient intake between the Spanish children with and without FH. The Spanish FH group had a higher intake of total fat, monounsaturated fatty acids, cholesterol and fibre, but a lower intake of PUFAs compared to the Norwegian FH group.

Food group consumption

The intake of different food groups was studied in the two FH groups and the Spanish control group (Table 3). Norwegian children with FH consumed more cereals, whole grain bread, jam, processed meat, cheese, butter, margarine, rapeseed oil, precooked food, natural juices, beverages with sugar and beverages with artificial sweeteners, while the Spanish children with FH consumed more potatoes, fried potatoes, nuts, legumes, meat, fatty fish, eggs, yogurt, olives, olive oil and snacks.

Correlations between diet components and lipid profiles

The correlations to the lipid profiles of children with FH and the E% supplied by macronutrients, sorted by country, are shown in Table 4.

In general, all correlations were weak. In the Norwegian cohort, we found that the E% from fats, MUFAs, PUFAs and SFAs were positively associated with TC, LDL-C and ApoB. The intake of cholesterol was associated with lower HDL-C. In the Spanish cohort, the E% from fats and MUFAs were positively associated with HDL-C and apolipoprotein A1 (ApoA1) and negatively associated with LDL-C and ApoB. In the Spanish cohort, the intake of cholesterol was positively associated with ApoA1. The correlations between the food groups and lipid profiles are shown in supplementary table 2 and 3

The stepwise linear regression analysis shows no impact of diet composition on total and LDL-C levels when Norwegian and Spanish groups were analysed separately. When Norwegian and Spanish FH groups were pooled, LDL-C was weakly predicted by carbohydrates and protein calories percentage (inverse effect; coefficient (p-value); −0.06(0.03), −0.18(0.001) respectively).

Discussion

We report the characteristics in the intake of diets consumed by children with FH in Norway and Spain. The diet is recognized as a cornerstone factor in controlling and improving the lipid profile, but there are different diet patterns according to habits and cultures, and each of them has different nuances. The present study compared two cohorts from different countries as well as two types of diets. The Nordic and Mediterranean diets have been studied separately, but our study shows the similarities and differences in how these two diets impact lipids in children with FH.

In general terms, Spanish children eat more total fat and MUFAs, approximately double the amount of cholesterol, and approximately 25% more fibre, while Norwegian children eat more PUFAs. No significant differences in E% supplied by carbohydrates and proteins were observed. Although this study focuses on children with FH, the diet pattern differences were similar in both control groups (apart from a higher carbohydrate intake in Norwegian children without FH), suggesting that our results reflect

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Table 2 Diet composition of children with and without FH.

	NORWEGIAN COHORT (n = 52)				SPANISH COHORT (n = 207)				FH	Non FH
	Recommendations in Norway[a]	FH (n = 29)	Non FH (n = 23)	*P*	Recommendations in Spain[b]	FH (n = 85)	Non FH (n = 122)	*P*	Norwegian vs Spanish cohort (n = 114)	Norwegian vs Spanish cohort (n = 145)
Carbohydrates (E%)	50–60	47.5 (45.7–54.4)	52.5 (46.3–54.8)	0.23	50–55	48.9 (44.9–50.9)	47.1 (44.8–50.1)	0.18	0.54	**0.01***
Protein (E%)	15	17.2 (15.6–18.8)	16.0 (15.0–17.2)	0.12	12–15	16.6 (15.5–17.6)	16.8 (15.5–18.7)	0.23	0.21	0.10
Fats (E%)	25–35	30.1 (28.4–33.8)	29.5 (26.2–32.5)	0.44	30–35 (<30)	34.8 (32.6–37.7)	35.4 (32.5–37.8)	0.49	**<0.001***	**<0.001***
SFA (E%)	<7	12.0 (9.7–14.2)	12.0 (10.6–13.8)	0.52	<7-10	10.9 (9.1–12.5)	10.8 (9.4–12.3)	0.73	0.10	**0.06***
MUFA (E%)	>20	9.8 (8.9–11.1)	10.0 (8.6–11.2)	0.81	>20	14.4 (13.2–16.0)	14.7 (13.5–16.4)	0.32	**<0.001***	**<0.001***
PUFA (E%)	>10	5.8 (5.4–6.4)	4.7 (3.8–5.7)	**0.04***	>10	4.9 (4.1–5.8)	4.46 (4.42–5.86)	0.38	**0.04***	0.20
Cholesterol (mg)	<200	174 (129–213)	154.0 (94.0–220)	0.30	<200-300	349 (294–418)	360 (297–441)	0.39	**<0.001***	**<0.001***
Fibre (g)	20–30	16.0 (13.4–21.5)	14.8 (10.1–19.6)	0.17	26–38	20.2 (16.0–26.2)	21.0 (17.2–26.1)	0.45	**0.03***	**<0.001***

The results are shown in percentage of energy provided (E%) for all the macronutrients and in mg/day of cholesterol and g/day of fibre. Data are presented as the median (25th–75th percentile). Significant *p* values in bold. Differences between groups were obtained by Mann–Whitney U test or ANCOVA (after adjusting by age).
FH, familial hypercholesterolemia; E%, energy percent; SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids.
*P remains significant after adjusting by age.
[a] **Recommendations in Norway:** The nutrient composition of the diet recommended by the National Cholesterol Education Program (NCEP), the European Society of Cardiology, the European Atherosclerosis Society (EAS) and the Nordic Council of Ministers.
[b] **Recommendations in Spain:** The nutrient composition of the diet recommended by Spanish Society of Atherosclerosis (SEA), European Atherosclerosis Society (EAS) and Public Health Agency of Catalonia (ASPCAT).

general differences between Nordic and Mediterranean diets in children beyond lipid alterations.

Although there were some slight differences in recruitment and lipid measurement methods, both FH groups displayed similar metabolic profiles that were well within what could be expected for children with FH. On the other hand, there were some differences between the control groups without FH. The Spanish children without FH showed significantly higher cholesterol concentrations attributable to age differences and recruitment method. While the Norwegian control children were selected from a healthy cohort, the Spanish sample included children attending the lipid unit to rule out FH.

Although lipid alterations in children with FH are driven by a genetic defect, an impact of diet on clinical expression could not be ruled-out. The stepwise linear regression showed a weak invers effect of carbohydrates and protein intake on TC and LDL-C, pointing to a direct effect of fats, when both FH groups were analysed together, but this effect was not observed when the analysis was applied to both cohorts separately.

Correlations between food intake and lipid parameters was generally weak, however, there were some interesting associations, that are worth commenting. Total fat, mainly MUFAs, was associated with cholesterol levels in those who consumed the Norwegian diet, but with HDL-C in those who consumed the Spanish diet.

Some explanations for these differences can be speculated from the analysis of food groups and patterns (supplemental table 2 and 3). The intake of fruit and vegetables combined was not different between the groups, but the Spanish children with FH consumed more vegetables, nuts and legumes, which may have contributed to the higher fibre intake [41]. The consumption of nuts was higher in the Spanish FH group [22,41], than in Norway. Interesting all groups show a lower fibre intake than recommendations, what should be emphasised and corrected. Although fibre consumption was lower in the Norwegian cohort, the intake of whole-grain bread was much higher, while white bread was most commonly consumed in Spain [8,12]. Whole-grain carbohydrates could explain the inverse correlation between carbohydrates and total and LDL-C observed in the Norwegian children [19].

The consumption of MUFAs was higher in both Spanish groups potentially due to the consumption of olive oil and olives. On the other hand, despite all groups show lower consumption of PUFA according recommendations, the FH Norwegian cohort, had higher PUFA consumption, driven by a higher intake of rapeseed oil. In fact, Norway dietary guidelines emphasise the use of PUFA-enriched foods like oils and margarines, particularly in FH patients [42]. SFA consumption was higher in the Norwegian cohort, particularly in children without FH. This was most likely caused by an increased consumption of cheese, butter, margarine and precooked meals compared to the Spanish cohort. The use of more processed food in the Norwegian cohort may be due to a fast lunch, whereas in

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Table 3 Intake of food groups (g/10 MJ) in FH children sorted by countries.

Intake, g/10MJ	NORWEGIAN COHORT		SPANISH COHORT		FH Norwegian vs Spanish cohort (n = 114)
	Recommendations[a]	FH (n = 29)	Recommendations[b]	FH (n = 85)	
Cereal	Change for whole grain	214 (147–302)	Change for whole grain	177 (121–225)	**0.03**
White bread	Change for whole grain	6.7 (0.0–27.5)	Better to choose whole grains	99.3 (58.5–156.2)	**<0.001**
Middle whole grain bread	Better to choose whole grains	0.0 (0.0–0.1)	Not recommended	–	–
Whole grain bread	↑+	70.6 (0.0–134.4)	↑	0.0 (0.0–0.0)	**<0.001**
Cereal breakfast with sugar	Change it for without sugar	0.0 (0.0–9.9)	Change it for without sugar	1.90 (0.0–13.7)	0.11
Cereal breakfast without sugar	Oats, barley and rye	0.0 (0.0–17.1)	Whole grain	0.0 (0.0–0.0)	**0.01**
Potatoes	↑+	37.6 (22.8–68.7)	↑	83.3 (41.1–98.7)	**0.05**
Fried potatoes	Not recommended	0.0 (0.0–0.0)	Not recommended	9.7 (0.0–19.8)	**<0.001**
Other potatoes	↑	–	3 times/week	65.8 (29.8–81.5)	–
Vegetables	↑	106 (71.21–175)	↑	148 (92.8–252)	0.13
Fruits	↑	187 (97.36–385)	↑	247 (130–392)	0.63
Jam	80% fruit	6.6 (0.0–24.9)	Not recommended	0.0 (0.0–0.7)	**<0.001**
Nuts	Recommended as healthy snacks (30gr)	0.0 (0.0–0.0)	↑+ (30 g/day)	4.7 (1.5–13.8)	**<0.001**
Olives	↑	0.0 (0.0–0.0)	↓	5.7 (0.0–14.5)	**<0.001**
Legumes	Not insist too much	0.0 (0.0–0.0)	↑+ (3–4 times/week)	17.8 (10.3–22.3)	**<0.001**
Meat	Choose lean meat	64.1 (43.7–100.2)	Choose lean meat	119 (88.2–143)	**<0.001**
Red meat	↓	56.2 (13.8–73.5)	↓ (1 time/week)	41.3 (28.9–59.5)	0.47
White meat	Moderate	0.0 (0.0–35.0)	Moderate	70.3 (55.5–91.4)	**<0.001**
Game	↑	0.0 (0.0–0.0)	Not used	–	–
Processed red meat	Not recommended	70.9 (38.0–151.2)	Not recommended	27.6 (16.0–35.7)	**<0.001**
Processed white meat	↓	0.0 (0.0–14.8)	↓	10.4 (3.9–13.9)	**0.01**
Fish	↑	55.5 (19.6–93.8)	↑	76.0 (46.0–105.5)	0.15
Fat fish	↑+	5.7 (0.0–30.4)	↑	21.0 (12.2–33.0)	**0.03**
Shellfish	↓	0.0 (0.0–0.0)	Only insists in culinary technique	14.5 (3.8–26.4)	<**0.001**
Lean fish	↑	0.0 (0.0–39.3)	↑	24.5 (14.0–58.6)	<**0.001**
Eggs	1-2 times/week	0.0 (0.0–33.6)	Not limit. Only insist in culinary technic	18.7 (9.7–23.8)	**0.06**
Total dairy	Choose low-fat and skimmed milk	483 (284–647)	Without sugar. Not change the milk type	346 (245–496)	**0.05**
Milk	2-3 times/day	305 (151–540)	2-3 times/day	236 (165–412)	0.47
Whole milk	↓	0.0 (0.0–0.0)	Not change milk	0.0 (0.0–0.0)	0.70
Low fat milk		128 (8.6–330)	Not change milk	153.1 (0.0–354)	0.33
Skimmed milk	↑+	0.0 (0.0–185)	Not change milk	0.0 (0.0–93.2)	0.31
Yoghurt	Without sugar	0.0 (0.0–84.1)	Without sugar	70.5 (35.9–101)	**0.03**
Cheese	Moderate	32.9 (15.7–62.9)	Moderate	18.9 (7.6–26.9)	**0.05**
Low-fat cheese	Choose this kind of cheese	3.9 (0.0–14.7)	Choose this kind of cheese	0.0 (0.0–15.5)	0.44
Cheese >30%	↓	0.0 (0.0–22.7)	↓	7.6 (0.0–17.5)	0.41
Fat dairy products	Not recommended	18.4 (0.0–35.7)	Not recommended	15.8 (7.3–41.3)	0.66
Total fat cooking	Rapeseed oil or liquid margarine	20.1 (11.3–34.3)	Olive oil	23.0 (18.6–27.1)	0.96
Butter	↓	3.5 (0.0–5.8)	Not recommended	0.0 (0.0–0.0)	**<0.001**
Margarine	↓	3.8 (1.3–9.1)	Not recommended	0.0 (0.0–0.0)	**<0.001**
Low-fat margarine	Choose this type of margarine	1.4 (0.0–12.5)	Not recommended	–	–
Rapeseed oil	↑+	0.0 (0.0–0.0)	Not used	0.0 (0.0–0.0)	**0.01**
Olive oil	Not used	0.0 (0.0–0.0)	↑+	20.3 (17.5–24.4)	**<0.001**
Others	–	–	–	0.0 (0.0–0.6)	
Dressings	PUFA mayonnaise	0.0 (0.0–10.4)	↓	2.3 (0.5–4.2)	0.68
Sweet products	Not recommended	64.2 (33.9–109)	Not recommended	55.7 (36.7–67.1)	0.13
Snacks	Not recommended	0.0 (0.0–9.6)	Not recommended	5.5 (2.9–10.5)	**0.01**
Precooked food and fast food	Not recommended	112 (65.5–165)	Not recommended	20.7 (12.7–27.5)	**<0.001**
Pizza	↓	0.0 (0.0–52.3)	↓	18.8 (12.0–26.7)	0.18
Prepared soups	↓	0.0 (0.0–0.0)	↓	0.0 (0.0–0.5)	0.92
Beverages	Change beverages by water	345 (141–595)	Change beverages by water	100 (60.9–161)	<**0.001**
With sugar	↓	147 (24.4–289)	↓	69.4 (0.0–134)	**0.05**

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Table 3 (*continued*)

Intake, g/10MJ	NORWEGIAN COHORT		SPANISH COHORT		FH Norwegian vs Spanish cohort (n = 114)
	Recommendations[a]	FH (n = 29)	Recommendations[b]	FH (n = 85)	
With artificial-sweeteners	↓	0.0 (0.0–68.6)	↓	0.0 (0.0–0.0)	**0.03**
Natural fruit juices	Without sugar	47.1 (0.0–162.7)	Not recommended	0.0 (0.0–26.8)	**0.06**
Tea/coffee	Without sugar (without caffeine in young children)	0.0 (0.0–21.4)	Without sugar and caffeine	0.0 (0.0–0.0)	0.13
Alcohol	Avoid	0.0 (0.0–0.0)	Avoid	0.0 (0.0–0.0)	0.09

Significant *p* values in bold.
MJ, mega joule; ↑, increase consumption; ↑+, more emphasis on increasing consumption than the other country; ↓, decrease consumption.
[a] **Recommendations in Norway:** The nutrient composition of the diet recommended by the National Cholesterol Education Program (NCEP), the European Society of Cardiology, the European Atherosclerosis Society (EAS) and the Nordic Council of Ministers.
[b] **Recommendations in Spain:** The nutrient composition of the diet recommended by Spanish Society of Atherosclerosis (SEA), European Atherosclerosis Society (EAS) and Public Health Agency of Catalonia (ASPCAT).

Table 4 Heatmap showing the correlations between standard lipid profile and consumption of macronutrients in FH children sorted by countries. All values were adjusted by age and gender.

	% CH		% PROTEIN		% FAT		% MUFA		% PUFA		% SFA		Fiber		Cholesterol	
	Norwegian	Spanish	Norwegian	Spanish	Norwegian	Spanish	Norwegian	Spanish	Norwegian	Spanish	Norwegian	Spanish	Norwegian	Spanish	Norwegian	Spanish
TC	-0.35	-0.05	-0.16	-0.18	**0.51**	0.12	**0.561**	0.04	0.16	0.05	0.27	0.16	-0.08	0.02	0.07	-0.06
LDL-C	-0.34	0.01	-0.12	-0.16	**0.47**	0.05	**0.514**	-0.03	0.19	0.02	0.22	0.16	-0.06	0.00	0.10	-0.10
HDL-C	-0.07	-0.22	-0.13	-0.03	0.14	**0.26**	0.145	**0.23**	-0.16	0.14	0.21	0.10	-0.26	-0.04	**-0.44**	0.06
TG	-0.15	0.07	-0.07	-0.09	0.18	-0.04	0.232	-0.19	0.06	-0.10	0.05	-0.18	0.29	0.11	0.29	0.11
ApoA	0.02	-0.30	-0.09	0.05	0.05	**0.34**	0.067	**0.25**	-0.05	0.20	0.07	0.14	-0.14	0.05	-0.36	**0.24**
ApoB	-0.33	0.07	-0.13	-0.14	**0.46**	-0.03	**0.519**	-0.08	0.21	0.03	0.20	0.06	-0.02	0.05	0.19	-0.12

Significant *P* values ($P < 0.05$) in bold and were obtained by partial correlations adjusted by age and gender.CH, carbohydrates; MUFA, Mono-unsaturated fatty acids; PUFA, Polyunsaturated fatty acids; SFA, Saturated fatty acids; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; ApoB, apolipoprotein B100; ApoA, apolipoprotein A.

Spain, lunch is usually a controlled main course at school or at home.

A main strength of the study is that it is one of the first articles showing information about Nordic and Mediterranean pattern diets, including a comprehensive analysis of food groups (53 categories), and its association lipid profiles in children with FH; however, there are some limitations. This study has a limited sample size of children with FH and the use of different questionnaires for the collection of food data. Moreover, the information about physical activity was scarce precluding analysing its impact on lipoprotein profile as it has been previously reported [43–45]. Although the study was performed in average social class in both countries, we have not specific data about socioeconomic family status could impact diet.

In conclusion, the Spanish children with FH had a higher intake of total fats, mainly MUFAs, cholesterol and dietary fibre, while the Norwegian children with FH had a higher intake of PUFAs. This study provides an estimate of dietary intake in the FH children population. The observe differences at basal diet are driven by cultural habits rather than medical recommendations. This study is suited to 'say something about what FH children eat in Norway and Spain'. It doesn't guide treatment. Despite diet differences, the baseline lipid profiles were very similar in the Norwegian and Spanish children with FH. For this reason, our data suggest that dietary recommendations should be framed to dietary patterns more than macronutrients.

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Appendix A. Supplementary data

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References

[1] Brown MS GJ. A receptor-mediated pathway for cholesterol homeostasis. Science 1986;232:34–47. https://doi.org/10.1002/anie.198605833.
[2] Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease. Eur Heart J 2013;34:3478–90. https://doi.org/10.1093/eurheartj/eht273.
[3] Hobbs HH, Brown MS, Goldstein JL. Molecular genetics of the LDL receptor gene in familial hypercholesterolemia. Hum Mutat 1992;1:445–66. https://doi.org/10.1002/humu.1380010602.
[4] Wiegman A, Gidding SS, Watts GF, Chapman MJ, Ginsberg HN, Cuchel M, et al. Familial hypercholesterolæmia in children and adolescents: gaining decades of life by optimizing detection and treatment. Eur Heart J 2015;36:2425–37. https://doi.org/10.1093/eurheartj/ehv157.
[5] Sjouke B, Kusters DM, Kindt I, Besseling J, Defesche JC, Sijbrands EJ, et al. Homozygous autosomal dominant hypercholesterolaemia in The Netherlands: prevalence, genotype-phenotype relationship, and clinical outcome. Eur Heart J 2015;36:560–5. https://doi.org/10.1093/eurheartj/ehu058.
[6] Benn M, Watts GF, Tybjærg-Hansen A, Nordestgaard BG. Mutations causative of familial hypercholesterolaemia: screening of 98 098 individuals from the Copenhagen General Population Study estimated a prevalence of 1 in 217. Eur Heart J 2016;37:1384–94. https://doi.org/10.1093/eurheartj/ehw028.
[7] Zamora A, Masana L, Comas-Cufí M, Vila À, Plana N, García-Gil M, et al. Familial hypercholesterolemia in a European Mediterranean population—prevalence and clinical data from 2.5 million primary care patients. J Clin Lipidol 2017;11:1013–22. https://doi.org/10.1016/j.jacl.2017.05.012.
[8] Torvik K, Narverud I, Ottestad I, Svilaas A, Gran JM, Retterstøl K, et al. Dietary counseling is associated with an improved lipid profile in children with familial hypercholesterolemia. Atherosclerosis 2016;252:21–7. https://doi.org/10.1016/j.atherosclerosis.2016.07.913.
[9] Shafiq N, Singh M, Kaur S, Khosla P, Malhotra S. Dietary treatment for familial hypercholesterolaemia. Cochrane Database Syst Rev 2010. https://doi.org/10.1002/14651858.cd001918.pub2.
[10] Ulven SM, Leder L, Elind E, Ottestad I, Christensen JJ, Telle-Hansen VH, et al. Exchanging a few commercial, regularly consumed food items with improved fat quality reduces total cholesterol and LDL-cholesterol: a double-blind, randomised controlled trial. Br J Nutr 2016;116:1383–93. https://doi.org/10.1016/0002-9149(93)90010-A.
[11] Connor WE, Connor SL. Importance of diet in the treatment of familial hypercholesterolemia. Am J Cardiol 1993;72:42D–53D. https://doi.org/10.1016/0002-9149(93)90010-A.
[12] Glassman M, Spark A, Berezin S, Schwarz S, Medow M, Newman LJ. Treatment of type IIa hyperlipidemia in childhood by a simplified American Heart Association diet and fiber supplementation. Am J Dis Child 1990;144:973–6.

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[13] Cicero AFG, Fogacci F, Giovannini M, Bove M, Debellis G, Borghi C. Effect of quantitative and qualitative diet prescription on children behavior after diagnosis of heterozygous familial hypercholesterolemia. Int J Cardiol 2019;293:193–6. https://doi.org/10.1016/j.ijcard.2019.05.069.
[14] Zampelas A, Magriplis E. Dietary patterns and risk of cardiovascular diseases: a review of the evidence. Proc Nutr Soc 2020;79:68–75. https://doi.org/10.1017/S0029665119000946.
[15] Herforth A, Arimond M, Álvarez-Sánchez C, Coates J, Christianson K, Muehlhoff E. A global review of food-based dietary guidelines. Adv Nutr 2019;10:730. https://doi.org/10.1093/advances/nmy130.
[16] Borges CA, Slater B, Santaliestra-Pasías AM, Mouratidou T, Huybrechts I, Widhalm K, et al. Dietary patterns in European and Brazilian adolescents: comparisons and associations with socioeconomic factors. Nutrients 2018;10:57. https://doi.org/10.3390/nu10010057.
[17] Negele L, Schneider B, Ristl R, Stulnig TM, Willfort-Ehringer A, Helk O, et al. Effect of a low-fat diet enriched either with rapeseed oil or sunflower oil on plasma lipoproteins in children and adolescents with familial hypercholesterolaemia. Results of a pilot study. Eur J Clin Nutr 2015;69:337–43. https://doi.org/10.1038/ejcn.2014.234.
[18] Berild A, Holven KB, Ulven SM. Recommended Nordic diet and risk markers for cardiovascular disease. Tidsskr Nor Laegeforen 2017;137:721–6. https://doi.org/10.4045/tidsskr.16.0243.
[19] Adamsson V, Reumark A, Fredriksson IB, Hammarström E, Vessby B, Johansson G, et al. Effects of a healthy Nordic diet on cardiovascular risk factors in hypercholesterolaemic subjects: a randomized controlled trial (NORDIET). J Intern Med 2011;269:150–9. https://doi.org/10.1111/j.1365-2796.2010.02290.x.
[20] Liyanage T, Ninomiya T, Wang A, Neal B, Jun M, Wong MG, et al. Effects of the Mediterranean diet on cardiovascular outcomes-A systematic review and meta-analysis. PLoS One 2016;11:e0159252. https://doi.org/10.1371/journal.pone.0159252.
[21] Eleftheriou D, Benetou V, Trichopoulou A, La Vecchia C, Bamia C. Mediterranean diet and its components in relation to all-cause mortality: meta-analysis. Br J Nutr 2018;120:1081–97. https://doi.org/10.1017/S0007114518002593.
[22] Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med 2013;368:1279–90. https://doi.org/10.1056/NEJMoa1200303.
[23] Bach-Faig A, Berry EM, Lairon D, Reguant J, Trichopoulou A, Dernini S, et al. Mediterranean diet pyramid today. Science and cultural updates. Public Health Nutr 2011;14:2274–84. https://doi.org/10.1017/S1368980011002515.
[24] Shen J, Wilmot KA, Ghasemzadeh N, Molloy DL, Burkman G, Mekonnen G, et al. Mediterranean dietary patterns and cardiovascular health. Annu Rev Nutr 2015;35:425–49. https://doi.org/10.1146/annurev-nutr-011215-025104.
[25] Papadaki A, Scott JA. The impact on eating habits of temporary translocation from a Mediterranean to a Northern European environment. Eur J Clin Nutr 2002;56:455–67. https://doi.org/10.1038/sj.ejcn.1601337.
[26] Hoffman R, Gerber M. Evaluating and adapting the Mediterranean diet for non-Mediterranean populations: a critical appraisal. Nutr Rev 2013;71:573–84. https://doi.org/10.1111/nure.12040.
[27] Adamsson V, Reumark A, Cederholm T, Vessby B, Risérus U, Johansson G. What is a healthy Nordic diet? Foods and nutrients in the NORDIET study. Food Nutr Res 2012;56:1–8. https://doi.org/10.3402/fnr.v56i0.18189.
[28] Mithril C, Dragsted LO, Meyer C, Blauert E, Holt MK, Astrup A. Guidelines for the new Nordic diet. Public Health Nutr 2012;15:1941–7. https://doi.org/10.1017/S136898001100351X.
[29] Adamsson V, Cederholm T, Vessby B, Risérus U. Influence of a healthy Nordic diet on serum fatty acid composition and associations with blood lipoproteins - results from the NORDIET study. Food Nutr Res 2014;58:1–6. https://doi.org/10.3402/fnr.v58.24114.
[30] Uusitupa M, Hermansen K, Savolainen MJ, Schwab U, Kolehmainen M, Brader L, et al. Effects of an isocaloric healthy Nordic diet on insulin sensitivity, lipid profile and inflammation markers in metabolic syndrome - a randomized study (SYSDIET). J Intern Med 2013;274:52–66. https://doi.org/10.1111/joim.12044.
[31] Lemming EW, Byberg L, Wolk A, Michaëlsson K. A comparison between two healthy diet scores, the modified Mediterranean diet score and the Healthy Nordic Food Index, in relation to all-cause and cause-specific mortality. Br J Nutr 2018;119:836–46. https://doi.org/10.1017/S0007114518000387.
[32] Christensen JJ, Ulven SM, Retterstøl K, Narverud I, Bogsrud MP, Henriksen T, et al. Comprehensive lipid and metabolite profiling of children with and without familial hypercholesterolemia: a cross-sectional study. Atherosclerosis 2017;266:48–57. https://doi.org/10.1016/j.atherosclerosis.2017.09.021.
[33] Ibarretxe D, Rodríguez-Borjabad C, Feliu A, Bilbao JÁ, Masana L, Plana N. Detecting familial hypercholesterolemia earlier in life by actively searching for affected children: The DECOPIN project. Atherosclerosis 2018;278:210–6. https://doi.org/10.1016/j.atherosclerosis.2018.09.039.
[34] Júlíusson PB, Roelants M, Nordal E, Furevik L, Eide GE, Moster D, et al. Growth references for 0–19 year-old Norwegian children for length/height, weight, body mass index and head circumference. Ann Hum Biol 2013;40(3):220–7. https://doi.org/10.3109/03014460.2012.759276.
[35] Sobradillo B, Aguirre A, Aresti U, Bilbao A, Fernández-Ramos C, Lizárraga A, et al. Curvas y Tablas de Crecimiento. Estudios longitudinal y transversal. Bilbao: Fundación Faustino Orbegozo; 2004.
[36] Lillegaard ITL, Andersen LF. Validation of a pre-coded food diary with energy expenditure, comparison of under-reporters v. acceptable reporters. Br J Nutr 2005;94:998–1003. https://doi.org/10.1079/bjn20051587.
[37] Lillegaard ITL, Øverby NC, Andersen LF. Can children and adolescents use photographs of food to estimate portion sizes? Eur J Clin Nutr 2005;59:611–7. https://doi.org/10.1038/sj.ejcn.1602119.
[38] Lillegaard ITL, Løken EB, Andersen LF. Relative validation of a pre-coded food diary among children, under-reporting varies with reporting day and time of the day. Eur J Clin Nutr 2007;61:61–8. https://doi.org/10.1038/sj.ejcn.1602487.
[39] Andersen LF, Pollestad ML, Jacobs DR, Løvø A, Hustvedt B-E. Validation of a pre-coded food diary used among 13-year-olds: comparison of energy intake with energy expenditure. Public Health Nutr 2005;8:1315–21. https://doi.org/10.1079/phn2005751.
[40] Martin-Moreno JM, Boyle P, Gorgojo L, Maisonneuve P, Fernandez-Rodriguez JC, Salvini S. Development and validation of a food frequency questionnaire in Spain. Int J Epidemiol 1993;22:512–9.
[41] Agència de Salut Pública de Catalunya. Guia alimentària 'Petits canvis per menjar millor, Barcelona. 2019.
[42] Øyri LK, Johansen D, Holven KB, Bogsrud MP, Strøm EC. For deg med høyt kolesterol eller høye triglyserider Kostholdsråd ved høye blodlipider. 1st ed. Oslo: Nasjonal kompetansetjeneste for familiær hyperkolesterolemi, Lipidklinikken, Oslo universitetssykehus; 2016.
[43] Jago R, Nicklas T, Yang SJ, Baranowski T, Zakeri I, Berenson GS. Physical activity and health enhancing dietary behaviors in young adults: Bogalusa Heart Study. Prev Med 2005;41:194–202. https://doi.org/10.1016/j.ypmed.2004.09.045.
[44] Martino F, Puddu PE, Pannarale G, Colantoni C, Zanoni C, Martino E, et al. Metabolic syndrome among children and adolescents from Southern Italy: contribution from the Calabrian Sierras community study (CSCS). Int J Cardiol 2014;177:455–60. https://doi.org/10.1016/j.ijcard.2014.09.020.
[45] Martino F, Martino E, Versacci P, Niglio T, Zanoni C, Puddu PE. Lifestyle and awareness of cholesterol blood levels among 29159 community school children in Italy. Nutr Metabol Cardiovasc Dis 2019 Aug;29(8):802–7. https://doi.org/10.1016/j.numecd.2019.05.060.

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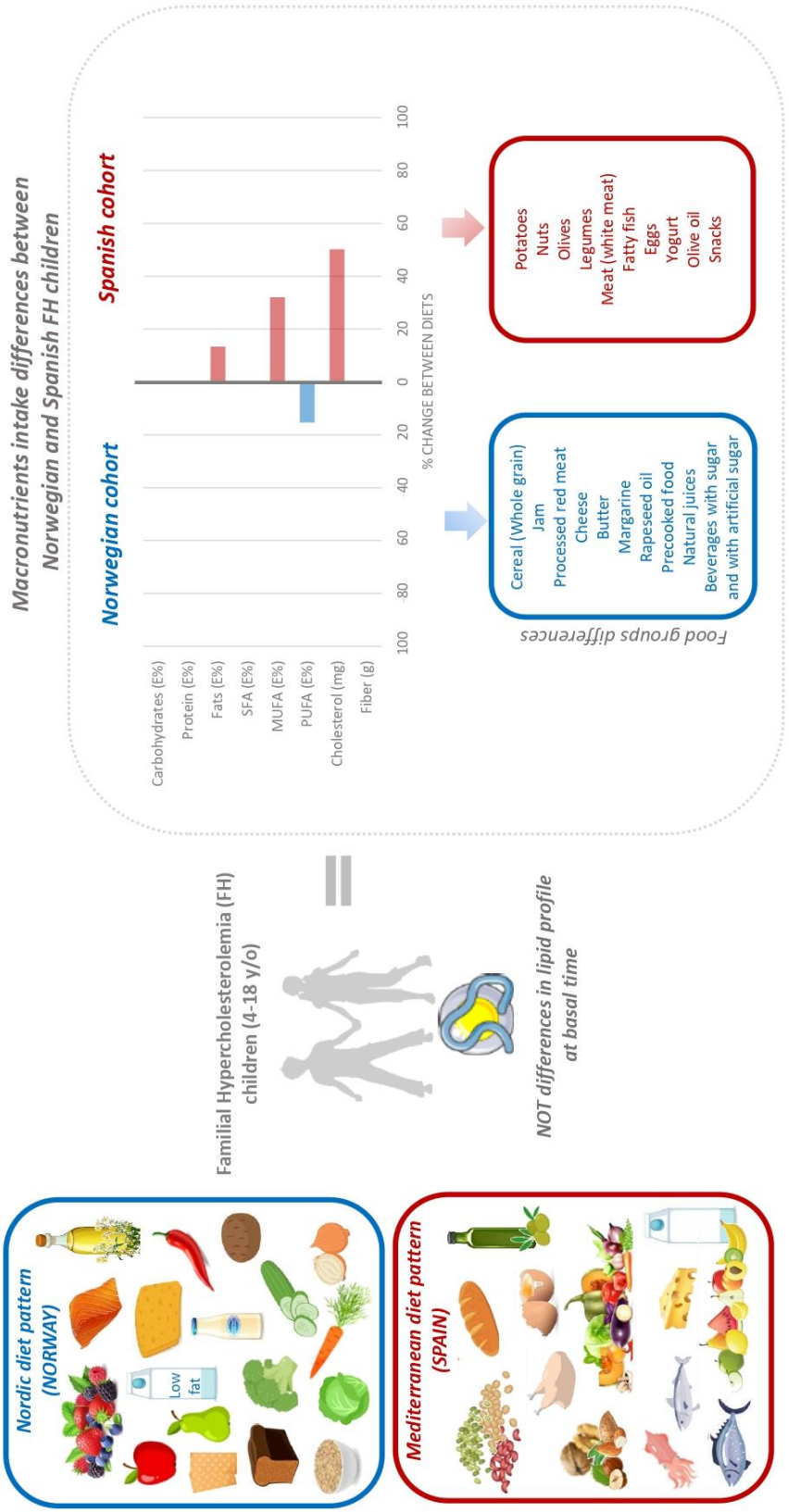

Macronutrients intake differences between Norwegian and Spanish FH children
Spanish cohort
Norwegian cohort
% CHANGE BETWEEN DIETS
100 80 60 40 20 0 20 40 60 80 100
Carbohydrates (E%)
Protein (E%)
Fats (E%)
SFA (E%)
MUFA (E%)
PUFA (E%)
Cholesterol (mg)
Fiber (g)
Food groups differences
Potatoes
Nuts
Olives
Legumes
Meat (white meat)
Fatty fish
Eggs
Yogurt
Olive oil
Snacks
Cereal (Whole grain)
Jam
Processed red meat
Cheese
Butter
Margarine
Rapeseed oil
Precooked food
Natural juices
Beverages with sugar and with artificial sugar
Familial Hypercholesterolemia (FH) children (4-18 y/o)
=
NOT differences in lipid profile at basal time
Nordic diet pattern (NORWAY)
Low fat
Mediterranean diet pattern (SPAIN)

Supplementary table 1. Description of the foods that composed each 20 food groups.

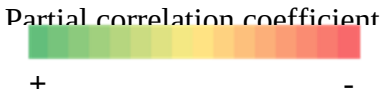
	The group include the following foods:
1.Cereal	Rice, pasta, breakfast cereal and all kind of bread.
1.1.White bread	*Bread with 100% of sifted flour.*
1.2. "Middle" whole grain bread	*Bread <50% total flour.*
1.3.Whole grain bread	*Bread >50% total flour.*
1.4.Cereal breakfast with sugar	--
1.5.Cereal breakfast without sugar	--
2.Potatoes	All kind of potatoes
2.1.Fried potatoes	*Potato fries and French fries*.
2.2.Other potatoes	
3.Vegetables	Chard, spinach, cabbage, cauliflower, broccoli, lettuce, endives, escarole, tomato, carrot, pumpkin, green beans, eggplant, zucchini, cucumber, pepper, paprika, asparagus, artichoke, leek, celery, onion, garlic and mushrooms.
4.Fruits	Orange, tangerine, grapefruit, banana, apple, pear, strawberry, peach, figs, apricot, nectarine, melon, watermelon, grapes, kiwi and berries.
5.Jam	All kind of jam.
6.Nuts	Almonds, walnuts, pistachios, hazelnut, pinions, cashews and raisins.
7.Olives	--
8. Legumes	Chickpeas, lentils, beans and peas.
9.Meat	Chicken, turkey, rabbit, pork, veal, cow, beef, sheep, lamb and goat.
9.1.Red meat	*Pork, veal, beef, sheep and mutton.*
9.2.White meat	*Chicken, turkey and rabbit.*
9.3.Game	*Goose, moose, deer, boar, quail…*
9.4.Processed red meat	*Turkey and chicken ham.*
9.5.Processed white meat	*Salami, serrano ham, liver pate, bacon, hamburger.*
10.Fish	All the types of fish: fresh fish, smoked fish, canned fish and seafood.
10.1.Fat fish	Salmon, herring, mackerel, tuna, sardine…
10.2.Sellfish	Prawns, mussels, clams, oysters…
10.3.Lean fish	Hake, cod, sole, monkfish, grouper, sea bream…
11.Eggs	Eggs and omelettes.
12.Total dairy	Milk, yoghurt and cheese.
12.1.Milk	*Whole, low fat and skimmed milk.*
12.1.1.Whole milk	--
12.1.2.Low fat milk	--

12.1.3.Skimmed milk	*--*
12.2.Yoghur	*All kind of yoghur*
12.3.Cheese	*All kind of cheese.*
12.3.1.Low-fat cheese	*Cheeses with less than 30% fat.*
12.3.2.High-fat cheese	*Cheeses with more than 30% fat.*
13.Fat dairy products	Cream, sour cream, ice cream and dairy desserts.
14.Total fat cooking	Butter, margarine, rapeseed oil, olive oil, soy oil, sunflower oil, corn oil and lard.
14.1.Butter	*--*
14.2.Margarine	*Normal margarine.*
14.2.1.Low-fat margarine	*Margarines with PUFA*
14.3.Rapeseed oil	*--*
14.4.Olive oil	*--*
14.5. Others oils and fats	*Sunflower, soybean oil and lard*
15.Dressings	Ketchup, mayonnaise and mustard.
16.Sweet products	Sugar, honey, chocolate, cocoa powder, cakes, pastries and cookies.
17.Snacks	Chips potatoes, pop corn, toasted corn, nachos, etc …
18.Precooked food and fast food	Pizza, kebab, taco, wraps, casserole dishes, spring rolls, etc
18.1.Pizza	*--*
18.2.Prepared soups	*Soups that are already prepared.*
19.Beverages	Natural juices or smoothies, juice and soft drinks with sugar and with artificial sweeteners, coffee and tea.
18.1.With sugar	*Juice and soft drinks with sugar*
18.2.Artificial-sweeteners	*Soft drink with artificial sweeteners.*
18.3.Natural fruit juices	*Natural juices or smoothies without sugar.*
18.4.Tea/coffee	*Coffee and tea.*
20.Alcohol	Wine, beer and alcohol drinks.

Supplementary table 2. The heatmap about correlations between food groups and normal lipid profile in Norwegian FH children.

	TC	LDL-C	HDL-C	TG	ApoA	ApoB
Cereal (all)	-0,177	-0,121	-0,071	-0,176	-0,012	-0,123
¤*White bread*	0,180	0,249	**-0,428**	0,112	-0,378	0,273
¤*Middle whole grain bread*	0,009	0,021	0,008	-0,037	0,044	0,029
¤*Whole grain bread*	-0,124	-0,110	-0,052	-0,023	-0,016	-0,089
¤*Cereal breakfast with sugar*	-0,247	-0,248	0,012	-0,135	-0,027	-0,274
¤*Cereal breakfast without sugar*	-0,123	-0,057	-0,174	-0,130	-0,228	-0,061
Potatoes	-0,068	-0,056	0,061	-0,099	0,021	-0,079
¤*Fried potatoes*	-0,085	-0,068	-0,178	-0,026	-0,244	-0,033
Vegetables	-0,225	-0,236	0,203	-0,142	0,271	-0,249
Legumes	0,072	0,049	0,045	0,010	0,006	0,083
Fresh fruits	-0,205	-0,199	0,073	-0,208	-0,014	-0,187
Jam	-0,160	-0,138	0,067	-0,276	-0,062	-0,194
Nuts	0,142	0,125	-0,106	0,146	0,017	0,152
Meat	-0,212	-0,260	0,365	-0,344	0,318	-0,272
¤*White meat*	-0,196	-0,188	0,083	-0,266	-0,039	-0,186
¤*Red meat*	-0,111	-0,167	0,340	-0,211	0,360	-0,180
¤*Red processed meat*	0,260	0,258	0,226	-0,175	0,180	0,205
¤White processed meat	-0,148	-0,172	0,004	0,181	0,081	-0,147
Game	0,094	0,147	-0,178	-0,059	-0,237	0,098
Fish	**-0,404**	**-0,466**	**0,429**	-0,273	**0,475**	**-0,446**
¤*Fatty fish*	-0,141	-0,210	**0,465**	-0,143	**0,427**	-0,200
¤*Lean fish*	-0,268	-0,273	0,223	-0,216	0,301	-0,284
¤*Sellfish*	-0,028	-0,105	0,339	-0,032	0,228	-0,078
Eggs	-0,276	-0,287	0,028	-0,058	-0,008	-0,288
Total dairy	-0,085	-0,078	0,164	-0,320	0,093	-0,054
¤*Milk*	-0,030	-0,026	0,118	-0,168	0,105	0,020
¤¤*Whole milk*	-0,161	-0,131	-0,132	-0,161	-0,227	-0,116
¤¤*Low-fat milk*	0,132	0,084	**0,392**	-0,165	**0,402**	0,095
¤¤*Skimmed milk*	-0,208	-0,138	**-0,383**	0,031	**-0,402**	-0,092
¤*Yogurt*	-0,060	-0,082	0,117	-0,136	0,048	-0,085
¤*Cheese*	**-0,540**	**-0,538**	0,089	-0,246	0,037	**-0,550**
¤¤*Low fat cheese*	-0,219	-0,216	0,026	0,019	0,144	-0,207
¤¤*High fat cheese*	-0,296	-0,342	0,341	-0,278	0,349	-0,311
Fat dairy products	0,238	0,281	-0,058	-0,200	-0,123	0,222
Fat	-0,277	-0,286	0,253	-0,311	0,254	-0,279
¤*Butter*	-0,234	-0,285	**0,470**	-0,339	**0,479**	-0,317
¤*Margarine*	-0,057	0,018	-0,099	-0,235	-0,266	-0,018
¤¤*Low fat margarine*	-0,231	-0,270	0,117	-0,014	0,285	-0,231
¤*Rapessed oil*	-0,253	-0,245	-0,047	-0,066	-0,122	-0,252
¤*Olive oil*	0,002	0,008	-0,144	0,082	-0,167	0,040

Dressings	-0,008	-0,005	-0,127	0,173	-0,141	0,035
Sweet products	0,184	0,184	-0,067	0,087	-0,029	0,196
Snacks	0,068	0,098	-0,205	0,064	-0,117	0,074
Precooked food and fast food	-0,323	-0,259	**-0,406**	-0,028	**-0,392**	-0,217
¤*Pizza*	-0,011	0,003	-0,061	-0,037	0,031	0,012
¤*Prepared soups*	-0,331	-0,333	-0,090	-0,075	-0,147	-0,322
Beverages	-0,275	-0,269	0,109	-0,208	-0,008	-0,285
¤*Sugar sweetened*	0,058	0,042	0,299	-0,145	0,148	0,016
¤*Artificial_sweetened*	-0,269	-0,247	-0,212	0,098	-0,236	-0,244
¤*Natural fruit juices*	-0,052	-0,026	0,098	-0,297	-0,041	-0,055
¤*Tea/coffee*	**-0,455**	**-0,477**	0,127	-0,267	0,162	**-0,463**
Alcohol	-0,249	-0,225	-0,111	-0,094	-0,079	-0,191



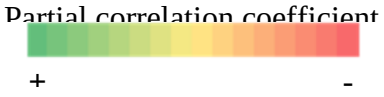
Significant *P* values (*P<0.05*) in bold and were obtained by partial correlations adjusted by age and gender.

CH, carbohydrates; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids; SFA, Saturated fatty acids; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; ApoB, apolipoprotein B100; ApoA, apolipoprotein A.

Supplementary table 3. The heatmap about correlations between food groups and normal lipid profile in Catalan FH children.

	TC	LDL-C	HDL-C	TG	ApoA	ApoB
Cereal (all)	-0,106	-0,086	-0,156	0,210	-0,188	0,010
¤*White bread*	-0,074	-0,059	-0,117	0,173	-0,138	0,011
¤*Whole grain bread*	0,000	-0,008	0,001	0,047	-0,015	0,013
¤*Cereal breakfast with sugar*	-0,020	0,000	-0,074	-0,004	-0,100	0,002
¤*Cereal breakfast without sugar*	0,023	0,025	0,012	-0,054	-0,040	0,028
Potatoes	0,051	0,050	0,107	-0,202	0,052	0,061
¤*Fried potatoes*	0,135	0,104	0,136	-0,078	0,145	0,116
¤*Other potatoes*	-0,011	0,005	0,054	-0,197	-0,013	0,011
Vegetables	-0,101	-0,091	0,031	-0,119	0,071	-0,054
Legumes	-0,130	-0,187	**0,294**	**-0,233**	0,207	**-0,252**
Fresh fruits	-0,034	-0,021	-0,091	0,107	-0,084	0,042
Jam	-0,007	0,063	-0,180	-0,120	-0,149	0,124
Nuts	0,006	0,014	-0,072	0,079	-0,042	0,055
Olives	0,019	0,024	-0,027	0,004	-0,124	0,055
Meat	-0,074	-0,052	-0,027	-0,131	0,024	-0,048
¤*White meat*	-0,177	-0,145	-0,065	-0,129	-0,018	-0,111
¤*Red meat*	0,059	0,061	0,022	-0,073	0,053	0,033
¤*Red processed meat*	-0,047	-0,121	**0,269**	0,002	**0,386**	-0,157
¤White processed meat	0,045	0,003	-0,081	**0,436**	-0,040	0,093
Fish	-0,193	-0,181	-0,048	0,008	-0,010	-0,082
¤*Fatty fish*	-0,096	-0,085	0,000	-0,079	0,081	-0,033
¤*Lean fish*	-0,150	-0,121	-0,133	0,053	-0,150	-0,019
¤*Sellfish*	-0,179	-0,191	0,041	0,022	0,063	-0,145
Eggs	-0,202	-0,172	-0,099	-0,017	-0,064	-0,151
Total dairy	0,059	0,104	-0,078	-0,164	-0,187	0,031
¤*Milk*	0,022	0,065	-0,082	-0,135	-0,183	-0,003
¤¤*Whole milk*	-0,034	0,003	-0,074	-0,112	-0,071	-0,043
¤¤*Low-fat milk*	0,080	0,096	0,006	-0,143	-0,084	0,036
¤¤*Skimmed milk*	-0,115	-0,089	-0,131	0,127	-0,131	-0,060
¤*Yogurt*	0,138	0,159	0,025	**-0,220**	-0,065	0,098
¤*Cheese*	0,128	0,166	-0,194	0,112	-0,176	0,195
¤¤*Low fat cheese*	-0,054	-0,036	-0,146	0,163	-0,176	0,055
¤¤*High fat cheese*	0,175	0,204	-0,112	0,012	-0,071	0,174
Fat dairy products	0,169	0,162	0,022	-0,007	0,130	0,128
Fat	0,017	-0,010	0,140	-0,084	0,100	-0,009
¤*Butter*	**0,278**	**0,217**	0,172	0,059	**0,291**	0,143
¤*Margarine*	0,099	0,086	0,079	-0,073	0,082	0,046
¤*Olive oil*	-0,086	-0,105	0,104	-0,058	0,040	-0,079
Dressings	-0,069	-0,097	0,185	-0,179	0,249	-0,128
Sweet products	-0,027	-0,039	0,064	-0,047	0,068	-0,129
Snacks	0,046	0,057	0,020	-0,124	-0,034	0,005

Precooked food and fast food	-0,176	-0,212	0,133	-0,023	0,200	**-0,271**
¤*Pizza*	-0,194	**-0,231**	0,131	-0,010	0,197	**-0,294**
¤*Prepared soups*	0,167	0,154	0,056	-0,043	-0,059	0,216
Beverages	0,010	0,044	-0,137	0,048	-0,132	0,050
¤*Sugar sweetened*	0,043	0,091	-0,171	0,020	-0,145	0,124
¤*Artificial_sweetened*	-0,080	-0,111	0,027	0,145	0,167	-0,046
¤*Natural fruit juices*	-0,010	-0,013	0,003	0,005	-0,077	-0,072
¤*Tea/coffee*	-0,051	-0,051	0,029	-0,065	-0,050	-0,084
Alcohol	0,156	0,149	-0,063	0,168	-0,063	0,115



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IMPACT OF NORDIC AND MEDITERRANEAN DIETS ON LIPOPROTEIN PHENOTYPE ASSESSED BY ^{1}H-NMR IN CHILDREN WITH FAMILIAL HYPERCHOLESTEROLEMIA

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Abbreviations:

^{1}H-NMR: proton-nuclear magnetic resonance

Apo A: apolipoprotein ApoA1

Apo B: apolipoprotein B100

BMI: body mass index

CVD: cardiovascular disease

CVR: cardiovascular risk

FH: familial hypercholesterolemia

HDL-C: high-density lipoprotein cholesterol

HeFH: heterozygous familial hypercholesterolemia

IQR: interquartile range

L_: large

LDL-C: low-density lipoprotein cholesterol

LDLR: low-density lipoprotein receptor

M_: medium

MUFA: monounsaturated fatty acid

PUFA: polyunsaturated fatty acid

S_: small

SD: standard deviation

TC: total cholesterol

TRL: triglyceride-rich lipoproteins

VLDL-C: very-low-density lipoprotein cholesterol

XL_: extra large

XS_: extra small

XXL_: extremely large

Z-score: standard deviation scores

ABSTRACT:

Background: Different dietary patterns could modulate the lipoprotein phenotype in familial hypercholesterolemia. Both Nordic and Mediterranean diets are considered healthy despite their striking differences. It is important to study the impact of these various diets on children with familial hypercholesterolemia (FH).

Aim: To determine the impact of Nordic and Mediterranean diets on the lipoprotein profile assessed by nuclear magnetic resonance (RMN) in children with heterozygous FH.

Methods: This was a case-control cross-sectional study performed in the Lipid Units at Sant Joan University Hospital in Reus (Spain) and Oslo University (Norway).
We studied 256 children (mean age 10 y/o; approximately 50% girls): 85 Spanish and 29 Norwegian FH children and 142 non-FH healthy controls (119 from Spain and 23 from Norway). An FH-associated genetic variant was present in 81% of Spanish children with FH and all Norwegian children with FH. A ^{1}H-NMR based advanced lipoprotein test (Nightingale®) providing information on the particle number, size and lipid composition of 14 lipoprotein subclasses was performed and correlated to the dietary components.

Results: Standard LDL-C, HDL-C and triglycerides were not significantly different between the Nordic and Mediterranean FH groups. Spanish children with FH had more LDL particles, mainly of the large and medium LDL subclasses, than Norwegian children. Spanish FHs also had more HDL particles, mainly medium and small, than Norwegian FHs. The mean LDL size of Spanish FH patients was larger, while the HDL size was smaller than that of the Norwegian patients. The HDL particle number and size were the main determinants of differences between the two groups. In Norwegian

children with FH, dietary total fat and MUFAs showed a significant correlation with all apolipoprotein B-containing lipoproteins and LDL size. A weaker association pattern was observed in Spanish children.

Conclusions: The lipoprotein profiles of Spanish and Norwegian children showed differences when studied by ^{1}H-NMR. These differences are in part associated with differences in diet patterns.

Key words: Familial hypercholesterolemia, Children, Nordic diet, Mediterranean diet, NMR lipid profile

INTRODUCTION

Heterozygous familial hypercholesterolemia (HeFH) is a relatively common inherited disorder caused by genetic variants in the low-density lipoprotein (LDL) receptor or functionally related genes (1). Children born with this genetic condition are at increased risk for atherosclerotic cardiovascular disease (CVD). During childhood, HeFH is asymptomatic and is detected by high cholesterol levels and family history. Implementing screening strategies for familial hypercholesterolemia (FH) detection in children leads to early diagnosis and appropriate treatment, resulting in a better prognosis (2,3).

Although LDL-C elevation is unequivocally the main determinant of cardiovascular risk (CVR) in FH, a significant variability in the incidence of CVD events has been reported in FH patients, even among those carrying the same genetic mutations and comparable LDL-C levels (4,5). Lipid and lipoprotein metabolism is very complex, and the usual clinical approach to assess lipoprotein profiles, based on the direct biochemical determination of total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) and triglycerides (TGs) and the calculation of low-density lipoprotein cholesterol (LDL-C), is limited. In recent years, proton-nuclear magnetic resonance (^{1}H-NMR) techniques have been implemented to determine the number and size of lipoproteins (6,7). These techniques allow us to obtain more extensive and detailed information on lipoprotein metabolism. It has been shown that knowing the amount and size of lipoprotein particles provides more accurate information about CVR (8–10). Current guidelines reinforce the importance of lipoprotein number on atherosclerosis pathogenesis and recommend the measurement of apolipoprotein B

(ApoB), a surrogate of atherogenic particle number, for a better CVR definition (8,9,11).

Even though the high cholesterol levels in HeFH children are driven by gene variants, environmental factors could modulate the lipoprotein phenotype. The influence of diet on CVR was well established many years ago (12–14). Dietary factors influence the development of CVD either directly or through their action on traditional CVR factors. There are several dietary components involved in lipoprotein quantity and quality. Among them, the total amount and type of fat in the diet appears to be an important determinant. Both Nordic and Mediterranean diets have been proposed to improve cardiovascular risk despite striking differences between them. In a previous study (15), we showed that Norwegian children consumed more polyunsaturated fatty acids (PUFAs) and, in contrast, Spanish children consumed more monounsaturated fatty acids (MUFAs), cholesterol and fiber. The aim of this study was to assess the differences in the lipoprotein subclass particle number and size as evaluated by ^{1}H-NMR in children with FH from Norway and Spain and to evaluate the correlations of Nordic and Mediterranean diet components with the lipoprotein profile.

MATERIALS AND METHODS

Study design and participants

This is a cross-sectional study. Children with and without FH from two established cohorts from Spain and Norway were selected for the study. We obtained information

about the full lipoprotein profile as assessed by ^{1}H-NMR (7) and dietary intakes of these FH children and compared the differences between the Spanish and Norwegian children.

The recruitment method and clinical characteristics of the study participants have been described previously (16–18). Two hundred fifty-six children with and without FH (N=114 and N=142, respectively), aged 4 to 18, were recruited from the lipid clinics at Oslo University Hospital (Oslo, Norway) and the "Sant Joan" University Hospital (Reus, Spain). Twenty-nine untreated Norwegian HeFH children with FH-associated genetic variants were recruited between September and December 2013. Healthy children (non-FH) included in the Stork-child study (2014-2015) with full diet composition data available were included as controls (n=23)_. Eighty-five Spanish children with FH were recruited from the DECOPIN Project (The Early Familial Hypercholesterolemia Detection Project) (18) from March 2013 to May 2019. The diagnosis criteria were a positive genetic test (81%) or LDL-C >4.14 mmol/L and one parent with definite FH if a genetic test was unavailable. Children evaluated for suspected FH who did not meet the FH criteria and had an LDL-C below 135 mg/dl composed the Spanish non-FH group (n=122). The exclusion criteria were the presence of secondary hyperlipidemia, severe chronic disease and other conditions that could alter nutrition or lipid metabolism.

Data on anthropometry, medical antecedents and physical examination were obtained from medical records. Standard biochemical measurements and plasma lipid profiles by ^{1}H-NMR were performed. A comprehensive study of the participants' diets was also performed (see below for details).

All participants were lipid-lowering therapy naive. Before study participation, one of the parents (or the child, if older than 16 years old) signed the informed consent

form. The study was approved by the Regional Committee for Medical and Health Research Ethics (East region of Norway) and by the Ethics Committee of the Hospital Research Institute in Reus (Spain). The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Standard laboratory studies

In the Norwegian group, the nonfasting blood samples for biochemical tests were obtained, while in the Spanish group, analyses were performed in samples obtained after overnight fast. TC and TG levels were determined by enzymatic colorimetric tests, HDL-C was measured by a direct enzymatic colorimetric method, and apolipoprotein levels were measured by immunoturbidimetric assays. LDL-C levels were calculated by the Friedewald equation for the Spanish samples and by a direct enzymatic method for the Norwegian group.

Advanced lipoprotein profiling by ^{1}H-NMR

Nightingale's Health ® (Finland) method was used to study the number and size of the different lipoprotein subclasses. This method gives a detailed metabolic profile, including the particle number of the main lipoprotein subclasses (VLDLP, IDLP, LDLP, and HDLP) and their mean size (VLDL-Z, LDL-Z, and HDL-Z). It also provides the particle number of 14 different lipoprotein subclasses: 6 VLDL [extremely large (XXL), very large (XL), large (L), medium (M), small (S) and extra small (XS)], 1 IDL, 3 LDL [L, M, S] and 4 HDL [XL, L, M, S]). The lipid cargo of all these fractions, including free and esterified cholesterol, triglycerides and phospholipids, was also determined. The method has been extensively described and used in several recent publications (17,19–22).

Dietary intake evaluation

The diet assessment method has been previously reported (15). In brief, the diet data were collected from children and families by registered nutritionists in both cohorts during the first medical visit. The diets of both countries were analyzed based on two validated food frequency questionnaires (FFQs). The Norwegian FFQ contains a list of 277 food items (23–26), and the Spanish FFQ contains a list of 137 food items (27–30).

In the Norwegian cohort, the daily intake of energy and nutrients was computed using the AE-14 food database and the "kost beregnings system" software (version 7.1, year 2014).

In the Spanish cohort, the frequencies of consumption were reported on an incremental scale with nine levels (never or almost never, 1-3 times per month, once per week, 2-4 times per week, 5-6 times per week, once per day, 2-3 times per day, 4-6 times per day and more than 6 times per day). The reported frequencies of food consumption were converted to the number of intakes per day and multiplied by the weight of the portion size indicated.

Validated photographic booklets were used for the estimation of portion size in both cohorts. To compare the diet components in both cohorts, the macronutrient composition of the diets was calculated from the questionnaire information.

Statistical analysis

Continuous data are shown as the median (25^{th}-75^{th} percentile) for skewed data or as frequencies (percentage) for categorical data. Kolmogorov-Smirnov tests were used to ensure normality. The Mann-Whitney test was used to detect significant differences between groups. The Chi square test was used to compare categorical variables. The lipid profile and lipoprotein particle number and size comparisons between groups were analyzed by ANCOVA and adjusted by age. The associations between lipoprotein profile and diet components were analyzed using a partial correlation adjusted for age. We considered a significant partial correlation coefficient to be >0.400 of clinical interest.

To assess the relationships between lipid variables and patient provenance, we carried out a series of multivariate models. To do so, we divided our dataset into a training set consisting of 80% of the patients in which models were constructed and a test set consisting of the other 20% of the patients to evaluate model performance. The models included three types: logistic regressions regularized via elastic net, random forests and boosted models. For each scenario, we trained several versions of each type of model, adjusted their parameters via 5-fold cross-validation and chose the model that performed best. This model was then evaluated on the test set, and its performance is the one that we report.

To assess the effect of the predictive variables on the target, we carried out a dual approach. Random forests and boosted models proved to be more accurate models for such complex scenarios (31) and furthermore allowed us to assess the relative importance of each variable against all others via out-of-bag accuracy before and after variable permutation (32). Both models are slightly different in variable selection but

close enough to be able to combine them to draw meaningful conclusions. A receiver operating characteristic (ROC) curve based on a model including the particle number of all lipoprotein subclasses was performed to estimate the differences between both cohorts.

All analyses were performed using the SPSS 25.0 statistical package for Windows (SPSS, IBM®, Chicago, IL) and the R statistical program version 4.0 (R Core Team, 2014). A *p*-value <0.05 was considered statistically significant in all analyses.

RESULTS

In Table 1, we show the baseline characteristics of Norway and Spain FH and non-FH children. Spanish children with FH were younger than the other groups. No significant differences were observed in sex or z-score body mass index (z-score BMI). Regarding the standard lipid profile, nonsignificant differences were observed between Norwegian and Spanish FH children. Significantly higher concentrations of total, LDL and HDL cholesterol were observed in the non-FH Spanish children than in the non-FH Norwegian group (p<0,0001, p<0,0001 and p=0,024, respectively).

The lipoprotein particle number and size distribution sorted by country and diagnosis are shown in Table 2. Despite showing no differences in the standard lipid profile, the Spanish FH children had a significantly higher number of LDL particles (p=0,038), mainly the L_LDL (p=0,032) and M_LDL subclasses (p=0,031). They also showed a higher number of HDL particles (p<0,0001), mainly the M_HDL (p=0,004) and

S_HDL subclasses (p<0,0001). Overall, the LDL medium size was smaller in the Norwegian FH children (p<0,0001), while they had larger HDLs (p=0,003).

The abovementioned differences were further confirmed by a gradient boosting analysis (figure 1), where the HDLP number, mainly the smaller HDLP, was the main determinant of lipoprotein metabolism differences between Norwegian and Spanish FH cohorts (Figure 2). An ROC curve based on a model, including only the particle number of VLDL, LDL and HDL and their subclasses, discriminated both cohorts with a significant specificity and sensitivity (AUC: 0.856) (Figure 3). A huge amount of information was obtained from the lipid cargo of lipoprotein subfractions; however, all data derived from them were aligned with the particle number, not providing any additional information (Supplementary Table 1).

Figure 4 illustrates the correlations between lipoprotein particle number and size and the macronutrients (total percentage of fat, MUFAs, PUFAs and daily consumption of fiber and cholesterol) that we had already identified as significantly different between cohorts in a previous study (15). While the association between nutrients and lipoprotein parameters was rather weak in the Spanish groups, total fat, MUFAs and fiber showed robust associations with several lipoprotein parameters in the Norwegian FH group. Total fat and MUFAs show a relatively strong direct association (“r” value above 0.400) with all ApoB-containing lipoprotein particle numbers [VLDL (mainly smaller subfractions), IDL and all LDL subfractions]. Fiber consumption was directly associated with VLDL size and inversely associated with mean HDL size and M_HDL.

DISCUSSION

In this study, we provide new information on the lipoprotein profile as assessed by 1H-NMR in children with FH from two different environments, Nordic and Mediterranean. Beyond standard techniques, ^{1}H-NMR quantifies the particle number, size and lipid composition of the main lipoprotein families and up to 14 particle subclasses. The first observation is that despite a similar standard profile, ^{1}H-NMR analysis detected significant differences between both populations. Both the Norwegian and Spanish FH children, as expected, had a higher absolute number of LDL particles than the non-FH children (33). These differences were observed in all subclasses, including S_LDL. However, there were no differences in the overall distribution within subclasses (34). These data do not support that the LDL receptor (LDLR) dysfunction has a stronger impact on S_LDL particles. These kids had more S_LDL particles because they had more LDL overall (supplementary figure 1). In fact, the mean LDL size is significantly higher in children with FH than in children without FH in both countries, supporting that the vascular effects in FH patients are driven by an increased number of all LDL subclasses rather than specific accumulation of more atherogenic subclasses. On the other hand, we also observed a higher level of VLDL and IDL particle number in FH children, which was not detected by standard measurements. Taking into account that LDLR also takes part in remnant and IDL clearance, this result should not be surprising despite not usually being captured by standard analyses. The higher concentration of triglyceride-rich lipoproteins (TRL), even at subclinical levels in children, could account for an added CVR in FH. Recently, several studies have confirmed the impact of cholesterol carried by TRL on CVR (35,36). Furthermore, the Copenhagen study, using

the same NMR method, showed that that VLDL particles are more atherogenic than LDL in terms of particle to particle (37).

When comparing the Norwegian and Spanish FH groups, after age adjustment, a significant difference in LDL particle number was observed. Spanish children had more LDL particles of all subclasses according to differences in LDL-C, although nonsignificant was higher in the Spanish group. Interestingly, this difference was not observed in the S_LDL; moreover, the mean diameter of LDL was larger in the Spanish group. An important difference between the Norwegian and Spanish children with FH was the HDL particle number concentration. The Spanish children with FH had a nonsignificant trend toward high HDL-C and ApoA concentrations (Table 1); however, the HDL particles, particularly M_HDL and S_HDL, and the mean HDL diameter were significantly higher (Table 2 and Figure 2). In the random forest analysis, the main identifier of the Spanish vs, Norwegian FH groups was the concentration of HDL particles and S_HDL, as confirmed by the gradient boosting model (figure 1). According to the ROC curve, a model based on lipoprotein subclasses predicted either the Norwegian or Spanish FH groups with a significant sensitivity and specificity (AUC= 0.856) (figure 3). Again, the HDL particle number plays the main role in this calculation. Although have more HDL and large LDL particles could be associated with a better vascular prognosis, the reasons and clinical significance of these differences remain to be clarified.

Several differences were observed between the non-FH groups from both countries. However, the recruitment differences in both countries (general population in Norway and attending the lipid clinic in Spain) preclude the discussion of these differences.

One aim of our study was to assess the impact of two diverse diets (Nordic and Mediterranean) on the lipid profile of children with FH. We reported that Norwegian children consumed more PUFAs, while Spanish children had more MUFAs, fiber and cholesterol in their diets. The discrepancy between these two dietary patterns could contribute to the lipoprotein profile differences. However, when we studied the associations between macronutrients and advanced lipoprotein profile components, we observed several robust correlations only in the Norway FH group and only weak associations in the Spanish cohort. After adjusting for age, total fat, but mainly MUFAs, was directly correlated with all ApoB-containing lipoproteins. The direct correlation between MUFAs and LDL-C was recently reported in a study based on UK Biobank data (38). This correlation was not observed in the Spanish FH group, where the association between MUFAs and atherogenic particles was very weak or even inverse. This discrepancy can probably be explained by the total amount of MUFAs consumed by Spanish kids. They consumed more MUFAs than Norwegian children, which could drive the differences in cholesterol levels between cohorts, but intragroup variation is probably not enough to determine differences in the lipid profile subclasses. Another explanation could be the type of foods providing fats in both dietary patterns. While in the Nordic diet, MUFAs are provided by foods also rich in saturated fats, in the Mediterranean diet, MUFAs are mainly provided by olive oil and nuts. On the other hand, in Norway, the amount of MUFAs is indirectly associated with PUFAs; therefore, these associations could indicate an inverse effect of PUFAs on atherogenic lipoprotein particles.

Although the association between macronutrients and lipoprotein profile components could provide some clues based on observed differences, the main conclusion to draw from our data is that, beyond isolated macronutrients, two perfectly defined different dietary patterns, Nordic and Mediterranean, have an impact on the lipoprotein profile of children with FH. Recent epidemiological data have confirmed that both European regions are considered low risk for cardiovascular disease based on standardized incidence data (39), suggesting that lifestyle factors, including nourishment habits, in both Norway and Spain are healthy.

Our work has several limitations and some unique strengths. The FH sample size is relatively small. The recruitment methods and sample collection were not equally designed between groups because we analyzed pre-existing cohorts. On the other hand, the comparison between FH of two different environments of Nordic and Mediterranean gives a unique strength to our results. The comprehensive diet composition analysis also provides robustness to our data. Finally, advanced lipoprotein analysis with ^{1}H-NMR gives a unique deeper view of lipoprotein metabolism in these FH groups.

In conclusion, children with FH exposed to different diets and environments, Nordic vs. Mediterranean, have subtle metabolic differences than are not catched by standard analysis, involving all atherogenic particles. HDL particles are also different between Spanish and Norwegian children with FH. The beneficial impact of Nordic and Mediterranean dietary patterns on CVR is probably mediated by different effects on the lipoprotein profile. Healthy diets based on food patterns rather than nutrient

proportions should be designed according to local cultural traditions to increase adherence and effectiveness.

References:

1. Wiegman A, Gidding SS, Watts GF, Chapman MJ, Ginsberg HN, Cuchel M, et al. Familial hypercholesterolæmia in children and adolescents: Gaining decades of life by optimizing detection and treatment. Eur Heart J. 2015;36:2425–37.

2. Luirink IK, Wiegman A, Kusters DM, Hof MH, Groothoff JW, De Groot E, et al. 20-Year follow-up of statins in children with familial hypercholesterolemia. N Engl J Med. 2019;381:1547–56.

3. Tada H, Takamura M, Kawashiri M. Familial Hypercholesterolemia: A Narrative Review on Diagnosis and Management Strategies for Children and Adolescents. Vasc Health Risk Manag. 2021;17:59-67.

4. Santos RD. Phenotype vs. genotype in severe familial hypercholesterolemia: What matters most for the clinician. Curr Opin Lipidol. 2017;28:130–5.

5. Bianconi V, Banach M, Pirro M. Why patients with familial hypercholesterolemia are at high cardiovascular risk? Beyond LDL-C levels. Trends Cardiovasc Med. 2021;31:205–15.

6. Mallol R, Amigó N, Rodríguez MA, Heras M, Vinaixa M, Plana N, et al. Liposcale: a novel advanced lipoprotein test based on 2D diffusion-ordered 1H NMR spectroscopy. J Lipid Res. 2015;56:737–46.

7. Soininen P, Kangas AJ, Würtz P, Suna T, Ala-Korpela M. Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. Circ Cardiovasc Genet.2015;8:192–206.

8. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J. 2019;41:111-118.

9. Jellinger PS, Handelsman Y, Rosenblit PD, Bloomgarden ZT, Fonseca VA, Garber AJ, et al. American Association of Clinical Endocrinologists and American College of Endocrinology guidelines for management of dyslipidemia and prevention of cardiovascular disease. Endocr Pract. 2017;23:1–87.

10. Mora S, Buring JE, Ridker PM. Discordance of low-density lipoprotein (LDL) cholesterol with alternative LDL-related measures and future coronary events. Circulation. 2014;129:553-61.

11. Standards of Medical Care in Diabetes—2021. Diabetes Care. 2021;44:S1.

12. Dalen JE, Devries S. Diets to prevent coronary heart disease 1957-2013: What have we learned?. The American Journal of Medicine. 2014;127:364-369.

13. Eckel RH, Jakicic JM, Ard JD, De Jesus JM, Houston Miller N, Hubbard VS, et al. 2013 AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: A report of the American College of cardiology/American Heart Association task force on practice guidelines. Circulation. 2014;129:S76-S99.

14. Dai H, Much AA, Maor E, Asher E, Younis A, Xu Y, et al. Global, regional, and national burden of ischaemic heart disease and its attributable risk factors, 1990–2017: results from the Global Burden of Disease Study 2017. Eur Hear J. 2020; qcaa076.

15. Rodríguez-Borjabad C, Narveud I, Christensen JJ, Ulven SM, Malo AI, Ibarretxe D, et al. Dietary intake and lipid levels in Norwegian and Spanish children with familial hypercholesterolemia. Nutr Metab Cardiovasc Dis. 2021;31:1299-1307.

16. Torvik K, Narverud I, Ottestad I, Svilaas A, Gran JM, Retterstøl K, et al. Dietary counseling is associated with an improved lipid profile in children with familial hypercholesterolemia. Atherosclerosis. 2016;252:21–7.

17. Christensen JJ, Ulven SM, Retterstøl K, Narverud I, Bogsrud MP, Henriksen T, et al. Comprehensive lipid and metabolite profiling of children with and without familial hypercholesterolemia: A cross-sectional study. Atherosclerosis. 2017;266:48–57.

18. Ibarretxe D, Rodríguez-Borjabad C, Feliu A, Bilbao J, Masana L, Plana N. Detecting familial hypercholesterolemia earlier in life by actively searching for affected children: The DECOPIN project. Atherosclerosis. 2018;278.

19. Soininen P, Kangas AJ, Würtz P, Suna T, Ala-Korpela M. Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. Circ Cardiovasc Genet. 2015;8:192–206.

20. Würtz P, Havulinna AS, Soininen P, Tynkkynen T, Prieto-Merino D, Tillin T, et al. Metabolite profiling and cardiovascular event risk: A prospective study of 3 population-based cohorts. Circulation. 2015;131:774–85.

21. Kujala UM, Mäkinen VP, Heinonen I, Soininen P, Kangas AJ, Leskinen TH, et al. Long-term leisure-time physical activity and serum metabolome. Circulation. 2013;127:340–8.

24. Wang Q, Würtz P, Auro K, Mäkinen VP, Kangas AJ, Soininen P, et al. Metabolic profiling of pregnancy: Cross-sectional and longitudinal evidence. BMC Med. 2016;14:1–14

23. Lillegaard ITL, Andersen LF. Validation of a pre-coded food diary with energy expenditure, comparison of under-reporters v. acceptable reporters. Br J Nutr. 2005;94:998–1003.

24. Lillegaard ITL, Øverby NC, Andersen LF. Can children and adolescents use photographs of food to estimate portion sizes? Eur J Clin Nutr. 2005;59:611–7.

25. Lillegaard ITL, Løken EB, Andersen LF. Relative validation of a pre-coded food diary among children, under-reporting varies with reporting day and time of the day. Eur J Clin Nutr. 2007;61:61–8.

26. Andersen LF, Pollestad ML, Jacobs DR, Løvø A, Hustvedt B-E. Validation of a pre-coded food diary used among 13-year-olds: comparison of energy intake with energy expenditure. Public Health Nutr.2005;8:1315–21.

27. Martin-Moreno JM, Boyle P, Gorgojo L, Maisonneuve P, Fernandez-Rodriguez JC SS. Development and Validation of a Food Frequency Questionnaire in Spain. Int J Epidemiol. 1993;22:512–9.

28. Vázquez C, Alonso R, Garriga M, de Cos A, de la Cruz JJ, Fuentes-Jiménez F, et al. Validation of a food frequency questionnaire in Spanish patients with familial hypercholesterolaemia. Nutr Metab Cardiovasc Dis.2012;22:836–42.

29.Vioque J, Garcia-de-la-Hera M, Gonzalez-Palacios S, Torres-Collado L, Notario-Barandiaran L, Oncina-Canovas A, et al. Reproducibility and Validity of a Short Food

Frequency Questionnaire for Dietary Assessment in Children Aged 7–9 Years in Spain. Nutrients. 2019;11:933.

30. Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med. 2013;368:1279–90.

31.Couronné R, Probst P, Boulesteix AL. Random forest versus logistic regression: a large-scale benchmark experiment. BMC Bioinformatics.2018;19:270.

32. Breiman, L. Random Forests. Machine Learning. 2001;45:5–32.

33. Rodríguez-Borjabad C, Malo AI, Ibarretxe D, Girona J, Heras M, Ferré R, et al. Efficacy of therapeutic lifestyle changes on lipid profiles assessed by NMR in children with familial and non-familial hypercholesterolemia. Clin e Investig en Arterioscler. 2020;32:49–58.

34. Rodríguez-Borjabad C, Ibarretxe D, Girona J, Ferré R, Feliu A, Amigó N, et al. Lipoprotein profile assessed by 2D-1H-NMR and subclinical atherosclerosis in children with familial hypercholesterolaemia. Atherosclerosis. 2018;270:117–22.

35. Castañer O, Pintó X, Subirana I, Amor AJ, Ros E, Hernáez Á, et al. Remnant cholesterol, not LDL cholesterol, is associated with incident cardiovascular disease. J Am Coll Cardiol. 2020;76:2712–24.

36. Balling M, Nordestgaard BG, Langsted A, Varbo A, Kamstrup PR, Afzal S. Small Dense Low-Density Lipoprotein Cholesterol Predicts Atherosclerotic Cardiovascular Disease in the Copenhagen General Population Study. J Am Coll Cardiol .2020;75:2873–5.

37. Johansen MØ, Vedel-Krogh S, Nielsen SF, Afzal S, Davey Smith G, Nordestgaard BG. Per-Particle Triglyceride-Rich Lipoproteins Imply Higher Myocardial Infarction Risk Than Low-

Density Lipoproteins: Copenhagen General Population Study. Arterioscler Thromb Vasc Biol. 2021;41:2063-2075.

38.Kelly RK, Watling CZ, Tong TYN, Piernas C, Carter JL, Papier K, et al. Associations between macronutrients from different dietary sources and serum lipids in 24 639 UK Biobank Study participants. Arterioscler Thromb Vasc Biol. 2021;41:2190-2200

39.SCORE2 working group and ESC Cardiovascular risk collaboration. SCORE2 risk prediction algorithms: new models to estimate 10-year risk of cardiovascular disease in Europe. Eur Heart J. 2021;42:2439-2454.

Table 1. Comparison of demographic characteristics and biochemical measurement about Norwegian and Spanish cohort in children with and without FH.

	NORWEGIAN COHORT (n=52)			SPANISH COHORT (n=204)			FH Norway vs Spain	NoFH Norway vs Spain
	FH (n=29)	Non FH (n=23)	*P*	FH (n=85)	Non FH (n=119)	*P*		
Demographic characteristics								
Age (y)	11,00(8,00-13,00)	10,50(7,80-12,20)	0,448	9,00(6,00-12,00)	11,00(8,00-13,00)	**0,006**	**0,024**	0,706
Gender (% girls)	55,2	52,2	0,520	47,05	55,17	0,589	0,589	0,825
Z-score-BMI	0,32(-0,24-0,91)	0,17(-0,51-0,53)	0,587	0,27(-0,42-0,83)	-0,07(-0,65-0,67)	0,129	0,552	0,408
Biochemical measurements								
TC (mmol/L)	5,80(5,20-7,10)	3,80(3,40-4,50)	**<0,0001**	6,81(5,93-7,82)	4,90(4,35-5,28)	**<0,0001**	0,153	**<0,0001**
LDL-C (mmol/L)	4,10(3,40-5,20)	2,00(1,62-2,52)	**<0,0001**	4,82(3,86-5,70)	2,73(2,33-3,16)	**<0,0001**	0,249	**<0,0001**
HDL-C (mmol/L)	1,40(1,30-1,70)	1,50(1,30-1,80)	0,644	1,53(1,32-1,73)	1,71(1,40-1,97)	**0,001**	0,538	**0,024**
TG (mmol/L)	0,80(0,60-1,10)	0,70(0,50-1,00)	0,389	0,72(0,56-0,99)	0,67(0,52-0,84)	**0,035**	0,190	0,217
ApoB(g/L)	1,20(0,90-1,40)	-	-	1,33(1,15-1,56)	0,89(0,74-0,97)	**0,001**	0,066	-
ApoA (g/L)	1,4(1,3-1,6)	-	-	1,46(1,30-1,57)	1,55(1,39-1,73)	**<0,0001**	0,422	-
Lp(a) (mg/dL)	293,00(154,00-581,00)	-	-	156,26(64,26-675,87)	129,18(45,84-425,03)	0,194	0,934	-

Data are presented as the median (25^{th}-75^{th} percentile). We used Mann-Whitney U test and Chi square.

P-values of biochemical measurements adjusted by age (ANCOVA). All the p-values in bold.

FH, familial hypercholesterolemia; z-score-BMI, z-score body mass index; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Triglycerides, TG; ApoB, apolipoprotein B100; ApoA, apolipoprotein A1; Lp(a), lipoprotein (a).

- Data not available.

Table 2. Lipoprotein particle number and size of Norwegian and Spanish non-FH and FH children.

	NORWEGIAN COHORT (n=52)			CATALAN COHORT (n=204)			FH Norway vs Spain	NoFH Norway vs Spain
	FH (n=29)	Non FH (n=23)	*p*	FH (n=85)	Non FH (n=119)	*p*		
Lipoprotein particle number								
VLDL (nmol/L)	175,00(157,00-235,00)	126,00(100,00-158,00)	**<0,0001**	191,97(167,31-236,43)	154,70(128,07-188,42)	**<0,0001**	0,768	**0,009**
XXL-VLDL (nmol/L)	0,14(0,00-1,00)	0,02(0,01-1,07)	1,000	0,01(0,00-0,52)	0,01(0,00-0,34)	1,000	1,000	1,000
XL-VLDL (nmol/L)	2,55(1,74-4,64)	1,90(1,16-3,50)	0,274	2,56(1,72-3,87)	1,78(0,91-3,08)	**0,005**	1,000	1,000
L-VLDL (nmol/L)	8,96(6,11-13,70)	6,69(5,01-11,20)	0,316	8,66(6,50-12,67)	7,22(4,56-10,44)	**0,013**	1,000	1,000
M-VLDL (nmol/L)	49,50(43,00-65,50)	31,20(22,50-38,80)	**<0,0001**	58,16(47,10-71,91)	43,33(33,59-52,51)	**<0,0001**	0,226	**<0,0001**
S-VLDL (nmol/L)	44,90(39,10-57,50)	34,10(26,50-43,70)	**0,002**	47,77(41,04-60,18)	40,40(32,92-52,42)	**<0,0001**	0,589	**0,017**
XS-VLDL (nmol/L)	73,30(65,30-91,40)	51,90(45,90-62,20)	**<0,0001**	77,29(64,64-88,41)	63,49(50,77-73,55)	**<0,0001**	0,657	**0,004**
IDL (nmol/L)	405,00(355,00-530,00)	256,00(220,00-297,00)	**<0,0001**	479,00(386,55-558,37)	364,17(291,70-425,00)	**<0,0001**	0,397	**<0,0001**
LDL (nmol/L)	1535,75(1300,49-1995,76)	928,00(779,00-1149,56)	**<0,0001**	1859,52(1517,26-2160,51)	1361,62(1153,34-1691,57)	**<0,0001**	**0,038**	**<0,0001**
L-LDL (nmol/L)	883,00(739,00-1121,59)	502,00(425,00-642,00)	**<0,0001**	1086,94(867,68-1265,81)	781,25(657,19-968,41)	**<0,0001**	**0,032**	**<0,0001**
M-LDL (nmol/L)	396,00(344,00-543,00)	240,00(205,00-305,00)	**<0,0001**	492,75(406,48-563,44)	359,83(30,30-443,01)	**<0,0001**	**0,031**	**<0,0001**
S-LDL (nmol/L)	253,00(220,00-319,00)	177,00(147,00-199,00)	**<0,0001**	290,97(245,08-332,20)	225,54(191,76-268,59)	**<0,0001**	0,126	**<0,0001**
HDL (µmol/L)	21,23(20,14-22,68)	21,23(20,14-22,68)	0,212	25,53 (23,42-27,60)	25,85(23,31-27,98)	0,423	**<0,0001**	**<0,0001**
XL-HDL (µmol/L)	0,44(0,38-0,50)	0,37(0,31-0,41)	**<0,0001**	0,40(0,33-0,46)	0,35(0,28-0,46)	0,215	0,074	0,356
L-HDL (µmol/L)	2,57(2,21-3,12)	2,51(1,84-2,81)	0,102	2,61(2,08-2,98)	2,39(1,96-3,33)	0,446	0,783	0,112
M-HDL (µmol/L)	5,20(4,84-6,12)	5,50(4,97-5,98)	0,565	6,05(5,41-6,88)	6,34(5,56-7,46)	**0,023**	**0,004**	**<0,0001**
S-HDL (µmol/L)	13,49(12,79-14,21)	12,86(12,03-14,24)	0,185	16,28(14,82-17,39)	15,84(14,77-17,54)	0,739	**<0,0001**	**<0,0001**
Lipoprotein size								
VLDL-Z (nm)	38,01(37,47-38,64)	37,68(37,09-38,64)	0,780	38,06(37,64-38,48)	37,82(37,38-38,45)	**0,040**	0,497	0,814
LDL-Z (nm)	23,72(23,68-23,77)	23,59(23,55-23,64)	**<0,0001**	23,77(23,74-23,80)	23,73(23,68-23,77)	**<0,0001**	**<0,0001**	**<0,0001**
HDL-Z (nm)	9,75(9,64-9,83)	9,77(9,65-9,82)	0,704	9,66(9,58-9,73)	9,67(9,59-9,80)	0,270	**0,003**	0,203

Data are presented as the median (25th-75th percentile). Significant p values in bold and were obtained by ANCOVA (adjusted by age).

FH, familial hypercholesterolemia; XXL, extremely large; XL, very large; L, large; M, medium; S, small; XS: extra small; VLDL, very low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Z, size.

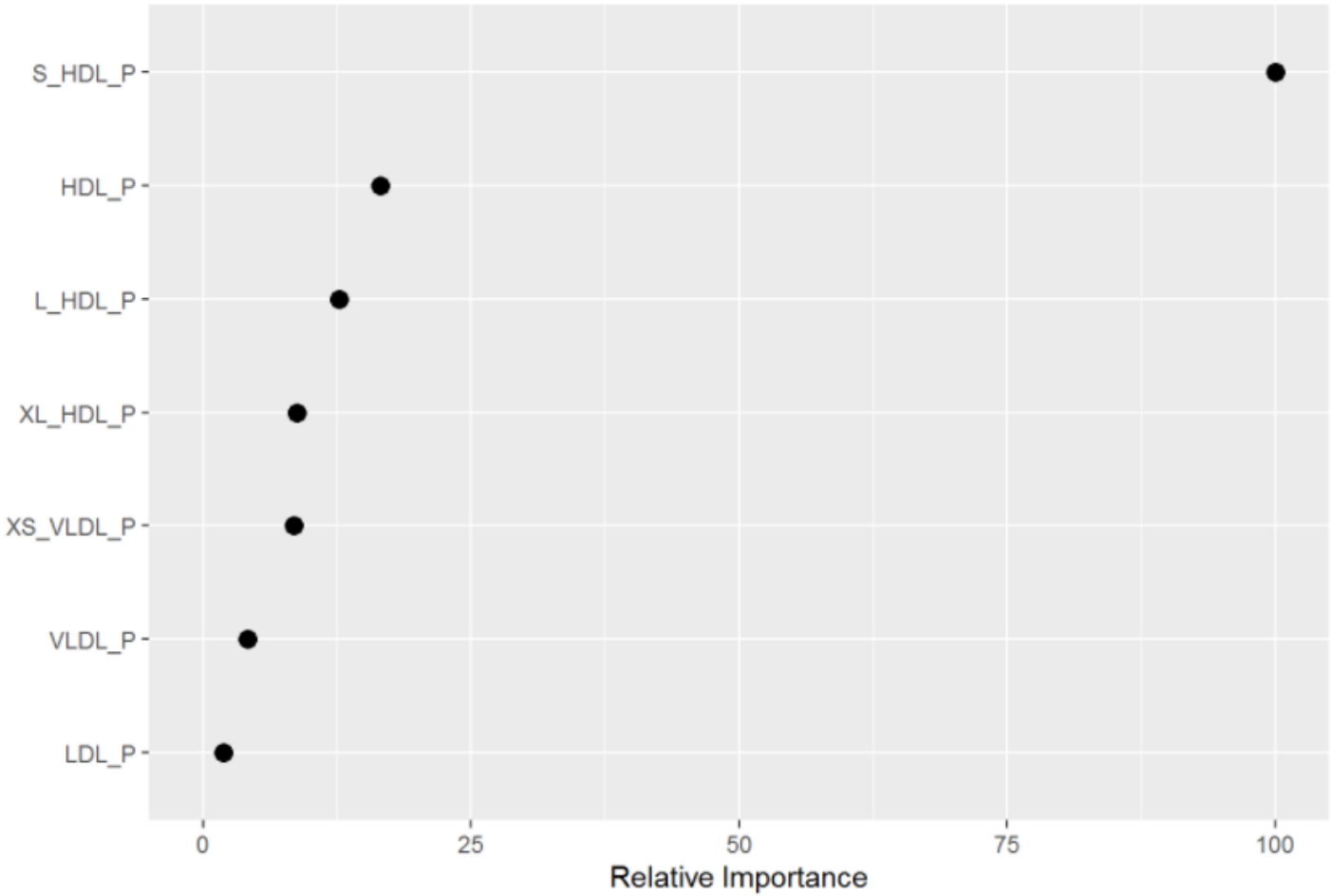


Figure 1. Seven top of the most influence variables from the gradient boosting model.

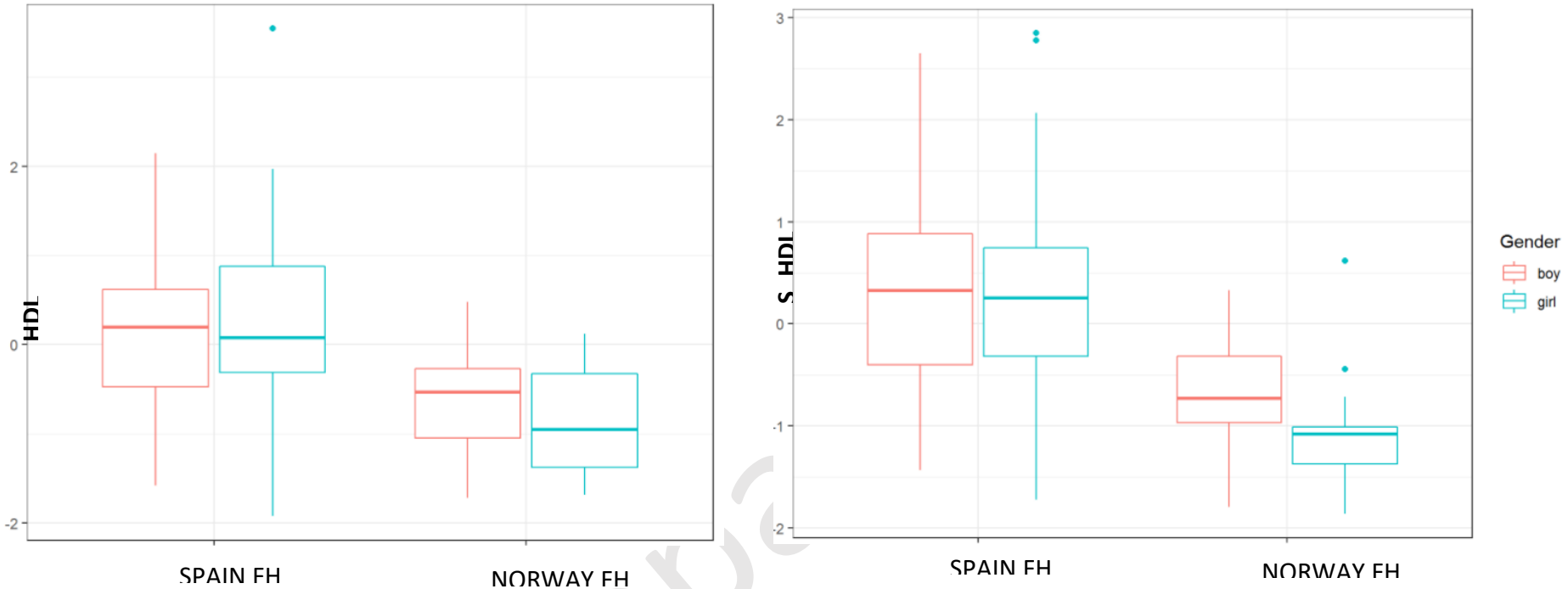


Figure 2. Box plot of HDL particles and S_HDL particles about Norwegian and Spanish FH children.

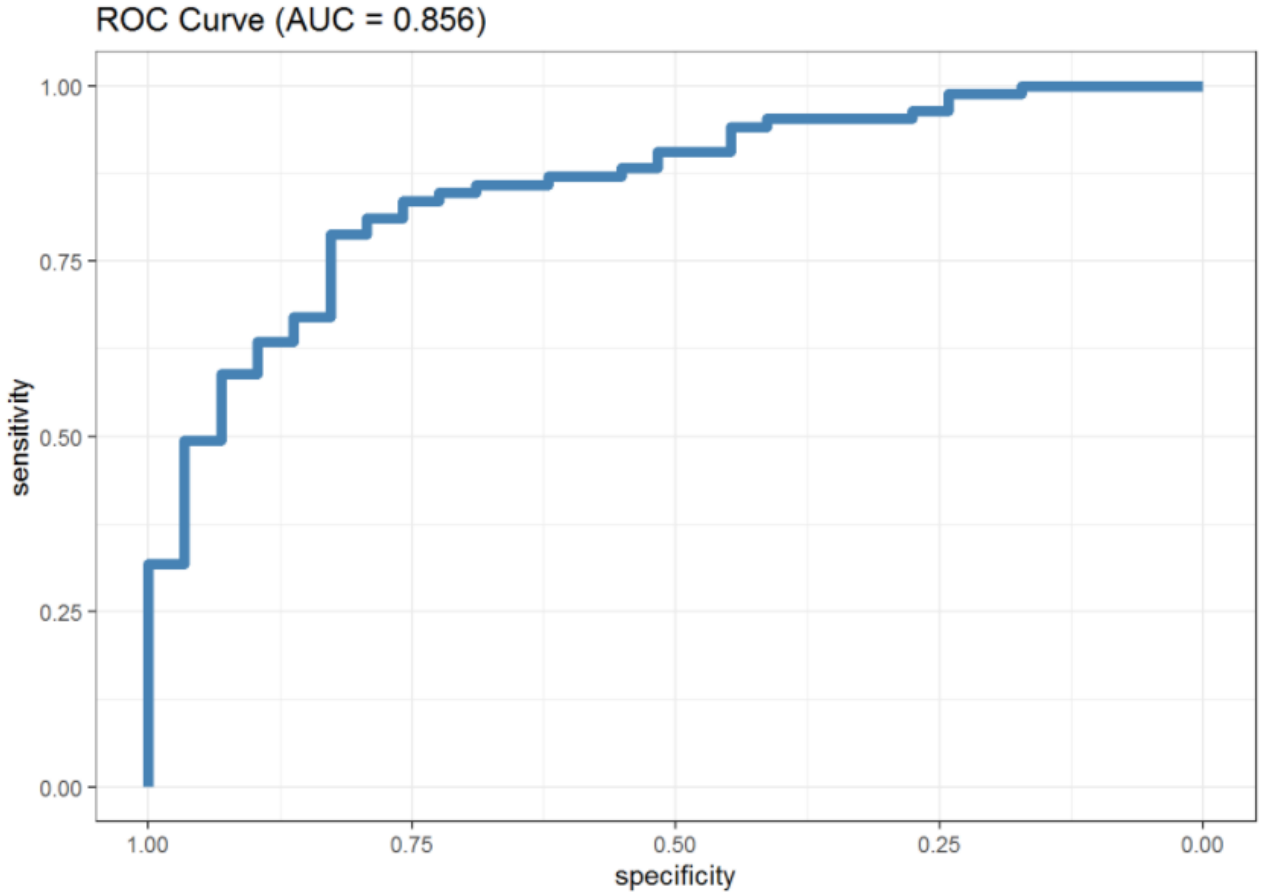


Figure 3. Receiver Operating Characteristic (ROC) curves. ROC curves analysis: Model 1 included VLDL (L_VLDL; S_VLDL; XS_VLDL), IDL, LDL and HDL (XL_HDL; L_HDL; M_HDL; S_HDL) particles. Area under the ROC curve was 0,856.

	NORWAY (N=52)					SPAIN (N=204)				
	% Fat	MUFA	PUFA	Fiber	Cholesterol	% Fat	MUFA	PUFA	Fiber	Cholesterol
VLDL (nmol/L)	**0,289**	0,300	**0,279**	0,145	0,176	0,013	-0,027	0,005	-0,051	-0,047
XXL-VLDL (nmol/L)	0,117	0,156	0,119	0,259	0,179	-0,021	-0,077	-0,111	-0,008	-0,057
XL-VLDL (nmol/L)	0,180	0,222	0,174	0,228	0,197	-0,012	-0,060	-0,081	-0,044	-0,054
L-VLDL (nmol/L)	0,189	0,234	0,156	0,211	0,176	-0,001	-0,050	-0,081	-0,044	-0,052
M-VLDL (nmol/L)	**0,292**	**0,299**	**0,306**	0,160	0,190	-0,013	-0,057	0,012	-0,042	-0,042
S-VLDL (nmol/L)	**0,278**	**0,291**	0,220	0,142	0,140	0,015	-0,023	-0,018	-0,052	-0,051
XS-VLDL (nmol/L)	**0,300**	**0,294**	**0,307**	0,066	0,154	0,045	0,021	0,062	-0,052	-0,034
IDL (nmol/L)	**0,303**	**0,288**	**0,347**	0,076	0,175	0,015	-0,012	0,081	-0,030	-0,019
LDL (nmol/L)	**0,293**	**0,284**	**0,342**	0,122	0,179	-0,019	-0,054	0,053	-0,031	-0,027
L-LDL (nmol/L)	**0,296**	**0,284**	**0,341**	0,107	0,170	-0,015	-0,048	0,058	-0,031	-0,024
M-LDL (nmol/L)	**0,280**	0,276	**0,336**	0,138	0,178	-0,027	-0,064	0,043	-0,029	-0,028
S-LDL (nmol/L)	**0,298**	0,292	**0,348**	0,153	0,213	-0,024	-0,059	0,048	-0,037	-0,037
HDL (µmol/L)	0,041	0,073	-0,042	-0,178	-0,260	**0,145**	0,106	0,003	0,071	0,079
XL-HDL (µmol/L)	0,221	0,180	0,169	-0,076	0,141	0,125	0,128	0,066	-0,017	0,002
L-HDL (µmol/L)	0,101	0,072	-0,007	-0,216	-0,079	**0,163**	**0,157**	0,044	0,018	0,043
M-HDL (µmol/L)	-0,025	0,008	-0,168	-0,292	-0,347	**0,180**	**0,151**	-0,004	0,072	0,089
S-HDL (µmol/L)	0,019	0,067	0,015	-0,022	-0,197	0,054	0,005	-0,019	0,074	0,063
VLDL-Z (nm)	0,031	0,083	0,109	0,191	0,145	-0,054	-0,082	-0,091	-0,019	-0,053
LDL-Z (nm)	0,180	0,148	**0,328**	0,021	0,040	0,016	0,013	0,013	0,015	0,048
HDL-Z (nm)	0,089	0,043	-0,050	-0,230	-0,023	**0,152**	**0,163**	0,045	0,004	0,011

	NORWAY non-FH (N=23)					SPAIN non-FH (N=119)					NORWAY FH (N=29)					SPAIN FH (N=85)				
	% Fat	MUFA	PUFA	Fiber	Cholesterol	% Fat	MUFA	PUFA	Fiber	Cholesterol	% Fat	MUFA	PUFA	Fiber	Cholesterol	% Fat	MUFA	PUFA	Fiber	Cholesterol
VLDL (nmol/L)	0,146	0,181	0,194	-0,259	-0,004	-0,008	0,001	0,093	-0,059	-0,114	**0,398**	**0,455**	0,093	0,050	0,194	0,075	-0,024	-0,052	-0,013	0,081
XXL-VLDL (nmol/L)	0,105	0,180	0,190	-0,045	0,056	-0,002	0,033	-0,009	0,016	-0,171	0,133	0,176	0,044	0,299	0,266	-0,032	-0,192	-0,222	-0,031	0,067
XL-VLDL (nmol/L)	0,124	0,200	0,214	-0,206	0,049	-0,009	0,026	0,014	-0,030	-0,154	0,218	0,270	0,063	0,287	0,294	0,005	-0,142	-0,174	-0,048	0,081
L-VLDL (nmol/L)	0,122	0,199	0,206	-0,229	0,038	0,012	0,047	0,006	-0,041	-0,164	0,238	0,290	0,039	0,285	0,278	0,002	-0,147	-0,168	-0,035	0,095
M-VLDL (nmol/L)	0,175	0,236	0,240	-0,305	0,002	-0,033	-0,040	0,120	-0,043	-0,090	**0,405**	**0,454**	0,096	0,058	0,212	0,049	-0,042	-0,060	-0,010	0,068
S-VLDL (nmol/L)	0,122	0,157	0,159	-0,279	-0,048	0,013	0,036	0,050	-0,070	-0,131	0,380	0,432	0,037	0,082	0,167	0,043	-0,068	-0,067	-0,006	0,084
XS-VLDL (nmol/L)	0,116	0,072	0,106	-0,158	-0,007	-0,006	-0,010	0,127	-0,065	-0,065	**0,441**	**0,503**	0,140	-0,125	0,117	0,153	0,113	0,057	-0,006	0,064
IDL (nmol/L)	0,168	0,154	0,177	-0,204	0,063	-0,027	-0,060	0,165	-0,038	-0,014	**0,462**	**0,512**	0,156	-0,158	0,123	0,114	0,100	0,063	0,018	0,046
LDL (nmol/L)	0,179	0,222	0,235	-0,289	-0,041	-0,056	-0,083	0,163	-0,026	-0,022	**0,437**	**0,480**	0,125	-0,064	0,169	0,062	0,021	-0,002	-0,002	0,033
L-LDL (nmol/L)	0,182	0,215	0,226	-0,282	-0,051	-0,052	-0,083	0,163	-0,027	-0,014	0,447	0,488	0,126	-0,097	0,149	0,068	0,035	0,011	0,001	0,031
M-LDL (nmol/L)	0,143	0,201	0,218	-0,298	-0,058	-0,061	-0,083	0,162	-0,020	-0,031	0,420	0,464	0,121	-0,023	0,188	0,049	-0,004	-0,030	-0,004	0,038
S-LDL (nmol/L)	0,233	0,278	0,288	-0,280	0,037	-0,060	-0,080	0,156	-0,030	-0,039	0,422	0,468	0,129	-0,008	0,213	0,057	0,005	-0,007	-0,011	0,028
HDL (µmol/L)	-0,255	-0,208	-0,300	-0,223	-0,369	**0,200**	0,138	0,032	0,006	0,026	0,241	0,285	-0,045	-0,274	-0,234	0,068	0,053	-0,050	0,163	0,153
XL-HDL (µmol/L)	0,118	-0,017	-0,153	0,045	0,136	0,106	0,085	-0,003	-0,057	0,001	0,313	**0,376**	0,119	-0,374	-0,022	0,172	0,213	0,201	0,057	0,021
L-HDL (µmol/L)	-0,024	-0,120	-0,264	0,000	-0,011	0,168	0,130	-0,026	-0,045	0,017	0,172	0,202	0,017	**-0,408**	-0,240	**0,156**	0,198	0,156	**0,121**	0,081
M-HDL (µmol/L)	-0,280	-0,255	-0,336	-0,142	-0,325	0,232	0,174	-0,014	-0,007	0,025	0,158	0,191	-0,065	-0,341	-0,346	0,098	0,100	-0,017	0,181	0,168
S-HDL (µmol/L)	-0,236	-0,131	-0,162	-0,263	-0,390	0,114	0,059	0,086	0,047	0,021	0,219	0,258	-0,053	-0,046	-0,077	-0,016	-0,061	-0,149	0,116	0,125
VLDL-Z (nm)	0,085	0,177	0,153	-0,280	0,035	-0,010	0,014	-0,022	-0,019	-0,121	-0,031	-0,003	0,017	**0,479**	0,336	-0,104	-0,213	-0,180	-0,004	0,065
LDL-Z (nm)	0,048	0,030	0,003	-0,170	-0,218	-0,023	-0,037	0,031	-0,011	0,073	0,313	0,337	0,237	-0,271	**-0,039**	0,112	0,138	0,036	0,095	0,078
HDL-Z (nm)	0,058	-0,061	-0,168	0,094	0,126	0,152	0,124	-0,055	-0,060	0,003	0,113	0,113	0,031	**-0,399**	-0,216	0,149	0,220	0,201	0,097	0,013

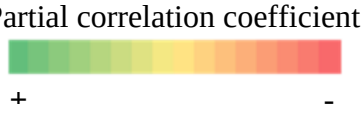


Figure 4, Heatmap showing the correlations between lipoprotein particle number and size and consumption of diferent macronutrients, All values were adjusted by age.

Supplementary table 1. Lipid cargo of 14 lipoprotein subclasses assessed by ^{1}H-NMR.

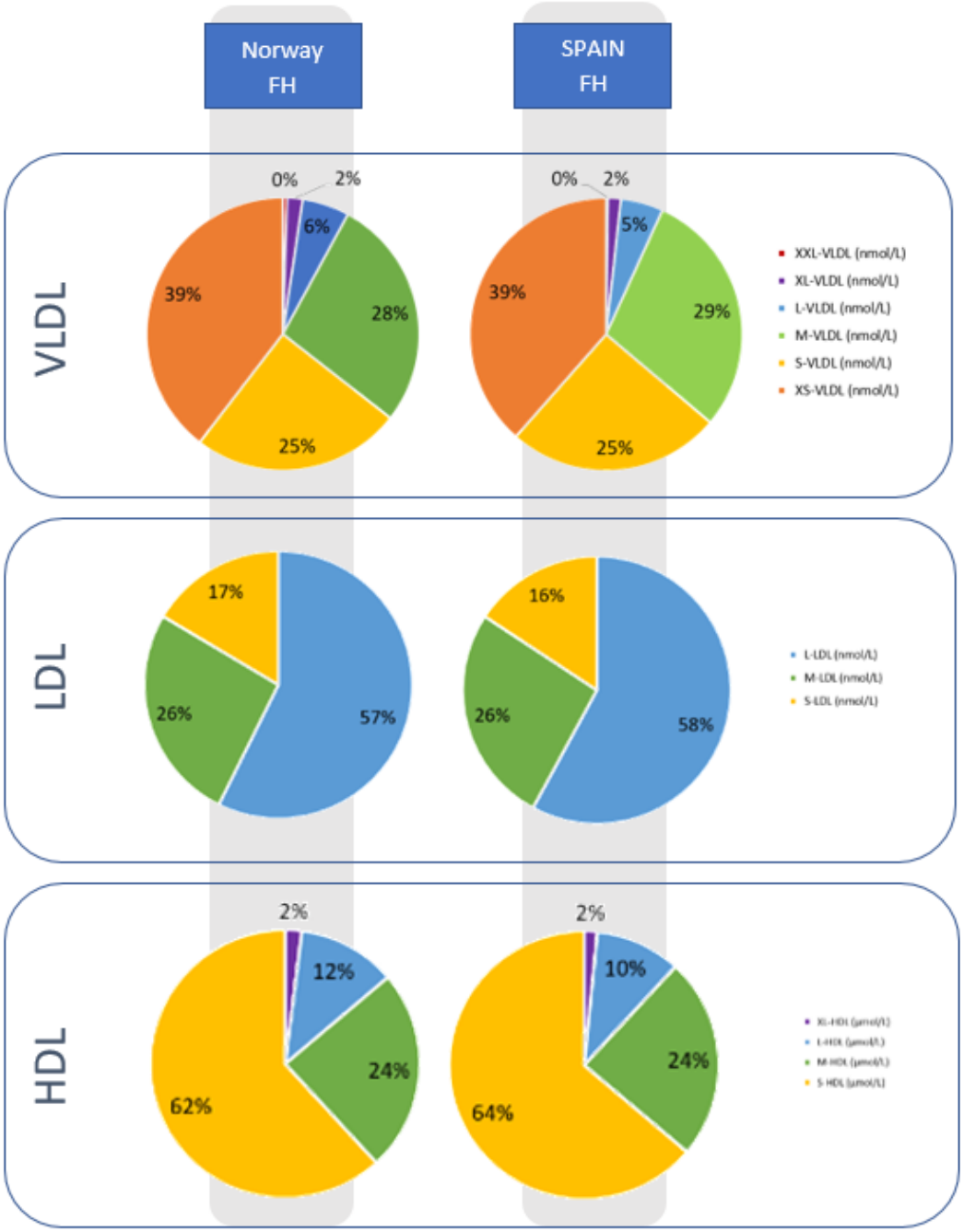
		µmol/L	NORWEGIAN COHORT (n=52) FH (n=29)	NORWEGIAN COHORT (n=52) Non FH (n=23)	*p*	SPANISH COHORT (n=204) FH (n=85)	SPANISH COHORT (n=204) Non FH (n=119)	*p*	FH Norway vs Spain	Non FH Norway vs Spain
VLDL	XXL_VLDL	XXL_VLDL_TL	21,1(0-95,96)	12,22(0,33-109,69)	0,919	6,13(0-59)	5,32(0-38,16)	0,876	0,119	0,112
		XXL_VLDL_PL	0,06(0-6,76)	0,10(0,05-12,09)	0,453	0,04(0-2,69)	0,08(0-1,35)	0,622	0,200	0,120
		XXL_VLDL_C	4,77(0-19,17)	1,01(0,11-22,53)	0,919	0,14(0-20,09)	0,1(0-12,9)	0,908	0,251	0,117
		XXL_VLDL_CE	4,73(0-15,72)	0,92(0,08-14,55)	0,963	0,08(0-15,63)	0,05(0-10,36)	0,841	0,311	0,143
		XXL_VLDL_FC	0,06(0-5,68)	0,09(0,02-10,14)	0,732	0,02(0-3,93)	0,03(0-2,22)	0,898	0,155	0,058
		XXL_VLDL_TG	16,3(0-65,26)	11,21(0,12-72,75)	0,875	2,37(0-36,06)	3,86(0-22,48)	0,912	0,080	0,082
	XL_VLDL	XL_VLDL_TL	120,13(85,06-222,46)	85,98(46,24-153,21)	0,085	121,13(84,17-184,59)	84,69(44,65-139,54)	**0,000**	0,637	0,628
		XL_VLDL_PL	16,42(11,2-32,86)	11,90(7,17-24,34)	0,103	15,8(9,82-27,15)	10,13(4,08-20,71)	**0,000**	0,378	0,297
		XL_VLDL_C	42,15(35,32-71,39)	26,56(18,11-40,41)	**0,002**	49,16(36,44-68,98)	33,82(20,85-48,53)	**0,000**	0,899	0,262
		XL_VLDL_CE	31,23(24,98-50,79)	18,10(12,44-25,30)	**0,000**	35,03(27,79-46,49)	24,43(15,63-33,79)	**0,000**	0,596	0,031
		XL_VLDL_FC	13,86(10,03-26,65)	8,45(4,99-17,47)	**0,040**	13,59(9,09-22,65)	9,1(4,41-16,6)	**0,000**	0,543	0,672
		XL_VLDL_TG	63,07(35,8-106,38)	48,83(20,25-87,82)	0,333	56,88(37,15-83,85)	41,03(21-67,74)	**0,001**	0,472	0,291
	L_VLDL	L_VLDL_TL	237,56(169,04-376,45)	176,26(121,23-287,99)	0,120	235,15(180,31-337,67)	187,95(118,71-270,5)	**0,001**	0,848	0,883
		L_VLDL_PL	38(26,69-61,3)	28,58(19,79-48,50)	0,148	38,61(27,68-57,94)	29,95(17,13-47,85)	**0,002**	0,868	0,733
		L_VLDL_C	77(62,31-121,87)	51,01(36,67-78,38)	**0,008**	86,09(65,86-120,3)	66,53(40,26-95,92)	**0,000**	0,878	0,323
		L_VLDL_CE	46,57(37,81-75,22)	30,66(20,61-43,19)	**0,000**	53,04(41,16-72,02)	38,86(24,76-55,34)	**0,000**	0,843	0,103
		L_VLDL_FC	31,52(23-50,61)	22,25(14,34-37-04)	0,075	34,14(24,36-49,34)	26,05(14,91-39,21)	**0,001**	0,940	0,849
		L_VLDL_TG	122(81,06-179,21)	100,93(60,48-154,62)	0,434	112,27(82,68-149,4)	93,09(62,7-129,75)	**0,003**	0,587	0,427
	M_VLDL	M_VLDL_TL	615,75(507,84-816,37)	414,25(284,39-497,87)	**0,000**	692,53(554,8-842,12)	520,75(400,08-641,33)	**0,000**	0,251	**0,002**
		M_VLDL_PL	133(117,94-182,13)	84,37(60,06-104,21)	**0,000**	160,91(129,82-196,25)	119,81(91,56-145,16)	**0,000**	0,124	**0,000**
		M_VLDL_C	247,76(206,98-345,97)	120,80(94.01-173,23)	**0,000**	310,34(242,81-375,8)	211,48(160,78-266,98)	**0,000**	0,135	**0,000**
		M_VLDL_CE	145,81(119-202,03)	64,99(51,15-94,01)	**0,000**	177,12(141,07-220,48)	121,49(93,06-149,86)	**0,000**	0,150	**0,000**
		M_VLDL_FC	102,79(88,96-145,76)	61,70(44,17-76,31)	**0,000**	128,11(99,8-152,97)	89,48(66,69-110,9)	**0,000**	0,169	**0,000**
		M_VLDL_TG	219,56(157,76-299,95)	170,40(124,03-251,18)	0,107	219(175,85-284,71)	184,79(139,98-232,69)	**0,001**	0,899	0,688
	S_VLDL	S_VLDL_TL	380,06(339,21-502,55)	278,40(213,63-341,42)	**0,000**	418,47(369,63-525,29)	341,34(271,27-431,09)	**0,000**	0,240	**0,002**
		S_VLDL_PL	93,74(84,53-130,85)	66,59(50,52-77,05)	**0,000**	110,16(95,52-136,7)	86,45(69,72-106,18)	**0,000**	0,086	**0,000**
		S_VLDL_C	182,3(159,73-263,74)	115,39(90,71-143,67)	**0,000**	214,08(182,03-268,24)	158,94(126,66-200,71)	**0,000**	0,157	**0,000**
		S_VLDL_CE	107,66(95,61-156,16)	67,33(53,12-85,26)	**0,000**	122,2(101,88-157,7)	93,67(70,98-118,83)	**0,000**	0,256	**0,000**
		S_VLDL_FC	73,57(66,26-107,2)	48,06(37,36-58,18)	**0,000**	92,29(76,32-111,4)	68(53,8-83,78)	**0,000**	0,071	**0,000**

		S_VLDL_TG	111,09(85,17-127,79)	92,29(73,02-120,87)	0,392	101,54(81,34-135,43)	95,47(75,29-121,95)	0,241	0,632	0,801
	XS_VLDL	XS_VLDL_TL	385,55(338,68-480,84)	269,00(243,01-321,95)	**0,000**	391,38(324,79-444,38)	314,32(247,96-360,39)	**0,000**	0,539	0,054
		XS_VLDL_PL	96,94(85,65-123,35)	71,53(64,44-82,84)	**0,000**	96,92(81,73-110,74)	78,74(64,82-89,96)	**0,000**	0,312	0,246
		XS_VLDL_C	241,24(207,89-307,67)	155,85(137,96-187,05)	**0,000**	247,84(203,91-285,54)	190,34(149,62-214,13)	**0,000**	0,605	**0,012**
		XS_VLDL_CE	171,46(147,28-220,83)	109,04(95,07-128,26)	**0,000**	178,99(144,39-208,39)	134,13(103,62-152,73)	**0,000**	0,646	**0,007**
		XS_VLDL_FC	69,32(61,01-88,11)	46,81(42,45-55,30)	**0,000**	69,43(56,59-80,97)	54,52(42,54-64,17)	**0,000**	0,464	0,074
		XS_VLDL_TG	44,38(40,22-52,52)	42,84(39,54-52,64)	0,445	44,9(35,53-52,94)	43,48(36,75-51,41)	0,528	0,513	0,918
IDL	ILDL	IDL_TL	1484,85(1329,51-1890,56)	1037,38(880,88-1180,97)	**0,000**	1722,59(1418,46-1958,37)	1353,84(1135,6-1560)	**0,000**	0,185	**0,000**
		IDL_PL	350,38(316,23-443,43)	252,00(214,37-285,72)	**0,000**	402,7(334,72-460,07)	318,98(271,82-364,55)	**0,000**	0,208	**0,000**
		IDL_C	1060,51(941,47-1378,8)	716,29(605,30-821,09)	**0,000**	1212,74(1022,29-1423,49)	956,43(803,73-1114,24)	**0,000**	0,167	**0,000**
		IDL_CE	761,7(674,7-991,18)	510,96(430,60-587,09)	**0,000**	874,31(735,77-1030,42)	684,72(578,97-807,76)	**0,000**	0,142	**0,000**
		IDL_FC	298,82(266,77-387,62)	205,32(175,61-231,09)	**0,000**	339,83(286,78-393,07)	267,33(225,27-309,27)	**0,000**	0,233	**0,000**
		IDL_TG	77,58(69,28-85,91)	69,09863,99-77,26)	**0,022**	79,32(69,86-91,98)	74,78(66,28-82,32)	**0,003**	0,517	**0,059**
LDL	L_TLDL	L_TLDL_TL	1997,45(1787,9-2526,4)	1330,17(1144,93-1568,88)	**0,000**	2408,73(2023,35-2779,05)	1846,2(1613,37-2219,66)	**0,000**	0,016	**0,000**
		L_TLDL_PL	440(403,88-557,34)	300,50(266,06-352,57)	**0,000**	540,73(458,49-605,63)	421,38(375,43-498,21)	**0,000**	**0,006**	**0,000**
		L_TLDL_C	1472,55(1310,63-1892,68)	954,78(817,28-1156,73)	**0,000**	1790,95(1487,78-2078,83)	1337,57(1165,7-1618,74)	**0,000**	**0,022**	**0,000**
		L_TLDL_CE	1034,39(915,69-1317,78)	685,39(575,49-822,58)	**0,000**	1287,39(1044,64-1467,98)	946,07(811,07-1154,24)	**0,000**	**0,024**	**0,000**
		L_TLDL_FC	438,16(390,65-574,91)	281,20(243,25-337,20)	**0,000**	530,85(440,68-605,61)	407,95(352,73-480,75)	**0,000**	**0,018**	**0,000**
		L_TLDL_TG	75,73(71,67-87,34)	67,18(61,79-75,58)	**0,001**	84,18(77,01-97,08)	77,28(68,59-87,29)	**0,000**	0,067	**0,000**
	M_TLDL	M_TLDL_TL	807,12(705,7-1096,7)	518,96(452,71-633,25)	**0,000**	1001,42(834,12-1138,78)	744,79(634,73-898,59)	**0,000**	**0,015**	**0,000**
		M_TLDL_PL	199,89(175,64-256,89)	132,89(116,45-157,25)	**0,000**	248,5(207,43-280,51)	186,66(163,96-226,9)	**0,000**	**0,007**	**0,000**
		M_TLDL_C	582,65(504,09-796,36)	368,36(317,29-454,42)	**0,000**	725,2(603-832,66)	534,23(450,6-649,64)	**0,000**	**0,016**	**0,000**
		M_TLDL_CE	396,45(343,66-543,1)	247,99(206,72-311,82)	**0,000**	493,33(407,36-567,19)	359,92(304,34-441,86)	**0,000**	**0,021**	**0,000**
		M_TLDL_FC	191,15(163,59-239,26)	119,11(103,93-144,86)	**0,000**	230,93(194,92-259,49)	172,3(151,59-206,91)	**0,000**	**0,008**	**0,000**

		M_TLDL_TG	25,85(22,72-29,68)	21,58(18,97-25,07)	**0,002**	27,27(24,63-31,3)	25,15(21,68-28,24)	**0,000**	0,103	**0,002**
	S_TLDL	S_TLDL_TL	381,38(336,57-483,54)	257,78(228,14-303,33)	**0,000**	445,89(373,99-499,54)	339,91(295,42-401,99)	**0,000**	**0,046**	**0,000**
		S_TLDL_PL	110,2(95,19-132,42)	75,66(69,88-85,59)	**0,000**	120,56(104,55-137,77)	95,25(83,27-109,76)	**0,000**	0,083	**0,000**
		S_TLDL_C	262,27(228,18-337,44)	170,42(152,30-208,18)	**0,000**	310,31(260,87-350,72)	234,6(200,9-278,91)	**0,000**	**0,033**	**0,000**
		S_TLDL_CE	176,42(156,53-231,47)	120,10(102,88-140,24)	**0,000**	211,39(178,39-241,35)	161,49(138,84-194,54)	**0,000**	**0,043**	**0,000**
		S_TLDL_FC	85,89(71,65-99,08)	53,19(49,43-61,46)	**0,000**	95,22(81,54-108,91)	72,02(63,62-85,16)	**0,000**	**0,023**	**0,000**
		S_TLDL_TG	11,12(9,6-13,04)	9,49(7,71-11,69)	**0,008**	11,53(10,17-13,5)	10,42(8,89-11,8)	**0,001**	0,578	0,056
HDL	XL_HDL	XL_HDL_TL	225,34(202,31-244,27)	195,77(166,81-225,38)	**0,017**	194,66(159,18-225,87)	180,42(138,14-222,09)	0,206	**0,002**	0,450
		XL_HDL_PL	99,57(85,06-115,61)	93,29(72,77-108,19)	0,138	82,92(65,81-98,59)	81,06(56,51-106,94)	0,647	**0,001**	0,224
		XL_HDL_C	114,92(104,54-123,87)	99,09(87,50-110,04)	**0,001**	104,68(89,37-119,95)	94,3(77,03-116,23)	**0,039**	**0,016**	0,844
		XL_HDL_CE	81,88(75,45-91,48)	72,84(62,87-80,17)	**0,003**	78,57(66,22-91,56)	71,15(58,11-90,95)	0,073	0,171	0,559
		XL_HDL_FC	30,43(29,09-32,83)	28,71(23,83-29,87)	**0,001**	25,76(22,15-28,8)	22,68(19,58-26,65)	**0,003**	**0,000**	**0,003**
		XL_HDL_TG	5,61(4,69-6,55)	4,43(3,70-5,42)	**0,004**	4,79(4,01-6,17)	4,71(3,75-5,75)	0,158	**0,034**	0,609
	L_HDL	L_HDL_TL	796,49(651,88-951,08)	806,04(621,77-877,70)	0,501	812,39(657,82-912,16)	765,35(639,45-1033,5)	0,636	0,848	0,371
		L_HDL_PL	379,54(302,49-442,33)	383,89(311,04-428,58)	0,804	369,75(295,11-415,35)	355,75(298,28-493,96)	0,352	0,539	0,578
		L_HDL_C	399,81(337,08-470,28)	402,15(302,20-434,18)	0,333	412,9(347,22-480,21)	393,3(321,6-536,91)	0,988	0,684	0,192
		L_HDL_CE	309,23(263,28-353,75)	316,68(237,08-338,38)	0,381	323,52(274,02-379)	314,44(255,04-425,32)	0,915	0,522	0,133
		L_HDL_FC	90,58(78,45-104,52)	84,71(65,13-96,93)	0,124	88,44(71,08-102,08)	79,91(67-111,59)	0,675	0,378	0,505
		L_HDL_TG	21,9(18,92-27)	20,50(17,02-23,83)	0,412	16,06(12,88-21,5)	18,8(13,25-24,15)	0,125	**0,005**	0,239
	M_HDL	M_HDL_TL	972,91(896,6-1107,43)	1050,61(943,00-1113,02)	0,176	1100,57(986,61-1245,14)	1175,2(1033,21-1329,37)	**0,019**	**0,001**	**0,000**
		M_HDL_PL	449,74(397,38-491,79)	470,61(427,18-514,78)	0,148	477,07(433,47-549,64)	519,42(461,3-586,99)	**0,010**	**0,020**	**0,006**
		M_HDL_C	489,04(456,94-591,62)	532,34(490,47-568,99)	0,188	600,96(531,5-675,08)	631,11(542,88-714,54)	0,050	**0,000**	**0,000**
		M_HDL_CE	406,26(363,94-484,43)	438,45(406,11-470,35)	0,124	500,76(441,65-559,52)	526,57(452,14-590,54)	**0,034**	**0,000**	**0,000**
		M_HDL_FC	87,11(80,62-104,3)	90,40(80,73-98,55)	0,993	102,02(90,61-116,29)	104,69(90,46-124,05)	0,179	**0,002**	**0,000**
		M_HDL_TG	31,64(23,94-37,27)	31,14(24,71-42,69)	0,456	27,62(20,85-34,48)	30,73(24,08-37,34)	**0,026**	0,171	0,257
	S_H	S_HDL_TL	1085,12(1023,7-1134,76)	1078,35(990,83-1186,58)	0,775	1250,72(1168,89-1314,87)	1238,93(1170,45-1357,8)	0,777	**0,000**	**0,000**

		S_HDL_PL	605,07(556,88-634,35)	600,20(553,33-659,60)	0,847	674,59(632,66-715,74)	684,46(644,43-745,01)	0,250	**0,000**	**0,000**
		S_HDL_C	448,71(426,87-471,13)	430,52(405,54-480,51)	0,265	536,03(494,49-571,35)	525,07(494,22-575,67)	0,618	**0,000**	**0,000**
		S_HDL_CE	331,26(314,86-350,68)	320,78(301,94-361,87)	0,562	400,84(368,25-428,77)	394,63(371,81-430,24)	0,879	**0,000**	**0,000**
		S_HDL_FC	117,54(110,55-126,33)	110,88(103,60-119,97)	**0,046**	133,6(125,38-139,94)	130,39(122,35-141,28)	0,171	**0,000**	**0,000**
		S_HDL_TG	33,34(27,58-37,7)	32,00(28,24-39,76)	0,789	31,86(26,51-38,12)	31,17(27,17-38,39)	0,757	0,772	0,530

Data are presented as the median (25th-75th percentile). Significant p values in bold and were obtained by Mann-Whitney U test.
XXL, extremely large; XL, very large; L, large; M, medium; S, small; XS: extra small; VLDL, very low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TL, total lipid; PL, phospholipids; C, cholesterol; CE, cholesteryl esters; FC, free cholesterol, TG, triglycerides.



Supplementary figure 1. The percentage of different subfractions in VLDL, LDL and HDL of FH children.

7. Summary of results

7. SUMMARY OF RESULTS

The following results can be extracted from the papers presented in the doctoral thesis:

1. The implementation of active strategies to detect FH in children, in close collaboration with primary care paediatricians, provides a high-performance method for early FH detection. Both direct and inverse cascade screening show a high FH detection yield [in our hands, in two years: 87 FH children (84% genetically positive) and 41 parents (63% genetically positive) diagnosed].

2. Children with FH can be detected early by active screening, thus allowing them to be referred to specialized lipids units to start treatment as soon as possible with TLSC and, in some cases, statins.

3. According to ROC curves, total cholesterol >245 mg/dL or LDL-C >170 mg/dL showed the highest sensitivity and specificity associated with the presence of a positive genetic FH-associated variant. The parameter that shows the best relationship between sensitivity and specificity is an ApoB/ApoA (ROC AUC:0.820).

4. IDOL and PCSK9 plasma levels were higher in FH children [IDOL 116.77(77.55-238.12) pg/mL *p=0.007*; PCSK9 281.14(246.64-329.60) ng/mL *p=0.007*] than in the control group [IDOL 93.18(43.92-158.77) pg/mL; PCSK9 240.55(199.61-287.74) ng/mL]. In contrast, sLDLR levels showed higher concentrations in FH children but did not achieve statistical differences.

5. The absolute number of smaller LDL particles assessed by 1H-NMR was 33% higher in FH children compared with non-FH control children (*p<0.0001*). No differences were observed in the distribution between LDL particles subclasses.

6. The number of either total and smaller LDL particles were significantly associated with a thicker cIMT in the subgroup of adolescents with FH (r=0.407 *p=0.015*; r=0.507 *p=0.002* respectively).

7. A lifestyle education program aimed at younger children with FH and non-FH hypercholesterolemia provides great impact in healthy lifestyles [increase vegetables (*p=0.001*) and reduce sweet products (*p=0.022*) and precooked food (*p=0.029*); physical activity increase >150min/week (*p<0.0001*)] and improves lipid profile even in FH. The implementation of educational programs based on play activities is welcomed by children and families.

8. The educational TLSC strategies impacted positively on the lipid profile of children with FH (TC: -18.58 mg/dL *p=0.010*; LDL-C: -16.11 mg/dL *p=0.011*)

9. The main dietary differences between the Nordic and Mediterranean diets are high amounts of PUFA rich foods versus MUFA rich foods respectively. Spanish children eat more fats, MUFA, cholesterol and fibre and Norwegian children eat more PUFA.

10. The differences between Nordic and Mediterranean diet are not statistically associated with baseline LDL-C levels between Spanish and Norwegian FH children.

11. Correlations between food intake and lipoprotein parameters assessed by ^{1}H-NMR were generally weak. Norwegian FH children have less HDL and LDL particles than Spanish FH children.

8. General discussions

8. GENERAL DISCUSSION

FH is an autosomal dominant genetic disorder characterized by high LDL-C and is the most common monogenic disorder in human. Lifelong exposure to high LDL-C levels results in a markedly increased risk of premature CVD (321). Taking early action with FH children can delay CVD.

One of the biggest problems is that FH is underdiagnosed and undertreated (66). It is important to implement screening methods to detect children with FH. We report the impact of two screening strategies: the Ch-P and the P-Ch pathway. Whereas in the P-Ch pathway we studied the offspring of FH adults (the most common method in clinical practice), in the Ch-P pathway children with high LDL are first detected by paediatricians and are then referred to our specialized unit where we completed the study of their cholesterol. This screening was highly efficient at detecting genetically positive FH children (70% genetically positive test). This percentage is well above that obtained with other strategies and is cost-effective (131,172). Another interesting aspect of the Ch-P pathway is that it has allowed FH detection in entire families.

One issue is that it is sometimes difficult to detect FH because the values overlap and genetic testing is not widely available. Therefore, studying new markers can be useful in clinical practice. There are also two further problems. On the one hand, the detection of FH in the child based on the family history of CVD has low sensitivity and specificity (because the parents are still young and CVD has not yet been identified in them) (322), and on the other hand, the phenotype is very diverse (LDL-C ranging from 130 to >300 mg/dL. As a result, our objective was to search for an advanced lipid profile study that included 1H-NMR assessment of particles and classic and new biomarkers such as the ApoB/ApoA index, IDOL, sLDLR and PCSK9 to help us to between children more likely to be affected by FH.

We found the PURE study and InterHeart study, in which the ApoB/ApoA index was associated with higher CVR. This index is an estimate of the relationship between pro and antiatherogenic lipoprotein particles (323,324). Furthermore, this ratio has been found to be a sensitive and specific marker for the detection of FH; it

has been described with values as low as 0.68 (325) and in our case the value was 0.82. The ratio also showed a positive correlation with cIMT (326).

In recent years, total PCSK9 plasma levels have been exhaustively studied but publications about IDOL and sLDLR are scarce. As expected, total PCSK9 plasma concentrations were higher in FH children due to gene upregulation secondary to low intracellular cholesterol levels. Similarly, IDOL, which is the inducible degrader of LDLR, also showed higher levels in FH children compared with controls. The problem with IDOL is that its role in the circulation and its secretion mechanism are unknown. The putative role of circulating IDOL in FH diagnosis needs more research. On the other hand, sLDLR did not show significant differences between groups. However, a surprising trend was observed of higher levels in FH children and a positive correlation with LDL-associated lipid parameters. This may be considered a paradox because FH is characterized by lower LDLR activity. One explanation could be that although FH children produce fewer functioning receptors, there is a compensatory synthesis rate because of lower intrahepatic cholesterol concentrations. Alternatively, higher LDLR liberation from the liver membranes to plasma, dependent on a cell surface metalloproteinase by unknown regulating mechanisms, could also play a role.

In our study, these three parameters had a positive but low impact on predicting FH with positive gene variants.

Today we know that small LDL particles are the most atherogenic particles and provide us with more information about CVR (53). Cholesterol concentrations are only partially informative in terms of CVR associated with LDL-C. Individuals with a higher proportion of small LDL need more particles to transport the same amount of cholesterol. We observed that FH children had 33% more small LDL particles than the control group. This explains the CVR of these patients. Interestingly, if we look at the proportion of large, medium and small LDL particles, they are the same in both groups. They have more small particles because they have more particles. An association between cIMT and LDL was observed in a subgroup of children over 14 years of age but not in other age ranges.

PCSK9 and sLDLR were significantly correlated with LDL particles subclasses according to NMR data. These associations have been described by our group (327,328).

In addition to looking for new biomarkers to better diagnose FH children, it is important to start treating these children. TLSC are the initial phase of treatment and are essential for management of the disease. TLSC implemented in children with genetically driven hypercholesterolemia has an overall beneficial effect on diet and PA, leading to improvement in the lipoprotein profile. Education is key to changing habits and improving the diagnosis. The best thing about starting TLSC in children is that the changes are easier to assimilate and last over time (66,169). The introduction of a dietitian-nutritionist into clinical practice is of great benefit.

The percentages of fats and SFA were reduced in FH children and the percentage of fibre was increased in the control group. These facts have been associated with an improvement in lipid profile. For many years, importance has been given to the study of macronutrients. However, today it is well known that the most important thing is healthy patterns and that food choices are better than macronutrients because foods do not contain isolated nutrients. In our study we observed an increase in the consumption of fruits, legumes, nuts and fish.

The Mediterranean and Nordic diets have been proposed to improve CVR. These two diets are composed of different foods. Spanish children eat more fats, MUFA, cholesterol and fibre and Norwegian children eat more PUFA and whole grain cereals. Correlations between food intake and lipid parameters was generally weak, however, there were some interesting associations that are worth mentioning. Total fat, mainly MUFAs, was associated with high cholesterol levels in those who consumed the Norwegian diet, but with HDL-C in those who consumed the Spanish diet. Some explanations for these differences can be drawn from an analysis of food groups and patterns. Figure 39 shows these differences.

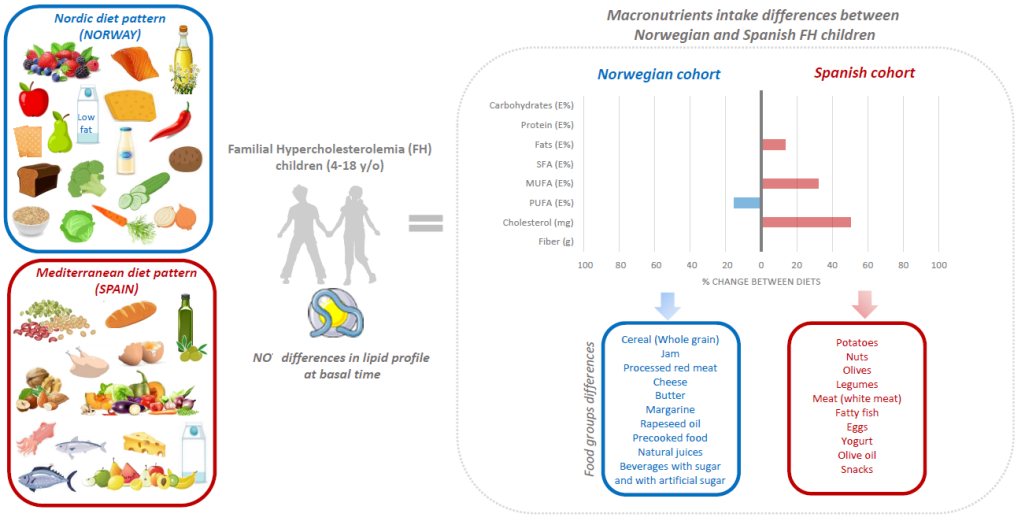


Figure 39: *Image showing differences in Mediterranean and Nordic diets in our FH children.*

PA also improved. Different guidelines recommend that children perform 60 min of PA every day. In our study, the number of children who performed 1hour/day of PA underwent significant increases of 4.6 fold in the FH group and of 4.8 fold in the control group. PA has been associated with an improvement in CVR.

A limitation of our study is the relatively small sample size (although it is the largest cohort of FH children to undergo an NMR lipoprotein profile), the ages ranges, the diagnosis of FH (we include children with clinical criteria of FH, even when their genetic study was negative; such an inclusion might be considered inappropriate) and diet assessments, which are always inaccurate (even though we used validated FFQ and the data acquisition was performed by the same nutritionist).

The strengths of our study is that it provides information about the entire lipid profile and new biomarkers in a huge cohort of FH children and information about the Mediterranean and Nordic dietary patterns, including comprehensive analysis of food groups and their association with lipid profiles in FH children.

9. Conclusions

9. CONCLUSIONS

The present thesis has the following conclusions:

1. FH, the most frequent monogenetic hereditary disorder, is underdiagnosed and undertreated.

2. Early identification of FH, in close collaboration with primary care paediatricians, is essential because of the increased risk of premature CVD. Establishing detection protocols involving paediatricians and lipid specialists is highly effective in early FH detection.

3. Detection of FH during childhood allows early intervention and childhood is the best time to implement healthy habits.

4. IDOL, PCSK9, soluble LDLR and the APOB/APOA ratio can be considered as additional biomarkers for improving the detection of FH-associated genetic variants in children suspected of FH.

5. According to 1H-NMR data, children with FH have a higher number of small LDL particles than control children. This difference is due to an increase in the absolute number of LDL particles and does not reflect a differential increase in the smaller LDL particles over the other subclasses as has been previously suggested.

6. The initial therapeutic approach towards young patients with FH should be TLSC. It is important to start treatment early to avoid complications in adulthood.

7. Educational strategies to implement TLSC in children lead to empowerment, increased adherence and overall metabolic improvement (standard lipid profile and advanced lipoprotein profiles) in hypercholesterolemic children, including those with FH.

8. The implementation of TLCS's educational programs based on play activities is welcomed by children and families and improves diet more than standard of care.

9. The Mediterranean diet and the Nordic diet are two different healthy diets. Our studies suggest that adherence to recommendations based on dietary patterns and healthy foods is more important than ensuring compliance with percentages of macronutrients.

10. These recommendations should be adapted to local dietary habits because eating practices are culturally rooted. Nutritional advice should emphasize the selection of healthy foods among locally prevalent foodstuffs and in line with local eating patterns.

11. The lipoprotein profiles assed by ^{1}H-NMR of Spanish and Norwegian children with FH showed differences in several lipoprotein subclasses that are not observe in standard lipid profile. Dietary differences could play a role even in genetically driven hypercholesterolemia.

In conclusion, early detection strategies and intensive treatment at an early age seem to be a good strategy for improving the prognosis of FH patients. Educational strategies to implement TLSC improve the standard and advanced lipid profile.

We must design common protocols together with paediatricians and harmonize the clinical care of FH children between different health levels to provide better treatment for these patients.

Our data show that healthy habits can be implemented in childhood and that this should lead to an improved prognosis in these patients.

10. Future perspectives

10. FUTURES PERSPECTIVES

Based on the results obtained in this doctoral thesis and, taking into account the scientific literature on the topics discussed, future research could consolidate the results obtained. Future lines of research to consider are detailed below.

Future investigations should use a larger sample of FH children to provide results with greater statistical power. In addition, it is important to follow these children to determine what risk they present in adulthood and if the changes in lifestyle improves their prognosis and reduce their CVR.

Future investigations should demonstrate the effectiveness of screening methods and implement them on a national level. Also, screening methods should be introduced into primary care training and networks should be created to improve detection of children with FH.

Future research should aim to improve current limited understanding of the clinical role of circulating proteins associated with LDLR function (soluble LDLR, IDOL and PCSK9).

There are many factors that influence the adherence to the diet. The present thesis only includes lifestyle, but other studies have also found that socioeconomic factors such as levels of formal education among parents are related to the diet.

Our results show that dietary treatment is important in children with FH to reduce the concentrations of LDL-C and ApoB and the numbers of atherogenic lipoprotein particles. Moreover, given that diet is the first line of therapeutic treatment until lipid-lowering therapy medications can start, more information on the effect of diet is necessary to ensure optimal treatment.

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11. References

11. REFERENCES

1. World Health Organitzation. WHO methods and data sources for the global burden of disease estimates 2000-2016. 2019.

2. Nichols M, Townsend N, Scarborough P, Rayner M. Cardiovascular disease in Europe: Epidemiological update. Eur Heart J. 2013;42: 3028–3034.

3. Timmis A, Townsend N, Gale C, Grobbee R, Maniadakis N, Flather M, et al. Eur Hear J. 2017;39:508–79.

4. Timmis A, Townsend N, Gale CP, Torbica A, Lettino M, Petersen SE, et al. European society of cardiology: Cardiovascular disease statistics 2019. Eur Heart J.2020;41:12–85.

5. Wilkins E, Wilson L, Wickramasinghe K, Bhatnagar P, Leal J, Luengo-Fernandez R, et al. European Cardiovascular Disease Statistics 2017. European Heart Network, Brussels. 2017.

6. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, et al. Global Burden of Cardiovascular Diseases and Risk Factors, 1990–2019: Update From the GBD 2019 Study. Journal of the American College of Cardiology. 2020;76:2982-3021.

7. Doyle JT, Helsin SA, Hilleboe HE, Formel PF KR. A prospective study of cardiovascular disease in Albany: report of three years' experience: ischemic heart disease. Am J Public Heal Nations Heal. 1957;47:25–32.

8. Chapman JM, Goerke LS, Dixon W, Loveland DB PE. Measuring the risk of coronary heart disease in adult population groups, IV: clinical status of a population group in Los Angeles under observation for two-three years. Am J Public Heal Nations Heal. 1957;47:33–42.

9. Keys A, Taylor HL, Blackburn HB, Brozek J, Anderson JT SE. Coronary heart disease among Minnesota business and professional men followed 15 years. Circulation. 1963;28:381–95.

10. Group TPPR. Relationship of blood pressure, serum cholesterol, smoking habit, relative weight and ECG abnormalities to the incidence of major coronary events: final report of the pooling Project. J Chronic Dis. 1978;31:201–306.

11. Dawber TR, Meadors GF MFJ. Epidemiological approaches to heart disease: the Framingham Study. Am J Public Heal Nations Heal. 1951;41:279–81.

12. Mahmood SS, Levy D, Vasan RS WT. The Framingham Heart Study and the epidemiology of cardiovascular disease: a historical perspective. Lancet. 2014;383:999–1008.

13. Brown JC, Gerhardt TE, Kwon E. Risk factors in coronary artery disease [Internet]. Treasure Island (FL). StatPearls Publishing, editor. Risk Factors in Coronary Artery Disease. 2021.

14. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement fromthe European Atherosclerosis Society Consensus Panel. Eur Heart J. 2017;38:2459–72.

15. Penson PE, Pirro M, Banach M. LDL-C: Lower is better for longer - Even at low risk. BMC Med.2020;18:320.

16. Institute for Health Metrics and Evaluation (IHME). Findings from the Global Burden of Disease Study 2017. Seattle, WA: IHME; 2018.

17. GBD 2015 Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016;388:1459–544.

18. Sotos-Prieto M, Bhupathiraju SN, Mattei J, Fung TT, Li Y, Pan A, et al. Association of changes in diet quality with total and cause-specific mortality. N Engl J Med. 2017;377:143–53.

19. Micha R, Peñalvo JL, Cudhea F, Imamura F, Rehm CD MD. Association Between Dietary Factors and Mortality From Heart Disease, Stroke, and Type 2 Diabetes in the United States. 2017;317:921–4.

20. Azadbakht L, Mirmiran P, Esmaillzadeh A, Azizi T, Azizi F. Beneficial Effects of a Dietary Approaches to Stop Hypertension Eating Plan on Features of the Metabolic Syndrome. Diabetes Care.2005;28:2823–31.

21. Zampelas A, Magriplis E. Dietary patterns and risk of cardiovascular diseases: A review of the evidence. Proc Nutr Soc. 2020;79:68-75.

22. Prasad DS, Das BC. Physical inactivity: a cardiovascular risk factor. Indian J Med Sci. 2009;63:33-42.

23. German C, Makarem N, Fanning J, Redline S, Elfassy T, McClain A, et al. Sleep, Sedentary Behavior, Physical Activity, and Cardiovascular Health: MESA. Med Sci Sports Exerc. 2021;53:724–31.

24. Katzmarzyk PT, Friedenreich C, Shiroma EJ, Lee IM. Physical inactivity and non-communicable disease burden in low-income, middle-income and high-income countries. Br J Sports Med. 2021:bjsports-2020-103640.

25. World Health Organization. WHO Global Action Plan on Physical Activity and Health 2018-2030. Geneva: The Head of Publishing, NHS Health Scotland, Woodburn House, Canaan Lane, Edinburgh, EH10 4SG; 2018.

26. Pratt M, Perez LG, Goenka S, Brownson RC, Bauman A, Sarmiento OL, et al. Can Population Levels of Physical Activity Be Increased? Global Evidence and Experience. Prog Cardiovasc Dis .2015;57:356–67.

27. Scott J. Pathophysiology and biochemistry of cardiovascular disease. Curr Opin Genet Dev. 2004;14:271–9.

28. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. Nature. 2011;473:317–25.

29. Bergheanu SC, Bodde MC, Jukema JW. Pathophysiology and treatment of atherosclerosis: Current view and future perspective on lipoprotein modification treatment. Netherlands Hear J. 2017;25:231–42.

30. Frayn KN ER. Human Metabolism: A Regulatory Perspective. Oxford. 4th, editor. Wiley-Blackwell; 2019.

31. I R. Recent advances in physiological lipoprotein metabolism. Clin Lab Med. 2014;52:1695–727.

32. Feingold KR GC. Introduction to Lipids and Lipoproteins. E, editor. South Dartmouth (MA): MDText.com; 2018.

33. van Leeuwen EM, Emri E, Merle BMJ, Colijn JM, Kersten E C-GA, Dammeier S, Meester-Smoor M, Pool FM, de Jong EK, Delcourt C R-B, E, Biarnés M, Luthert PJ, Ueffing M, Klaver CCW, Nogoceke E den HA, I L. A new perspective on lipid research in age-related macular degeneration. Prog Retin Eye Res. 2018;67:56–86.

34. D'Aquila T, Hung YH, Carreiro A, Buhman KK. Recent discoveries on absorption of dietary fat: Presence, synthesis, and metabolism of cytoplasmic lipid droplets within enterocytes. Biochim Biophys Acta - Mol Cell Biol Lipids. 2016;1861:730–47.

35. Mahmood H. Intestinal Lipid Absorption and Lipoprotein Formation. Curr Opin Lipidol. 2014;25:200–6.

36. Olivecrona G. Role of lipoprotein lipase in lipid metabolism. Curr Opin Lipidol. 2016;27:233–41.

37. Saeed A, Feofanova EV, Yu B, Sun W, Virani SS, Nambi V, Coresh J GC, Boerwinkle E, Ballantyne CM HR. Remnant-Like Particle Cholesterol, Low-Density Lipoprotein Triglycerides, and Incident Cardiovascular Disease. 2018;72:156-169.

38. Wang HH, Garruti G, Liu M, Portincasa P, Wang DQH. Cholesterol and lipoprotein metabolism and atherosclerosis: Recent advances in reverse cholesterol transport. Ann Hepatol.2017;16:s27–42.

39. Nordestgaard BG, Chapman MJ, Ray K, Borén J, Andreotti F, Watts GF, et al. Lipoprotein(a) as a cardiovascular risk factor: current status. Eur Heart J. 2010;31:2844-53.

40. Liu T, Yoon W-S, Lee S-R. Recent Updates of Lipoprotein(a) and Cardiovascular Disease. Chonnam Med J.2021;57:36.

41. Mineo C. Lipoprotein receptor signalling in atherosclerosis. Cardiovascular Research. Oxford University Press; 2021;116:1254–74.

42. Brown MS GJ. A receptor-mediated pathway for cholesterol homeostasis. Science.1986;232:34–47.

43. Murray W. Huff AD and HL. Biochemistry of Lipids, Lipoproteins and Membranes [Internet]. sixth. Elsevier; 2016 : 519–548

44. Benito-Vicente A, Uribe KB, Jebari S, Galicia-Garcia U, Ostolaza H, Martin C. Validation of LDLr activity as a tool to improve genetic diagnosis of familial hypercholesterolemia: A retrospective on functional characterization of LDLr variants. Int J Mol Sci. 2018;19:1676.

45. Soininen P, Kangas AJ, Würtz P, Suna T, Ala-Korpela M. Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. Circ Cardiovasc Genet. 2015;8:192–206.

46. Würtz P, Havulinna AS, Soininen P, Tynkkynen T, Prieto-Merino D, Tillin T, et al. Metabolite profiling and cardiovascular event risk: A prospective study of 3 population-based cohorts. Circulation. 2015;131:774–85.

47. Mallol R, Amigó N, Rodríguez MA, Heras M, Vinaixa M, Plana N, et al. Liposcale: a novel advanced lipoprotein test based on 2D diffusion-ordered 1H NMR spectroscopy. J Lipid Res.2015 ;56:737–46.

48. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J. 2020;41:111-188.

49. Jellinger PS, Handelsman Y, Rosenblit PD, Bloomgarden ZT, Fonseca VA, Garber AJ, et al. AMERICAN ASSOCIATION OF CLINICAL ENDOCRINOLOGISTS AND AMERICAN COLLEGE OF ENDOCRINOLOGY GUIDELINES FOR MANAGEMENT OF DYSLIPIDEMIA AND PREVENTION OF CARDIOVASCULAR DISEASE. Endocr Pract.2017;23:1–87.

50. Standards of Medical Care in Diabetes—2021. Diabetes Care. 2021;44:S1 LP-S2.

51. Cromwell WC, Otvos JD. Low-density lipoprotein particle number and risk for cardiovascular disease. Current Atherosclerosis Reports. Current Science Ltd; 2004;6:381-387.

52. Krauss RM. Lipoprotein subfractions and cardiovascular disease risk [Internet]. Vol. 21, Current Opinion in Lipidology. Curr Opin Lipidol; 2010

53. Blake GJ, Otvos JD, Rifai N, Ridker PM. Low-density lipoprotein particle concentration and size as determined by nuclear magnetic resonance spectroscopy as predictors of cardiovascular disease in women. Circulation.2002;106:1930–1937.

54. Ikezaki H, Lim E, Adrienne Cupples L, Liu CT, Asztalos BF, Schaefer EJ. Small dense low-density lipoprotein cholesterol is the most atherogenic lipoprotein parameter in the prospective framingham offspring study. J Am Heart Assoc.2021;10:1-15.

55. Ivanova EA, Myasoedova VA, Melnichenko AA, Grechko A V., Orekhov AN. Small Dense Low-Density Lipoprotein as Biomarker for Atherosclerotic Diseases. Oxid Med Cell Longev. 2017;2017:1273042.

56. Tribble DL, Holl LG, Wood PD, Krauss RM. Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. Atherosclerosis.1992;93:189-199.

57. de Graaf J, Hak-Lemmers HL, Hectors MP, Demacker PN, Hendriks JC, Stalenhoef AF. Enhanced susceptibility to in vitro oxidation of the dense low density lipoprotein subfraction in healthy subjects. Arterioscler Thromb a J Vasc Biol.1991;11:298–306.

58. Otvos JD, Mora S, Shalaurova I, Greenland P, MacKey RH, Goff DC. Clinical implications of discordance between low-density lipoprotein cholesterol and particle number. J Clin Lipidol.2011;5:105-113.

59. Raal FJ, Hovingh GK, Catapano AL. Familial hypercholesterolemia treatments: Guidelines and new therapies. Atherosclerosis. 2018;277:483–92.

60. Goldstein JL, Hobbs HH BM. Familial hypercholesterolemia. The Metabolic and Molecular Bases of Inhereted Disease, editor. New York: Scriver CR, Baudet AL, Sly WS, Valle D, Childs B, Kinzler KW, et al. ; 2001. 2863–2913 p.

61. Gidding SS, Ann Champagne M, de Ferranti SD, Defesche J, Ito MK, Knowles JW, et al. The Agenda for Familial Hypercholesterolemia. Circulation.2015

62. C. M. Xanthomata, hypercholesterolemia, angina pectoris. Acta Med Scand. 1938;89:75–84.

63. Langer T, Strober W LR. The metabolism of low density lipoprotein in familial type II hyperlipoproteinemia. J Clin Invest. 1972;51:1528–36.

64. Goldstein JL BM. Familial hypercholesterolemia: identification of a defect in the regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity associated with overproduction of cholesterol. Proc Natl Acad Sci U S A. 1973;70:2084–8.

65. Brown MS GJ. Familial hypercholesterolemia: defective binding of lipoproteins to cultured fibroblasts associated with impaired regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. Proc Natl Acad Sci U S A. 1974;71:788–92.

66. Wiegman A, Gidding SS, Watts GF, Chapman MJ, Ginsberg HN, Cuchel M, et al. Familial hypercholesterolæmia in children and adolescents: Gaining decades of life by optimizing detection and treatment. Eur Heart J. 2015;36:2425–37.

67. Hobbs HH, Brown MS, Goldstein JL. Molecular genetics of the LDL receptor gene in familial hypercholesterolemia. Hum Mutat. 1992;1:445–66.

68. Goldstein JL, Brown MS. History of Discovery : The LDL Receptor. Arter Thromb Vasc Biol.2009;29:431–8.

69. Ramaswami U, Cooper J, Humphries SE. The UK Paediatric Familial Hypercholesterolaemia Register: Preliminary data. Arch Dis Child. 2017;102:255–60.

70. Harada-Shiba M, Arai H, Ishigaki Y, Ishibashi S, Okamura T, Ogura M, et al. Guidelines for diagnosis and treatment of familial hypercholesterolemia 2017. J Atheroscler Thromb. 2018;25:751–70.

71. Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: Guidance for clinicians to prevent coronary heart disease. Eur Heart J. 2013;34:3478-90.

72. Goldberg AC, Hopkins PN, Toth PP, Ballantyne CM, Rader DJ, Robinson JG, et al. Familial Hypercholesterolemia: Screening, diagnosis and management of pediatric and adult patients. 2011;5:S1-8.

73. Watts GF, Gidding S, Wierzbicki AS, Toth PP, Alonso R, Brown WV, et al. Integrated guidance on the care of familial hypercholesterolaemia from the International FH Foundation. Int J Cardiol.2014;171:309-325.

74. Sniderman AD, Tsimikas S, Fazio S. The severe hypercholesterolemia phenotype: Clinical diagnosis, management, and emerging therapies. J Am Coll Cardiol. 2014;63:1935–47.

75. Masana L, Civeira F, Pedro-botet J, Castro I De, Pocoví M, Plana N, et al. Consenso de expertos sobre la detección y el manejo clínico de la hipercolesterolemia familiar. 2015;25:182–93.

76. Cuchel M, Bruckert E, Ginsberg HN, Raal FJ, Santos RD, Hegele RA, et al. Homozygous familial hypercholesterolaemia: new insights and guidance for clinicians to improve detection and clinical management. A position paper from the Consensus Panel

on Familial Hypercholesterolaemia of the European Atherosclerosis Society. Eur Heart J.2014;35:2146-2157.

77. Chemello KV, García-Nafría J, Gallo A, Martín C, Lambert G, Blom D. Lipoprotein metabolism in familial hypercholesterolemia. J Lipid Res.2021;62:100062.

78. Kamar A, Khalil A, Nemer G. The Digenic Causality in Familial Hypercholesterolemia: Revising the Genotype–Phenotype Correlations of the Disease.2021;11:572045.

79. Hobbs HH, Brown MS, Goldstein JL. Molecular genetics of the LDL receptor gene in familial hypercholesterolemia. Hum Mutat. 1992;1:445–66.

80. Hobbs HH, Russell DW, Brown MS GJ. The LDL receptor locus in familial hypercholesterolemia: mutational analysis of a membrane protein. Annu Rev Genet. 1990;24:133–70.

81. Sturm AC, Knowles JW, Gidding SS, Ahmad ZS, Ahmed CD, Ballantyne CM, et al. Clinical Genetic Testing for Familial Hypercholesterolemia: JACC Scientific Expert Panel. J Am Coll Cardiol. 2018;72:662-680.

82. Shah NP, Ahmed HM, Tang WHW. Familial hypercholesterolemia: Detect, treat, and ask about family. Cleve Clin J Med.2020;87:109-20.

83. Innerarity TL, Weisgraber KH, Arnold KS, Mahley RW, Krauss RM, Vega GL, et al. Familial defective apolipoprotein B-100: Low density lipoproteins with abnormal receptor binding. Proc Natl Acad Sci U S A. 1987;84:6919–23.

84. Soria LF, Ludwig EH, Clarke HR, Vega GL, Grundy SM MB. Association between a specific apolipoprotein B mutation and familial defective apolipoprotein B-100. Proc Natl Acad Sci U S A. 1989;86:587–91.

85. Abifadel M, Varret M, Rabès JP, Allard D, Ouguerram K, Devillers M, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat Genet. 2003;34:154–6.

86. Chora JR, Medeiros AM, Alves AC, Bourbon M. Analysis of publicly available LDLR, APOB, and PCSK9 variants associated with familial hypercholesterolemia: Application of ACMG guidelines and implications for familial hypercholesterolemia diagnosis. Genet Med.2018;20:591-8.

87. Harada-Shiba M, Takagi A, Miyamoto Y, Tsushima M, Ikeda Y YS, A Y. Clinical features and genetic analysis of autosomal recessive hypercholesterolemia. J Clin Endocrinol Metab. 2003;88:2541–7.

88. wan Z, Choi HY, Stitziel N, Ruel I, Bamimore MA, Husa R G, MH, Wang RH, Peloso GM, Hegele RA, Seidah NG KS, J G. APOE p.Leu167del mutation in familial hypercholesterolemia. Atherosclerosis. 2013;231:218–22.

89. Motazacker MM, Pirruccello J, Huijgen R, Do R, Gabriel S, Peter J, et al. Advances in genetics show the need for extending screening strategies for autosomal dominant hypercholesterolaemia. Eur Heart J.2012;33:1360-6.

90. Sharifi M, Futema M, Nair D, Humphries SE. Genetic Architecture of Familial Hypercholesterolaemia. Curr Cardiol Rep.2017;19:44.

91. Berberich AJ, Hegele RA. The complex molecular genetics of familial hypercholesterolaemia. Nat Rev Cardiol. 2019;16:9-20.

92. Goldstein JL, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG. Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. J Clin Invest.1973;52:1544-68.

93. Williams RR, Hunt SC, Schumacher MC, Hegele RA, Leppert MF, Ludwig EH, et al. Diagnosing heterozygous familial hypercholesterolemia using new practical criteria validated by molecular genetics. Am J Cardiol. 1993;72:171–6.

94. Sjouke B, Kusters DM, Kindt I, Besseling J, Defesche JC, Sijbrands EJG, et al. Homozygous autosomal dominant hypercholesterolaemia in the Netherlands:

Prevalence, genotype-phenotype relationship, and clinical outcome. Eur Heart J.2015;36:560-5.

95. Benn M, Watts GF, Tybjærg-Hansen A, Nordestgaard BG. Mutations causative of familial hypercholesterolaemia: Screening of 98 098 individuals from the Copenhagen General Population Study estimated a prevalence of 1 in 217. Eur Heart J. 2016;37:1384–94.

96. Zamora A, Masana L, Comas-Cufí M, Vila À, Plana N, García-Gil M, et al. Familial hypercholesterolemia in a European Mediterranean population—Prevalence and clinical data from 2.5 million primary care patients. J Clin Lipidol. 2017;11:1013–22.

97. Pang J, Martin AC, Mori TA, Beilin LJ, Watts GF. Prevalence of Familial Hypercholesterolemia in Adolescents: Potential Value of Universal Screening? J Pediatr. 2016;170:315–6.

98. Lehrman MA, Russell DW, Goldstein JL, Brown MS. Alu- Alu recombination deletes splice acceptor sites and produces secreted low density lipoprotein receptor in a subject with familial hypercholesterolemia. J Biol Chem. 1987;262:3354–61.

99. Meiner V, Landsberger D, Berkman N, Reshef A, Segal P, Seftel HC, et al. A common Lithuanian mutation causing familial hypercholesterolemia in Ashkenazi Jews. Am J Hum Genet. 1991;49:443–9.

100. Kotze MJ, Langenhoven E, Warnich L, du Plessis L, Retief AE. The molecular basis and diagnosis of familial hypercholesterolaemia in South African Afrikaners. Ann Hum Genet. 1991;55:115–21.

101. Leitersdorf E, Tobin EJ, Davignon J HH. Common low-density lipoprotein receptor mutations in the French Canadian population. J Clin Invest. 1990;85:1014–23.

102. Landsberger D, Meiner V, Reshef A, Levy Y, van der Westhuyzen DR, Coetzee GA, et al. A nonsense mutation in the LDL receptor gene leads to familial hypercholesterolemia in the Druze sect. Am J Hum Genet. 1992;50:427–33.

103. Koivisto UM, Turtola H, Aalto-Setälä K, Top B, Frants RR, Kovanen PT, et al. The familial hypercholesterolemia (FH)-North Karelia mutation of the low density lipoprotein receptor gene deletes seven nucleotides of exon 6 and is a common cause of FH in Finland. J Clin Invest. 1992;90:219–28.

104. Austin MA, Hutter CM, Zimmern RL, Humphries SE. Genetic Causes of Monogenic Heterozygous Familial Hypercholesterolemia: A HuGE Prevalence Review. Am J Epidemiol. 2004;160:407–20.

105. Lui DTW, Lee ACH, Tan KCB. Management of Familial Hypercholesterolemia: Current Status and Future Perspectives.Journal of the Endocrine Society. Endocrine Society. 2021;5:1-14.

106. Walzer S, Travers K, Rieder S, Erazo-Fischer E, Matusiewicz D. Homozygous familial hypercholesterolemia (HoFH) in Germany: An epidemiological survey. Clin Outcomes Res.2013;5:189-192.

107. Sánchez-Hernández RM, Civeira F, Stef M, Perez-Calahorra S, Almagro F, Plana N, et al. Homozygous Familial Hypercholesterolemia in Spain. CLINICAL PERSPECTIVE. Circ Cardiovasc Genet. 2016;9:504–10.

108. Neil HAW, Hammond T, Huxley R, Matthews DR, Humphries SE. Extent of underdiagnosis of familial hypercholesterolaemia in routine practice: Prospective registry study. Br Med J. 2000;321:148.

109. Civeira F. International Panel on Management of familial Hypercholesterolemia. Guidelines for the diagnosis of heterozygous familiar hyercholesterolemia. Atherosclerosis. 2004;173:55–68.

110. Vallejo-Vaz AJ, Ray KK. Epidemiology of familial hypercholesterolaemia: Community and clinical.Atherosclerosis. 2018:277:289-97.

111. Beheshti SO, Madsen CM, Varbo A, Nordestgaard BG. Worldwide Prevalence of Familial Hypercholesterolemia: Meta-Analyses of 11 Million Subjects. J Am Coll Cardiol. 2020;75:2553–66.

112. De Backer G, Besseling J, Chapman J, Hovingh GK, Kastelein JJ, Kotseva K, et al. Prevalence and management of familial hypercholesterolaemia in coronary patients: An analysis of EUROASPIRE IV, a study of the European Society of Cardiology. Atherosclerosis. 2015;241:169–75.

113. Oosterveer DM, Versmissen J, Yazdanpanah M, Hamza TH, Sijbrands EJG. Differences in characteristics and risk of cardiovascular disease in familial hypercholesterolemia patients with and without tendon xanthomas: A systematic review and meta-analysis. Atherosclerosis. 2009;207:311-7.

114. Civeira F, Castillo S, Alonso R, Meriño-Ibarra E, Cenarro A, Artied M, et al. Tendon Xanthomas in Familial Hypercholesterolemia Are Associated With Cardiovascular Risk Independently of the Low-Density Lipoprotein Receptor Gene Mutation. 2005;25:1960-5.

115. Ferriéres J, Lambert J, Lussier-Cacan S, Davignon J. Coronary artery disease in heterozygous familial hypercholesterolemia patients with the same LDL receptor gene mutation. Circulation. 1995;92:290–5.

116. Descamps OS, Leysen X, Van Leuven F, Heller FR. The use of Achilles tendon ultrasonography for the diagnosis of familial hypercholesterolemia. Atherosclerosis. 2001;157:514–8.

117. Fernández A, Sorokin A, Thompson PD. Corneal arcus as coronary artery disease risk factor.Atherosclerosis. 2007;193:235-40.

118. Koivunen-Niemela T, Viikari J, Niinikoski H, Simell O, Alanen A. Sonography in the detection of achilles tendon xanthomata in children with familial hypercholesterolaemia. Acta Paediatr Int J Paediatr. 1994;83:1178-81.

119. Pederiva C, Capra ME, Viggiano C, Rovelli V, Banderali G, Biasucci G. Early prevention of atherosclerosis: Detection and management of hypercholesterolaemia in children and adolescents. Life.2021;11:345.

120. Napoli C, Glass CK, Witztum JL, Deutsch R, D'Armiento FP, Palinski W. Influence of maternal hypercholesterolaemia during pregnancy on progression of early atherosclerotic lesions in childhood: Fate of Early Lesions in Children (FELIC) study. Lancet. 1999;354:1234–41.

121. Napoli C, D'Armiento FP, Mancini FP, Postiglione A, Witztum JL, Palumbo G, et al. Fatty streak formation occurs in human fetal aortas and is greatly enhanced maternal, hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atheroeclerotic lesions. J Clin Invest. 1997 ;100:2680–90.

122. Fahed AC, Habib RH, Nemer GM, Azar ST, Andary RR, Arabi MT, et al. Low-density lipoprotein levels and not mutation status predict Intima-media thickness in familial hypercholesterolemia. Ann Vasc Surg.2014;28:421–6.

123. Pauciullo P, Iannuzzi A, Sartorio R, Irace C, Covetti G, Di Costanzo A R, P. Increased intima-media thickness of the common carotid artery in hypercholesterolemic children. Arter Thromb. 1994;14:1075–9.

124. Tonstad S, Joakimsen O, Stensland-Bugge E, Leren TP, Ose L, Russell D BK. Risk factors related to carotid intima-media thickness and plaque in children with familial hypercholesterolemia and control subjects. Arter Thromb Vasc Biol. 1996;16:984–91.

125. Järvisalo MJ, Jartti L, Näntö-Salonen K, Irjala K, Rönnemaa T, Hartiala JJ, et al. Increased Aortic Intima-Media Thickness. Circulation. 2001;104:2943–7.

126. Wiegman A, De Groot E, Hutten BA, Rodenburg J, Gort J, Bakker HD, et al. Arterial intima-media thickness in children heterozygous for familial hypercholesterolaemia. Lancet. 2004;363:369–70.

127. De Jongh S, Lilien MR, Bakker HD, Hutten BA, Kastelein JJP, Stroes ESG. Family history of cardiovascular events and endothelial dysfunction in children with familial hypercholesterolemia. Atherosclerosis. 2002;163:193–7.

128. Kusters DM, Wiegman A, Kastelein JJP, Hutten BA. Carotid intima-media thickness in children with familial hypercholesterolemia. Circ Res. 2014;114:307–10.

129. Moráis López A, Lama More RA, Dalmau Serra J. Hipercolesterolemia. Abordaje terapéutico. An Pediatr. 2009;70:488–96.

130. Arroyo Díez FJ, Romero Albillos JA LVG. Dislipemias en edad pediátrica. Protoc diagn ter pediatr. 2019;1:125–40.

131. Kerr M, Pears R, Miedzybrodzka Z, Haralambos K, Cather M, Watson M, et al. Cost effectiveness of cascade testing for familial hypercholesterolaemia, based on data from familial hypercholesterolaemia services in the UK. Eur Heart J. 2017;38:1832–9.

132. Ascaso JF, Mata P, Arbona C, Civeira F, Valdivielso P, Masana L. Hipercolesterolemia familiar homocigota: adaptación a España del documento de posición del grupo de consenso sobre hipercolesterolemia familiar de la Sociedad Europea de Arteriosclerosis. Documento de Consenso de la Sociedad Española de Arteriosclerosis (SEA) y la Fundación Hipercolesterolemia Familiar (FHF). Clin Invest Arter.2015;27:80-96.

133. Bourbon M, Duarte MA, Alves AC, Medeiros AM, Marques L, Soutar AK. Genetic diagnosis of familial hypercholesterolaemia: The importance of functional analysis of potential splice-site mutations. J Med Genet.2009;46:352–7.

134. Wijers M, Kuivenhoven JA, Van De Sluis B. The life cycle of the low-density lipoprotein receptor: Insights from cellular and in-vivo studies. Current Opinion in Lipidology. Lippincott Williams and Wilkins. 2015;26:82-7.

135. Silverman MG, Ference BA, Im K, Wiviott SD, Giugliano RP, Grundy SM, et al. Association between lowering LDL-C and cardiovascular risk reduction among different therapeutic interventions: A systematic review and meta-analysis. JAMA. 2016;316:1289–97.

136. Zelcer N, Hong C, Boyadjian R, Tontonoz P. LXR regulates cholesterol uptake through idol-dependent ubiquitination of the LDL receptor. Science (80-). 2009;325:100–4.

137. Nelson JK, Cook ECL, Loregger A, Hoeksema MA, Scheij S, Kovacevic I, et al. Deubiquitylase inhibition reveals liver X receptor-independent transcriptional regulation of the E3 ubiquitin ligase IDOL and lipoprotein uptake. J Biol Chem. 2016;291:4813–25.

138. Zhang L, Reue K, Fong LG, Young SG, Tontonoz P. Feedback regulation of cholesterol uptake by the LXR-IDOL-LDLR axis.Arteriosclerosis, Thrombosis, and Vascular Biology. 2012;32:2541–6.

139. Teslovich TM, Musunuru K, Smith A V., Edmondson AC, Stylianou IM, Koseki M, et al. Biological, clinical and population relevance of 95 loci for blood lipids. Nature.2010;466:707–13.

140. Willer CJ, Schmidt EM, Sengupta S, Peloso GM, Gustafsson S, Kanoni S, et al. Discovery and refinement of loci associated with lipid levels. Nat Genet.2013;45:1274-85.

141. Sorrentino V, Fouchier SW, Motazacker MM, Nelson JK, Defesche JC, Dallinga-Thie GM, et al. Identification of a loss-of-function inducible degrader of the low-density lipoprotein receptor variant in individuals with low circulating low-density lipoprotein. Eur Heart J.2013;34:1292–7.

142. Zhang C ping, Tian Y, Zhang M, Tuo Q hui, Chen J xiong, Liao D fang. IDOL, inducible degrader of low-density lipoprotein receptor, serves as a potential therapeutic target for dyslipidemia. Med Hypotheses. 2016;86:138–42.

143. Ogura M. PCSK9 inhibition in the management of familial hypercholesterolemia Journal of Cardiology.2018;71:1-7.

144. Horton JD, Cohen JC, Hobbs HH. PCSK9: a convertase that coordinates LDL catabolism. J Lipid Res. 2009;50:S172-7.

145. Bergeron N, Phan BAP, Ding Y, Fong A, Krauss RM. Proprotein convertase subtilisin/kexin type 9 inhibition a new therapeutic mechanism for reducing cardiovascular disease risk. Circulation. 2015;132:1648–66.

146. Horton JD, Cohen JC, Hobbs HH. PCSK9: A convertase that coordinates LDL catabolism. Journal of Lipid Research.2009;50:S172–7.

147. van der Graaf A, Rodenburg J, Vissers MN, Hutten BA, Wiegman A, Trip MD, et al. Atherogenic lipoprotein particle size and concentrations and the effect of pravastatin in children with familial hypercholesterolemia. J Pediatr.2008;152:873–8.

148. Christensen JJ, Ulven SM, Retterstøl K, Narverud I, Bogsrud MP, Henriksen T, et al. Comprehensive lipid and metabolite profiling of children with and without familial hypercholesterolemia: A cross-sectional study. Atherosclerosis. 2017;266:48–57.

149. Jamal S, Alves AC, Medeiros AM, Bourbon M. sdLDL/LDL ratio in familial combined hyperlipidaemia patients and familial hypercholesterolemia patients. Atherosclerosis. 2014;235:e196.

150. Ayyobi AF, McGladdery SH, McNeely MJ, Austin MA, Motulsky AG, Brunzell JD. Small, dense LDL and elevated apolipoprotein B are the common characteristics for the three major lipid phenotypes of familial combined hyperlipidemia. Arterioscler Thromb Vasc Biol.2003;23:1289-94.

151. Balder JW, Lansberg PJ, Hof MH, Wiegman A, Hutten BA, Kuivenhoven JA. Pediatric lipid reference values in the general population: The Dutch lifelines cohort study. J Clin Lipidol. 2018;12:1208-1216.

152. Saltijeral A, Pérez de Isla L, Alonso R, Muñiz O, Díaz-Díaz JL, Fuentes F, et al. Attainment of LDL Cholesterol Treatment Goals in Children and Adolescents With Familial Hypercholesterolemia. The SAFEHEART Follow-up Registry. Rev Española Cardiol. 2017;70:444–50.

153. Martin AC, Gidding SS, Wiegman A, Watts GF. Knowns and unknowns in the care of pediatric familial hypercholesterolemia. J Lipid Res. 2017;58:1765–76.

154. Langslet G. Screening methods in the diagnosis and assessment of children and adolescents with familial hypercholesterolemia. 2013;11:1061–6.

155. Wilson JMG JG. Principles and practice of screening for disease. J R Coll Gen Pract. 1968;16:318.

156. Wald DS, Bestwick JP, Wald NJ. Child-parent screening for familial hypercholesterolaemia: Screening strategy based on a meta-analysis. Br Med J. 2007;335:599–603.

157. Kusters DM, Beaufort C De, Widhalm K, Guardamagna O, Bratina N, Ose L, et al. Paediatric screening for hypercholesterolaemia in Europe. 2012;97:272–6.

158. Klančar G, Grošelj U, Kovač J, Bratanič N, Bratina N, Trebušak Podkrajšek K, et al. Universal Screening for Familial Hypercholesterolemia in Children. J Am Coll Cardiol.2015;66:1250–7.

159. Groselj U, Kovac J, Sustar U, Mlinaric M, Fras Z, Podkrajsek KT, et al. Universal screening for familial hypercholesterolemia in children: The Slovenian model and literature review. Atherosclerosis.2018;277:383–91.

160. McKay AJ, Hogan H, Humphries SE, Marks D, Ray KK, Miners A. Universal screening at age 1–2 years as an adjunct to cascade testing for familial hypercholesterolaemia in the UK: A cost-utility analysis. Atherosclerosis.2018;275:434–43.

161. Ritchie SK, Murphy ECS, Ice C, Cottrell LA, Minor V, Elliott E, et al. Universal versus targeted blood cholesterol screening among youth: The CARDIAC project. Pediatrics. 2010;126:260–5.

162. Fábryová L, Hlavatá A, Cižmárová E, Šimurka P, Debreová M, Dukát A et al. Recommendations for diagnosis and treatment of dyslipidaemia in children and adolescents. Pediatr prax. 2011;12:3–9.

163. American Academy of Pediatrics. National Cholesterol Education Program: report of the Expert Panel of Blood Cholesterol Levels in Children and Adolescents Pediatrics. Pediatrics. 1992;89:525–84.

164. Kavey RE, Daniels SR, Lauer RM, Atkins DL, Hayman LL TK. American Heart Association. American Heart Association guidelines for primary prevention of atherosclerotic cardiovascular disease beginning in childhood. Circulation. 2003;107:1562–6.

165. Haney EM, Huffman LH, Bougatsos C, Freeman M, Steiner RD, Nelson HD. Screening and treatment for lipid disorders in children and adolescents: Systematic evidence review for the US Preventive Services Task Force. Pediatrics. 2007;120:e189-214.

166. Kreissl A, Walleczek N, Espina PR, Hallwirth U, Greber-Platzer S. Selective screening for familial hypercholesterolemia in Austrian children - First year results. BMC Pediatr. 2019;19:1–7.

167. Knowles JW, Rader DJ, Khoury MJ. Cascade screening for familial hypercholesterolemia and the use of genetic testing. JAMA.2017;318:381–2.

168. Daniels SR, Gidding SS, de Ferranti SD. Pediatric aspects of familial hypercholesterolemias: recommendations from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. J Clin Lipidol.2011;5:S30-7.

169. Descamps OS, Tenoutasse S, Stephenne X, Gies I, Beauloye V, Lebrethon MC, et al. Management of familial hypercholesterolemia in children and young adults : Consensus paper developed by a panel of lipidologists , cardiologists , paediatricians , nutritionists , gastroenterologists , general practitioners and a patient organization. Atherosclerosis. 2011;218:272–80.

170. Ibarretxe D, Rodríguez-Borjabad C, Feliu A, Bilbao JÁ, Masana L, Plana N. Detecting familial hypercholesterolemia earlier in life by actively searching for affected children:The DECOPIN project. Atherosclerosis. 2018;278:210–6.

171. Vinson A, Guerra L, Hamilton L, Wilson DP, FNLA. Reverse Cascade Screening for Familial Hypercholesterolemia. J Pediatr Nurs. 2019;44:50–5.

172. Pears R, Griffin M, Futema M, Humphries SE. Improving the cost-effectiveness equation of cascade testing for familial hypercholesterolaemia. Curr Opin Pediatr. 2015;26:162–8.

173. Farnier M, Civeira F, Descamps O, Kawashiri M, Grigore L, Vohnout B, et al. How to implement clinical guidelines to optimise familial hypercholesterolaemia diagnosis and treatment. Atheroscler Suppl. 2017;26:25–35.

174. Lambert CT, Sandesara P, Isiadinso I, Gongora MC, Eapen D, Bhatia N, Baer JT SL. Coronary Artery Disease Prevention Current Treatment of Familial Hypercholesterolaemia Current Treatment of Familial Hypercholesterolaemia. Eur Cardiol. 2014;9:76–81.

175. Catapano AL, Graham I, De Backer G, Wiklund O, Chapman MJ, Drexel H, et al. 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias. Eur Heart J. 2016.14;37:2999-3058.

176. Torvik K, Narverud I, Ottestad I, Svilaas A, Gran JM, Retterstøl K, et al. Dietary counseling is associated with an improved lipid profile in children with familial hypercholesterolemia. Atherosclerosis. 2016;252:21–7.

177. Liyanage T, Ninomiya T, Wang A, Neal B, Jun M, Wong MG, et al. Effects of the mediterranean diet on cardiovascular outcomes-a systematic review and meta-analysis. PLoS One. 2016;11:e0159252.

178. Eleftheriou D, Benetou V, Trichopoulou A, La Vecchia C, Bamia C. Mediterranean diet and its components in relation to all-cause mortality: Meta-analysis. Br J Nutr. 2018;120:1081–97.

179. Berild A, Holven KB, Ulven SM. Recommended Nordic diet and risk markers for cardiovascular disease. Tidsskr Nor Laegeforen. 2017;137:721-726.

180. Adamsson V, Reumark A, Fredriksson IB, Hammarström E, Vessby B, Johansson G, et al. Effects of a healthy Nordic diet on cardiovascular risk factors in hypercholesterolaemic subjects: A randomized controlled trial (NORDIET). J Intern Med. 2011;269:150–9.

181. Luirink, Ilse K, Hutten, Barbara A, Wiegman A. Optimizing Treatment of Familial Hypercholesterolemia in Children and Adolescents. Curr Cardiol Rep. 2015;17:78.

182. Vuorio A, Kuoppala J, Kovanen PT, Humphries SE, Tonstad S, Wiegman A DE. Statins for children with familial hypercholesterolemia. Cochrane Database Syst Rev. 2014;7:CD006401.

183. Civeira F, Plana N. Tratamiento de la hipercolesterolemia familiar heterocigota en la infancia y la adolescencia: un problema no resuelto. Rev Esp Cardiol. 2017 1;70:423–4.

184. Rosenson RS. Existing and emerging therapies for the treatment of familial hypercholesterolemia. J Lipid Res.2021 ;62:100060.

185. Plana N, Rodríguez-Borjabad C, Ibarretxe D, Masana L. Familial hypercholesterolemia in childhood and adolescents: A hidden reality. Clin e Investig en Arterioscler. 2017;29:129-140.

186. Ramaswami U, Humphries SE. Management of familial hypercholesterolaemia in childhood. Current opinion in pediatrics. 2020;32:633-40.

187. Luirink IK, Wiegman A, Kusters DM, Hof MH, Groothoff JW, De Groot E, et al. 20-Year follow-up of statins in children with familial hypercholesterolemia. N Engl J Med. 2019;381:1547–56.

188. Ramaswami U, Futema M, Bogsrud MP, Holven KB, Roeters van Lennep J, Wiegman A, et al. Comparison of the characteristics at diagnosis and treatment of children with heterozygous familial hypercholesterolaemia (FH) from eight European countries. Atherosclerosis.2020;292:178-87.

189. Anitschkow N CS. Ueber experimentelle Cholester-insteatose und ihre Bedeutung fuer die Entstehung einiger pathologischer Prozesse. Zentrbl Allg Pathol Pathol Anat. 1913;24:1–9.

190. Anitschkow N. Ueber die Veranderungen der Kaninchenaorta bei experimenteller Cholesterinsteatose. Beitr Pathol Anat. 1913:379–404.

191. Keys A, Anderson JT, Grande F. Serum cholesterol response to changes in the diet. IV. Particular saturated fatty acids in the diet. Metabolism.1965;14:776–87.

192. Hegsted DM, McGandy RB, Myers ML, Stare FJ. Quantitative effects of dietary fat on serum cholesterol in man. Am J Clin Nutr. 1965;17:281–95.

193. C M. Angina pectoris in hereditary xanthomatosis. Arch Intern Med. 1939;64:675–700.

194. Connor WE CS. Dietary treatment of familial hypercholesterolemia. Arteriosclerosis. 1989;9:91–105.

195. Dalen JE, Devries S. Diets to prevent coronary heart disease 1957-2013: What have we learned? American Journal of Medicine. 2014;127:364-9.

196. Eckel RH, Jakicic JM, Ard JD, De Jesus JM, Houston Miller N, Hubbard VS, et al. 2013 AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: A report of the American College of cardiology/American Heart Association task force on practice guidelines.Circulation. 2014;129:76–99.

197. Mente A, De Koning L, Shannon HS, Anand SS. A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. Archives of Internal Medicine. 2009 ;169:659–69.

198. McCaulley M. Association of dietary, circulating, and supplement fatty acids with coronary risk. Annals of internal medicine. 2014;161:456.

199. Casas R, Castro-Barquero S, Estruch R, Sacanella E. Nutrition and Cardiovascular Health. Int J Mol Sci. 2018;19:3988.

200. Forouhi NG, Krauss RM, Taubes G, Willett W. Dietary fat and cardiometabolic health: Evidence, controversies, and consensus for guidance. BMJ. 2018;361:K2139.

201. Shafiq N, Singh M, Kaur S, Khosla P, Malhotra S. Dietary treatment for familial hypercholesterolaemia. Cochrane Database Syst Rev. 2010; CD001918.

202. Chiavaroli L, Nishi SK, Khan TA, Braunstein CR, Glenn AJ, Mejia SB, et al. Portfolio Dietary Pattern and Cardiovascular Disease: A Systematic Review and Meta-analysis of Controlled Trials. Progress in Cardiovascular Diseases. 2018;61: 43–53.

203. Threapleton DE, Greenwood DC, Evans CEL, Cleghorn CL, Nykjaer C, Woodhead C, et al. Dietary fibre intake and risk of cardiovascular disease: Systematic review and meta-analysis.BMJ. 2013;347:f6879.

204. Hernáez Á, Castañer O, Goday A, Ros E, Pintó X, Estruch R, et al. The Mediterranean Diet decreases LDL atherogenicity in high cardiovascular risk individuals: a randomized controlled trial. Mol Nutr Food Res.2017;61:1601015.

205. Ulven SM, Leder L, Elind E, Ottestad I, Christensen JJ, Telle-Hansen VH, et al. Exchanging a few commercial, regularly consumed food items with improved fat quality reduces total cholesterol and LDL-cholesterol: A double-blind, randomised controlled trial. Br J Nutr. 2016;116:1383–93.

206. Connor WE, Connor SL. Importance of diet in the treatment of familial hypercholesterolemia. Am J Cardiol. 1993;72:42–53.

207. Negele L, Schneider B, Ristl R, Stulnig TM, Willfort-Ehringer A, Helk O, et al. Effect of a low-fat diet enriched either with rapeseed oil or sunflower oil on plasma lipoproteins in children and adolescents with familial hypercholesterolaemia. Results of a pilot study. Eur J Clin Nutr. 2015;69:337–43.

208. Mateo-Gallego R, Perez-Calahorra S, Cofán M, Baila-Rueda L, Cenarro A, Ros E, et al. Serum lipid responses to weight loss differ between overweight adults with familial hypercholesterolemia and those with Familial combined hyperlipidemia. J Nutr. 2014;144:1219–26.

209. Daniels SR, Gidding SS de FS. Pediatric aspects of familial Hypercholesteolemias: Recommendations from the National Lipid Association Expert Panel on Familial Hypercholesterolemias. J Clin Lipidol. 2011;5:S30–7.

210. Molven I, Retterstøl K, Andersen LF, Veierød MB, Narverud I, Ose L, et al. Children and young adults with familial hypercholesterolaemia (FH) have healthier food choices particularly with respect to dietary fat sources compared with non-FH children. J Nutr Sci.2013;2:1–8.

211. Burrows R, Correa-Burrows P, Reyes M, Blanco E, Albala C, Gahagan S. High cardiometabolic risk in healthy Chilean adolescents: associations with anthropometric, biological and lifestyle factors. Public Health Nutr.2016;19:486-93.

212. Minossi V, Pellanda LC. The “Happy Heart” educational program for changes in health habits in children and their families: protocol for a randomized clinical trial. BMC Pediat.2015;15:19.

213. Juonala M, Viikari JSA, Hutri-Kähönen N, Pietikäinen M, Jokinen E, Taittonen L, et al. The 21-year follow-up of the Cardiovascular Risk in Young Finns Study: Risk factor levels, secular trends and east-west difference.J Intern Med.2004;255:457–68.

214. Lughetti L, Predieri B, Balli F, Calandra S. Rational approach to the treatment for heterozygous familial hypercholesterolemia in childhood and adolescence: A review Journal of Endocrinological Investigation. 2007;30:700–19.

215. Daniels SR GFC on N. Lipid screening and cardiovascular health in Chilhood. Pediatrics. 2008;122:198–208.

216. Goldberg AC, Hopkins PN, Toth PP, Ballantyne CM, Rader DJ, Robinson JG, et al. Familial Hypercholesterolemia : Screening , Diagnosis and Management of Pediatric and Adult Patients Clinical Guidance from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. J Clin Lipidol. 2011;5:133–140.

217. Ramaswami U, Humphries SE, Priestley-barnham L, Green P, Wald DS, Capps N, et al. Current management of children and young people with heterozygous familial hypercholesterolaemia - HEART UK statement of care. Atherosclerosis 2019;290:1–8.

218. Berger S, Raman G, Vishwanathan R, Jacques PF, Johnson EJ. Dietary cholesterol and cardiovascular disease: A systematic review and meta-analysis. American Journal of Clinical Nutrition. 2015;102:276–94.

219. Griffin JD, Lichtenstein AH. Dietary Cholesterol and Plasma Lipoprotein Profiles: Randomized Controlled Trials.Current Nutrition Reports. 2013;2:274-82.

220. Nicklas TA, Dwyer J, Feldman HA, Luepker R V., Kelder SH, Nader PR. Serum cholesterol levels in children are associated with dietary fat and fatty acid intake. J Am Diet Assoc.2002;102:511–7.

221. Cheng HH, Wen YY, Chen C. Serum fatty acid composition in primary school children is associated with serum cholesterol levels and dietary fat intake. Eur J Clin Nutr 2003;57:1613–20.

222. Matthews VL, Wien M, Sabaté J. The risk of child and adolescent overweight is related to types of food consumed Nutr J.2011;10:1–7.

223. Haro-Mora JJ, García-Escobar E, Porras N, Alcázar D, Gaztambide J, Ruíz-Órpez A, et al. Children whose diet contained olive oil had a lower likelihood of increasing their body mass index Z-score over 1 year. Eur J Endocrinol. 2011;165:435–9.

224. O'Neil CE, Keast DR, Nicklas TA, Fulgoni VL. Out-of-hand nut consumption is associated with improved nutrient intake and health risk markers in US children and adults: National Health and Nutrition Examination Survey 1999-2004. Nutr Res.2012 ;32:185–94.

225. Thorsdottir I, Ramel A. Dietary Intake of 10- to 16-Year-Old Children and Adolescents in Central and Northern Europe and Association with the Incidence of Type 1 Diabetes. Ann Nutr Metab.2003;47:267–75.

226. Sánchez-Bayle M, Soriano-Guillén L. Influence of dietary intervention on growth in children with hypercholesterolaemia. Acta Paediatr Int J Paediatr. 2003;92:1043–6.

227. Astrup A, Dyerberg J, Elwood P, Hermansen K, Hu FB, Jakobsen MU, et al. The role of reducing intakes of saturated fat in the prevention of cardiovascular disease: Where does the evidence stand in 2010? In: American Journal of Clinical Nutrition.Am J Clin Nutr.2011: 684–8.

228. Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ, Willett WC. Trans Fatty Acids and Cardiovascular Disease. N Engl J Med.2006;354:1601–13.

229. Clifton PM, Keogh JB. A systematic review of the effect of dietary saturated and polyunsaturated fat on heart disease. Nutr Metab Cardiovasc Dis. 2017;27:1060–80.

230. Estruch R, Ros E, Salas-Salvadó J, Covas M-I, Corella D, Arós F, et al. Primary Prevention of Cardiovascular Disease with a Mediterranean Diet Supplemented with Extra-Virgin Olive Oil or Nuts. N Engl J Med. 2018;378:e34.

231. Ailhaud G, Guesnet P. Fatty acid composition of fats is an early determinant of childhood obesity: A short review and an opinion.Obesity Reviews. 2004:21–6.

232. Dehghan M, Mente A, Zhang X, Swaminathan S, Li W, Mohan V, et al. Associations of fats and carbohydrate intake with cardiovascular disease and mortality in 18 countries from five continents (PURE): a prospective cohort study. Lancet. 2017;390:2050–62.

233. Albertson AM, Thompson DR, Franko DL, Holschuh NM. Weight indicators and nutrient intake in children and adolescents do not vary by sugar content in ready-to-eat cereal: Results from National Health and Nutrition Examination Survey 2001-2006. Nutr Res.2011;31:229–36.

234. Williams LA, Wilson DP. Nutritional Management of Pediatric Dyslipidemia.2000

235. Pérez-Morales E, Bacardí-Gascón M, Jiménez-Cruz A. Ingesta de bebidas azucaradas antes de los seis anos y peso o imc en los niños mayores; una revisión sistemática de estudios prospectivos.Nutricion Hospitalaria. 2013;28: 47–51.

236. Morgan RE. Does consumption of high-fructose corn syrup beverages cause obesity in children?.Pediatric Obesity. 2013;8: 249–54.

237. Ambrosini GL, Oddy WH, Huang RC, Mori TA, Beilin LJ, Jebb SA. Prospective associations between sugar-sweetened beverage intakes and cardiometabolic risk factors in adolescents. Am J Clin Nutr. 2013;98:327-334.

238. Choumenkovitch SF, McKeown NM, Tovar A, Hyatt RR, Kraak VI, Hastings A V, et al. Whole grain consumption is inversely associated with BMI Z-score in rural school-aged children. Public Health Nutr .2013;16:212–8.

239. Hur Y, Reicks M. Relationship between whole-grain intake, chronic disease risk indicators, and weight status among adolescents in the National Health and Nutrition Examination Survey, 1999-2004. J Acad Nutr Diet. 2012;112:46–55

240. Bellisle F, Hébel P, Colin J, Reyé B, Hopkins S. Consumption of whole grains in French children, adolescents and adults. Br J Nutr .2014;112:1674–84.

241. Niinikoski H, Ruottinen S. Is carbohydrate intake in the first years of life related to future risk of NCDs? Nutr Metab Cardiovasc Dis. 2012;22:770–4.

242. Ventura EE, Davis JN, Alexander KE, Shaibi GQ, Lee W, Byrd-Williams CE, et al. Dietary intake and the metabolic syndrome in overweight Latino children. J Am Diet Assoc.2008;108:1355–9.

243. Yao B, Fang H, Xu W, Yan Y, Xu H, Liu Y, et al. Dietary fiber intake and risk of type 2 diabetes: A dose-response analysis of prospective studies. Eur J Epidemiol.2014;29:79–88.

244. Zhang Z, Xu G, Liu D, Zhu W, Fan X, Liu X. Dietary fiber consumption and risk of stroke. Eur J Epidemiol.2013;28:119–30.

245. Zhang B, Zhao Q, Guo W, Bao W, Wang X. Association of whole grain intake with all-cause, cardiovascular, and cancer mortality: A systematic review and dose-response meta-analysis from prospective cohort studies.European Journal of Clinical Nutrition. .2018;72:57–65.

246. Lutsey PL, Steffen LM, Feldman HA, Hoelscher DH, Webber LS, Luepker R V., et al. Serum homocysteine is related to food intake in adolescents: The child and adolescent trial for cardiovascular health. Am J Clin Nutr.2006;83:1380–6.

247. Eloranta AM, Lindi V, Schwab U, Kiiskinen S, Venäläinen T, Lakka HM, et al. Dietary factors associated with metabolic risk score in Finnish children aged 6-8 years: The PANIC study. Eur J Nutr.2014;53:1431–9.

248. Bradlee ML, Singer MR, Moore LL. Lean red meat consumption and lipid profiles in adolescent girls. J Hum Nutr Diet.2014;27:292–300.

249. Zheng J, Huang T, Yu Y, Hu X, Yang B, Li D. Fish consumption and CHD mortality: An updated meta-analysis of seventeen cohort studies. Public Health Nutr.2012;15:725–37.

250. Ozawa M, Yoshida D, Hata J, Ohara T, Mukai N, Shibata M, et al. Dietary Protein Intake and Stroke Risk in a General Japanese Population: The Hisayama Study. Stroke.2017;48:1478–86.

251. Bigornia SJ, LaValley MP, Moore LL, Northstone K, Emmett P, Ness AR, et al. Dairy intakes at age 10 years do not adversely affect risk of excess adiposity at 13 years. J Nutr.2014;144:1081–90.

252. Wiley AS. Dairy and milk consumption and child growth: Is BMI involved? An analysis of NHANES 1999-2004. Am J Hum Biol.2010;22:517–25.

253. Dixon LB, Pellizzon MA, Jawad AF, Tershakovec AM. Calcium and dairy intake and measures of obesity in hyper- and normocholesterolemic children. Obes Res.2005;13:1727–38.

254. Dror DK, Allen LH. Dairy product intake in children and adolescents in developed countries: Trends, nutritional contribution, and a review of association with health outcomes. Nutr Rev.2014;72:68–81.

255. Abreu S, Santos R, Moreira C, Santos PC, Vale S, Soares-Miranda L, et al. Relationship of milk intake and physical activity to abdominal obesity among adolescents. Pediatr Obes.2014;9:71–80.

256. Huang TT-K, McCrory MA. Dairy Intake, Obesity, and Metabolic Health in Children and Adolescents: Knowledge and Gaps. Nutr Rev.2005;63:71–80.

257. Hoppe C, Mølgaard C, Vaag A, Barkholt V, Michaelsen KF. High intakes of milk, but not meat, increase s-insulin and insulin resistance in 8-year-old boys. Eur J Clin Nutr.2005;59:393–8.

258. Lava SAG, Bianchetti MG, Simonetti GD. Salt intake in children and its consequences on blood pressure. Pediatric Nephrology. 2015;30:1389–96.

259. Juhola J, Magnussen CG, Viikari JSA, Kähönen M, Hutri-Kähönen N, Jula A, et al. Tracking of serum lipid levels, blood pressure, and body mass index from childhood to adulthood: The cardiovascular risk in young Finns study. J Pediatr.2011;159:584–90.

260. Kelishadi R, Gheisari A, Zare N, Farajian S, Shariatinejad K. Salt intake and the association with blood pressure in young Iranian children: first report from the middle East and north Africa. Int J Prev Med.2013;4:475–83.

261. Juhola J, Magnussen CG, Berenson GS, Venn A, Burns TL, Sabin MA, et al. Combined effects of child and adult elevated blood pressure on subclinical atherosclerosis: The international childhood cardiovascular cohort consortium. Circulation.2013;128:217–24.

262. Shi L, Krupp D, Remer T. Salt, fruit and vegetable consumption and blood pressure development: A longitudinal investigation in healthy children. Br J Nutr .2014;111:662–71.

263. He FJ, MacGregor GA. Importance of salt in determining blood pressure in children: Meta-analysis of controlled trials. Hypertension .2006;48:861–9.

264. Bach-Faig A, Berry EM, Lairon D, Reguant J, Trichopoulou A, Dernini S, et al. Mediterranean diet pyramid today. Science and cultural updates. Public Health Nutr. 2011;14:2274–84.

265. Shen J, Wilmot KA, Ghasemzadeh N, Molloy DL, Burkman G, Mekonnen G, et al. Mediterranean Dietary Patterns and Cardiovascular Health. Annu Rev Nutr. 2015;35:425–49.

266. Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F et al. Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med. 2013;368:1279–90.

267. Hoffman R, Gerber M. Evaluating and adapting the Mediterranean diet for non-Mediterranean populations: A critical appraisal. Nutr Rev. 2013;71:573–84.

268. Papadaki A, Scott JA. The impact on eating habits of temporary translocation from a Mediterranean to a Northern European environment. Eur J Clin Nutr. 2002;56:455–67.

269. Adamsson V, Reumark A, Cederholm T, Vessby B, Risérus U, Johansson G. What is a healthy Nordic diet? Foods and nutrients in the NORDIET study. Food Nutr Res. 2012;56:1–8.

270. Adamsson V, Cederholm T, Vessby B, Risérus U. Influence of a healthy Nordic diet on serum fatty acid composition and associations with blood lipoproteins - Results from the NORDIET study. Food Nutr Res. 2014;58:1–6.

271. Guardamagna O, Abello F, Baracco V, Federici G, Bertucci P, Mozzi A, et al. Primary hyperlipidemias in children: Effect of plant sterol supplementation on plasma lipids and markers of cholesterol synthesis and absorption. Acta Diabetol.2011;48:127–33.

272. Gylling H, Plat J, Turley S, Ginsberg HN, Ellegård L, Jessup W, et al. Plant sterols and plant stanols in the management of dyslipidaemia and prevention of cardiovascular disease Atherosclerosis.2014;232:346-60.

273. Wen YT, Dai JH, Gao Q. Effects of Omega-3 fatty acid on major cardiovascular events and mortality in patients with coronary heart disease: A meta-analysis of randomized controlled trials. Nutr Metab Cardiovasc Dis .2014;24:470–5.

274. Gulesserian T WK. Effect of a rapeseed oil substituting diet on serum lipids and lipoproteins in children and adolescents with familial hypercholesterolemia. J Am Coll Nutr. 2002;21:103–8.

275. McCrindle BW, Helden E CW. Garlic extract therapy in children with hypercholesterolemia. Arch Pediatr Adolesc Med. 1998;152:1089–94.

276. Widhalm K, Brazda G, Schneider B, Kohl S. Effect of soy protein diet versus standard low fat, low cholesterol diet on lipid and lipoprotein levels in children with familial or polygenic hypercholesterolemia. J Pediatr .1993;123:30–4.

277. Davidson MH, Dugan LD, Burns JH, Sugimoto D, Story K, Drennan K. A psyllium-enriched cereal for the treatment of hypercholesterolemia in children: A controlled, double-blind, crossover study. Am J Clin Nutr .1996;63:96–102.

278. Guardamagna O, Abello F, Baracco V, Stasiowska B, Martino F. The treatment of hypercholesterolemic children: Efficacy and safety of a combination of red yeast rice extract and policosanols. Nutr Metab Cardiovasc Dis. 2011;21:424–9.

279. Malhotra A, Shafiq N, Arora A, Singh M, Kumar R, Malhotra S. Dietary interventions (plant sterols, stanols, omega-3 fatty acids, soy protein and dietary fibers) for familial hypercholesterolaemia.Cochrane Database of Systematic Reviews. 2014 ;2014: CD001918.

280. Martin AC, Gidding SS, Wiegman A, Watts GF. Knowns and unknowns in the care of pediatric familial hypercholesterolemia. J Lipid .2017;58:1765–76.

281. Stergioulas A, Tripolitsioti A, Messinis D, Bouloukos A, Nounopoulos C. The effects of endurance training on selected coronary risk factors in children. Acta Paediatr.1998;87:401–4.

282. Civeira F, Pocoví M, Alegría E, Alonso R, Carmena R, Casasnovas JA, et al. Guidelines for the diagnosis and management of heterozygous familial hypercholesterolemia. Atherosclerosis. 2004;173:55–68.

283. Masana L, Civeira F, Pedro-Botet J, de Castro I, Pocoví M, Plana N, et al. Expert consensus on the detection and clinical management of familial hypercholesterolemia. Clin Investig Arterioscler .2013;25:182–93.

284. LeBlanc AG, Janssen I. Dose-response relationship between physical activity and dyslipidemia in youth. Can J Cardiol .2010;26:201-5.

285. Fernandes RA, Christofaro DGD, Casonatto J, Codogno JS, Rodrigues EQ, Cardoso ML, et al. Prevalence of dyslipidemia in individuals physically active during childhood, adolescence and adult age. Arq Bras Cardiol .2011;97:317–23.

286. Telford RD, Cunningham RB, Waring P, Telford RM, Olive LS, Abhayaratna WP. Physical Education and Blood Lipid Concentrations in Children: The LOOK Randomized Cluster Trial. PLoS One .2013;8.

287. Holmes MV, Dale CE, Zuccolo L, Silverwood RJ, Guo Y, Ye Z, et al. Association between alcohol and cardiovascular disease: Mendelian randomisation analysis based on individual participant data. BMJ. 2014;349:g4164.

288. Stockwell T, Zhao J, Panwar S, Roemer A, Naimi T, Chikritzhs T. Do “moderate” drinkers have reduced mortality risk? A systematic review and meta-analysis of alcohol consumption and all-cause mortality Journal of Studies on Alcohol and Drugs. 2016;77: 185–98.

289. Luepker R V. Outcomes of a Field Trial to Improve Children’s Dietary Patterns and Physical Activity. JAMA .1996;275:768.

290. Akioyamen LE, Genest J, Shan SD, Inibhunu H, Chu A, Tu J V. Anxiety, depression, and health-related quality of life in heterozygous familial hypercholesterolemia: A systematic review and meta-analysis. J Psychosom Res .2018;109:32–43.

291. Bagnasco MS. Psychological issues and cognitive impairment in adults with familial hypercholesterolemia. Fam Pract.2017;34:520–4.

292. Fundación Faustino Orbegozo Eizaguirre. Curvas y Tablas de crecimiento (estudios longitudinal y transversal). Instituto de Investigación sobre crecimiento y desarrollo. María Diaz.

293. Soininen P, Kangas AJ, Würtz P, Suna T, Ala-Korpela M. Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. Circ Cardiovasc Genet.2015;8:192–206.

294. Kujala UM, Mäkinen VP, Heinonen I, Soininen P, Kangas AJ, Leskinen TH, et al. Long-term leisure-time physical activity and serum metabolome. Circulation. 2013;127:340–8.

295. Wang Q, Würtz P, Auro K, Mäkinen VP, Kangas AJ, Soininen P, et al. Metabolic profiling of pregnancy: Cross-sectional and longitudinal evidence. BMC Med. 2016;14:1–14.

296. Junyent M, Gilabert R, Núñez I, Corbella E, Vela M, Zambón D, et al. Carotid ultrasound in the assessment of preclinical atherosclerosis. Distribution of intima-media thickness values and plaque frequency in a Spanish community cohort. Med Clin.2005;125:770–4.

297. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, et al. Use of Carotid Ultrasound to Identify Subclinical Vascular Disease and Evaluate Cardiovascular Disease Risk: A Consensus Statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force Endorsed by the Society for Vascular Medicine. . Journal of the American Society of Echocardiography. 2008;21: 93–111.

298. Martin-Moreno JM, Boyle P, Gorgojo L, Maisonneuve P, Fernandez-Rodriguez JC SS. Development and Validation of a Food Frequency Questionnaire in Spain. Int J Epidemiol. 1993;22:512–9.

299. Lillegaard ITL, Øverby NC, Andersen LF. Can children and adolescents use photographs of food to estimate portion sizes? Eur J Clin Nutr. 2005;59:611–7.

300. Lillegaard ITL, Løken EB, Andersen LF. Relative validation of a pre-coded food diary among children, under-reporting varies with reporting day and time of the day. Eur J Clin Nutr. 2007;61:61–8.

301. Lillegaard ITL, Andersen LF. Validation of a pre-coded food diary with energy expenditure, comparison of under-reporters v. acceptable reporters. Br J Nutr. 2005;94:998–1003.

302. Couronné R, Probst P, Boulesteix A-L. Random forest versus logistic regression: a large-scale benchmark experiment. BMC.2018;19:1–14.

303. Breiman L. Random Forests. Mach Learn.2001;45:5–32.

304. Diamond DM, Alabdulgader AA, de Lorgeril M, Harcombe Z, Kendrick M, Malhotra A, et al. Dietary Recommendations for Familial Hypercholesterolaemia: an Evidence-Free Zone. BMJ Evidence-Based Med . 2020 5;bmjebm-2020-111412.

305. Barkas F, Nomikos T, Liberopoulos E, Panagiotakos D. Diet and Cardiovascular Disease Risk Among Individuals with Familial Hypercholesterolemia: Systematic Review and Meta-Analysis. Nutrients.2020;12:2436.

306. Roy G, Boucher A, Couture P, Drouin-Chartier J-P. Impact of Diet on Plasma Lipids in Individuals with Heterozygous Familial Hypercholesterolemia: A Systematic Review of Randomized Controlled Nutritional Studies. Nutrients. 2021;13:235.

307. Lambert CT, Sandesara P, Isiadinso I, Gongora MC, Eapen D, Bhatia N, et al. Current treatment of familial hypercholesterolaemia. Eur Cardiol Rev.2014;9:76–81.

308. Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J, Ruiz-Gutiérrez V, Covas MI, et al. Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. Ann Intern Med.2006;145:1–11.

309. Sotos-Prieto M, Bhupathiraju SN, Mattei J, Fung TT, Li Y, Pan A, et al. Association of Changes in Diet Quality with Total and Cause-Specific Mortality. N Engl J Med .2017;377:143–53

310. Micha R, Peñalvo JL, Cudhea F, Imamura F, Rehm CD, Mozaffarian D. Association between dietary factors and mortality from heart disease, stroke, and type 2 diabetes in the United States. JAMA.2017;317:912–24.

311. Capra ME, Pederiva C, Viggiano C, De Santis R, Banderali G, Biasucci G. Nutritional Approach to Prevention and Treatment of Cardiovascular Disease in Childhood. Nutrients.2021;13:2359.

312. Kinnear FJ, Hamilton-Shield JP, Stensel DJ, Bayly G, Searle A, Thackray AE, et al. Nutrition and physical activity intervention for families with familial hypercholesterolaemia: protocol for a pilot randomised controlled feasibility study. Pilot Feasibility Stud.2020;6:42.

313. Black MM, Walker SP, Fernald LCH, Andersen CT, DiGirolamo AM, Lu C et al. Early childhood development coming of age: science through the life course. Lancet. 2017;389:77–90.

314. Jeong J, Franchett EE, Ramos de Oliveira C V, Rehmani K, Yousafzai AK. Parenting interventions to promote early child development in the first three years of life: A global systematic review and meta-analysis. PLOS Med .2021;18:e1003602.

315. Ibarretxe D, Rodríguez-Borjabad C, Feliu A, Bilbao J, Masana L, Plana N. Detecting familial hypercholesterolemia earlier in life by actively searching for affected children:The DECOPIN project. Atherosclerosis. 2018;278:210-216.

316. Vázquez C, Alonso R, Garriga M, de Cos A, de la Cruz JJ, Fuentes-Jiménez F, et al. Validation of a food frequency questionnaire in Spanish patients with familial hypercholesterolaemia. Nutr Metab Cardiovasc Dis. 2012;22:836–42.

317. Elosua R, Marrugat J, Molina L, Pons S, Pujol E. Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish men. The MARATHOM Investigators. Am J Epidemiol. 1994;139:1197–209.

318. Elosua R, Garcia M, Aguilar A, Molina L, Covas MI, Marrugat J. Validation of the Minnesota Leisure Time Physical Activity Questionnaire In Spanish Women. Investigators of the MARATDON Group. Med Sci Sports Exerc. 2000;32:1431–7.

319. Ravens-Sieberer U, Gosch A, Rajmil L, Erhart M, Bruil J, Duer W, et al. KIDSCREEN-52 quality-of-life measure for children and adolescents. Expert Rev Pharmacoecon Outcomes Res. 2005;5:353–64.

320. BaranowskiTom, RyanCourtney, Hoyos-CespedesAndrés, Shirong L. Nutrition Education and Dietary Behavior Change Games: A Scoping Review. .2019;8:153–76.

321. Kastelein JJP, Reeskamp LF, Hovingh GK. Familial Hypercholesterolemia: The Most Common Monogenic Disorder in Humans . Journal of the American College of Cardiology.2020;75:2567–9.

322. O'Loughlin J, Lauzon B, Paradis G, Hanley J, Lévy E, Delvin E, et al. Usefulness of the American Academy of Pediatrics recommendations for identifying youths with hypercholesterolemia. Pediatrics .2004113:1723–7

323. Yusuf PS, Hawken S, Ôunpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): Case-control study. Lancet. 2004;364:937–52.

324. Mente A, Dehghan M, Rangarajan S, McQueen M, Dagenais G, Wielgosz A, et al. Association of dietary nutrients with blood lipids and blood pressure in 18 countries: a cross-sectional analysis from the PURE study. Lancet Diabetes Endocrinol .2017;5:774–87.

325. Medeiros AM, Alves AC, Aguiar P, Bourbon M. Cardiovascular risk assessment of dyslipidemic children: Analysis of biomarkers to identify monogenic dyslipidemia. J Lipid Res.2014;55:947–55.

326. Juonala M, Viikari JSA, Kähönen M, Solakivi T, Helenius H, Jula A, et al. Childhood Levels of Serum Apolipoproteins B and A-I Predict Carotid Intima-Media Thickness and Brachial Endothelial Function in Adulthood. The Cardiovascular Risk in Young Finns Study. J Am Coll Cardiol. 2008;52:293–9.

327. Ibarretxe D, Girona J, Plana N, Cabré A, Ferré R, Amigó N, et al. Circulating PCSK9 in patients with type 2 diabetes and related metabolic disorders. Clin e Investig en Arterioscler.2016;28:71–8.

328. Guardiola M, Plana N, Ibarretxe D, Cabré A, González M, Ribalta J, et al. Circulating PCSK9 levels are positively correlated with NMR-assessed atherogenic dyslipidaemia in patients with high cardiovascular risk. Clin Sci.2015;128:877–82.

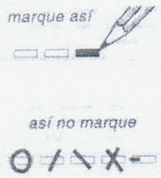
12. Supplementary material

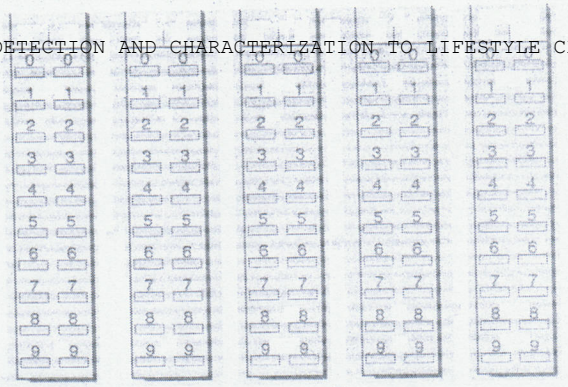
12. SUPPLEMENTARY MATERIAL

1. Food frequency questionnaire validated for Spanish population.
2. Informative brochure about lifestyle changes workshops.
3. Magazines covers used in the lifestyle changes workshops.
4. Photographs and blog about lifestyle changes workshops.
5. Diploma delivered at the end of the workshops.

1. Food frequency questionnaire validated for Spanish population.

01. Andalucía-Málaga
02. Andalucía-Sevilla-San Pablo
03. Andalucía-Sevilla-V. Rocío
04. Baleares
05. Catalunya-Barna Norte
06. Catalunya-Barna Sur
07. Catalunya-Reus-Tarragona
08. Madrid Norte
09. Madrid Sur
10. Navarra
11. País Vasco
12. Valencia





1

Por favor, marque una única opción para cada alimento.

Para cada alimento, marque el recuadro que indica la frecuencia de consumo **por término medio** durante el **año pasado**. Se trata de tener en cuenta también la variación verano/invierno. Por ejemplo, si toma helados 4 veces/semana sólo durante los 3 meses de verano, el uso promedio al año es 1/semana

CONSUMO MEDIO DURANTE EL AÑO PASADO

	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6+
I. LÁCTEOS									
1. Leche entera (1 taza, 200 cc)									
2. Leche semidesnatada (1 taza, 200 cc)									
3. Leche descremada (1 taza, 200 cc)									
4. Leche condensada (1 cucharada)									
5. Nata o crema de leche (1/2 taza)									
6. Batidos de leche (1 vaso, 200 cc)									
7. Yogurt entero (1, 125 gr.)									
8. Yogurt descremado (1, 125 gr.)									
9. Petit suisse (1, 55 gr.)									
10. Requesón o cuajada (1/2 taza)									
11. Queso en porciones o cremoso (1, porción 25 gr.)									
12. Otros quesos: curados, semicurados (Manchego, Bola, Emmental...) (50 gr.)									
13. Queso blanco o fresco (Burgos, cabra...) (50 gr.)									
14. Natillas, flan, puding (1, 130 cc)									
15. Helados (1 cucurucho)									

Un plato o ración de 100-150 gr, excepto cuando se indique otra cosa	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6+
II. HUEVOS, CARNES, PESCADOS									
16. Huevos de gallina (uno)									
17. Pollo o pavo CON piel (1 ración o pieza)									
18. Pollo o pavo SIN piel (1 ración o pieza)									
19. Carne de ternera o vaca (1 ración)									
20. Carne de cerdo (1 ración)									
21. Carne de cordero (1 ración)									
22. Conejo o liebre (1 ración)									
23. Hígado (ternera, cerdo, pollo) (1 ración)									
24. Otras vísceras (sesos, corazón, mollejas) (1 ración)									
25. Jamón serrano o paletilla (1 loncha, 30 gr.)									
26. Jamón York, jamón cocido (1 loncha, 30 gr.)									
27. Carnes procesadas (salchichón, chorizo, morcilla, mortadela, salchichas, butifarra, sobrasada, 50 gr.)									
28. Patés, foie-gras (25 gr.)									
29. Hamburguesa (una, 50 gr.), albóndigas (3 unidades)									
30. Tocino, bacon, panceta (50 gr.)									
31. Pescado blanco: mero, lenguado, besugo, merluza, pescadilla,... (1 plato, pieza o ración)									
32. Pescado azul: sardinas, atún, bonito, caballa, salmón (1 plato, pieza o ración 130 gr.)									
33. Pescados salados: bacalao, salazones (1 ración, 60 gr. en seco)									
34. Ostras, almejas, mejillones y similares (6 unidades)									
35. Calamares, pulpo, chipirones, jibia (sepia) (1 ración, 200 gr.)									
36. Crustáceos: gambas, langostinos, cigalas, etc. (4-5 piezas, 200 gr.)									
37. Pescados y mariscos enlatados al natural (sardinas, anchoas, bonito, atún) (1 lata pequeña o media lata normal, 50 gr.)									
38. Pescados y mariscos en aceite (sardinas, anchoas, bonito, atún) (1 lata pequeña o media lata normal, 50 gr.)									

Por favor, marque una única opción para cada alimento.

III. VERDURAS Y HORTALIZAS

Un plato o ración de 200 grs, excepto cuando se indique	CONSUMO MEDIO DURANTE EL AÑO PASADO								
	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6 +
39. Acelgas, espinacas									
40. Col, coliflor, brócoles									
41. Lechuga, endivias, escarola (100 gr.)									
42. Tomate crudo (1, 150 gr)									
43. Zanahoria, calabaza (100 gr.)									
44. Judías verdes									
45. Berenjenas, calabacines, pepinos									
46. Pimientos (150 gr.)									
47. Espárragos									
48. Gazpacho andaluz (1 vaso, 200 gr.)									
49. Otras verduras (alcachofa, puerro, cardo, apio)									
50. Cebolla (media unidad, 50 gr.)									
51. Ajo (1 diente)									
52. Perejil, tomillo, laurel, orégano, etc. (una pizca)									
53. Patatas fritas comerciales (1 bolsa, 50 gr.)									
54. Patatas fritas caseras (1 ración, 150 gr.)									
55. Patatas asadas o cocidas									
56. Setas, níscalos, champiñones									

IV. FRUTAS

Una pieza o ración	CONSUMO MEDIO DURANTE EL AÑO PASADO								
	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6 +
57. Naranja (una), pomelo (una), o mandarinas (dos)									
58. Plátano (uno)									
59. Manzana o pera (una)									
60. Fresas/fresones (6 unidades, 1 plato postre)									
61. Cerezas, picotas, ciruelas (1 plato de postre)									
62. Melocotón, albaricoque, nectarina (una)									
63. Sandía (1 tajada, 200-250 gr.)									
64. Melón (1 tajada, 200-250 gr.)									
65. Kiwi (1 unidad, 100 gr.)									
66. Uvas (un racimo, 1 plato postre)									
67. Aceitunas (10 unidades)									
68. Frutas en almíbar o en su jugo (2 unidades)									
69. Dátiles, higos secos, uvas-pasas, ciruelas-pasas (150 gr.)									
70. Almendras, cacahuetes, avellanas, pistachos, piñones (30 gr.)									
71. Nueces (30 gr.)									

72. ¿Cuántos días a la semana toma fruta como postre?	0	1	2	3	4	5	6	7

V. LEGUMBRES Y CEREALES

Un plato o ración (150 gr.)	CONSUMO MEDIO DURANTE EL AÑO PASADO								
	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6 +
73. Lentejas (1 plato, 150 gr. cocidas)									
74. Alubias (pintas, blancas o negras) (1 plato, 150 gr. cocidas)									
75. Garbanzos (1 plato, 150 gr. cocidos)									
76. Guisantes, habas (1 plato, 150 gr. cocidas)									
77. Pan blanco, pan de molde (3 rodajas, 75 gr.)									
78. Pan negro o integral (3 rodajas, 75 gr.)									
79. Cereales desayuno (30 gr.)									
80. Cereales integrales: muesli, copos avena, all-bran (30 gr.)									
81. Arroz blanco (60 gr. en crudo)									
82. Pasta: fideos, macarrones, espaguetis, otras (60 gr. en crudo)									
83. Pizza (1 ración, 200 gr.)									

así no marque
O / \ X -

Por favor, marque una única opción para cada alimento.

Una cucharada o porción individual. Para freír, untar, mojar en el pan, para aliñar, o para ensaladas, utiliza en total:

CONSUMO MEDIO DURANTE EL AÑO PASADO

	NUNCA O CASI NUNCA	AL MES 1 - 3	A LA SEMANA 1	A LA SEMANA 2 - 4	A LA SEMANA 5 - 6	AL DÍA 1	AL DÍA 2 - 3	AL DÍA 4 - 6	AL DÍA 6 +
VI. ACEITES Y GRASAS									
84. Aceite de oliva (una cucharada sopera)									
85. Aceite de oliva extra virgen (una cucharada sopera)									
86. Aceite de oliva de orujo (una cucharada sopera)									
87. Aceite de maíz (una cucharada sopera)									
88. Aceite de girasol (una cucharada sopera)									
89. Aceite de soja (una cucharada sopera)									
90. Mezcla de los anteriores (una cucharada sopera)									
91. Margarina (porción individual, 12 gr.)									
92. Mantequilla (porción individual, 12 gr.)									
93. Manteca de cerdo (10 gr.)									
94. Marca de aceite de oliva que usa habitualmente:	0 1 2 3 4 5 6 7 8 9 / 0 1 2 3 4 5 6 7 8 9								

No marque aquí

CONSUMO MEDIO DURANTE EL AÑO PASADO

	NUNCA O CASI NUNCA	AL MES 1 - 3	A LA SEMANA 1	A LA SEMANA 2 - 4	A LA SEMANA 5 - 6	AL DÍA 1	AL DÍA 2 - 3	AL DÍA 4 - 6	AL DÍA 6 +
VII. BOLLERÍA Y PASTELERÍA									
95. Galletas tipo María (4-6 unidades, 50 gr.)									
96. Galletas integrales o de fibra (4-6 unidades, 50 gr.)									
97. Galletas con chocolate (4 unidades, 50 gr.)									
98. Repostería y bizcochos hechos en casa (50 gr.)									
99. Croissant, ensaimada, pastas de té u otra bollería industrial comercial... (uno, 50 gr)									
100. Donuts (uno)									
101. Magdalenas (1-2 unidades)									
102. Pasteles (uno, 50 gr.)									
103. Churros, porras y similares (1 ración, 100 gr.)									
104. Chocolates y bombones (30 gr.)									
105. Cacao en polvo-cacaos solubles (1 cucharada de postre)									
106. Turrón (1/8 de barra, 40 gr.)									
107. Mantecados, mazapán (90 gr.)									

CONSUMO MEDIO DURANTE EL AÑO PASADO

	NUNCA O CASI NUNCA	AL MES 1 - 3	A LA SEMANA 1	A LA SEMANA 2 - 4	A LA SEMANA 5 - 6	AL DÍA 1	AL DÍA 2 - 3	AL DÍA 4 - 6	AL DÍA 6 +
VIII. MISCELÁNEA									
108. Croquetas, buñuelos, empanadillas, precocinados (una)									
109. Sopas y cremas de sobre (1 plato)									
110. Mostaza (una cucharadita de postre)									
111. Mayonesa comercial (1 cucharada sopera = 20 gr.)									
112. Salsa de tomate frito, ketchup (1 cucharadita)									
113. Picante: tabasco, pimienta, pimentón (una pizca)									
114. Sal (una pizca)									
115. Mermeladas (1 cucharadita)									
116. Azúcar (1 cucharadita)									
117. Miel (1 cucharadita)									
118. Snacks distintos de patatas fritas: gusanitos, palomitas, maíz, etc. (1 bolsa, 50 gr.)									
119. Otros alimentos de frecuente consumo:									
119.1									
119.2									
119.3									

Por favor, marque una única opción para cada alimento.

IX. BEBIDAS

	CONSUMO MEDIO DURANTE EL AÑO PASADO								
	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6 +
120. Bebidas carbonatadas con azúcar: bebidas con cola, limonadas, tónicas, etc. (1 botellín, 200 cc)									
121. Bebidas carbonatadas bajas en calorías, bebidas light (1 botellín, 200 cc)									
122. Zumo de naranja natural (1 vaso, 200 cc)									
123. Zumos naturales de otras frutas (1 vaso, 200 cc)									
124. Zumos de frutas en botella o enlatados (200 cc)									
125. Café descafeinado (1 taza, 50 cc)									
126. Café (1 taza, 50 cc)									
127. Té (1 taza, 50 cc)									
128. Mosto (100 cc)									
129. Vaso de vino rosado (100 cc)									
130. Vaso de vino moscatel (50 cc)									
131. Vaso de vino tinto joven, del año (100 cc)									
132. Vaso de vino tinto añejo (100 cc)									
133. Vaso de vino blanco (100 cc)									
134. Vaso de cava (100 cc)									
135. Cerveza (1 jarra, 330 cc)									
136. Licores, anís o anisetes... (1 copa, 50 cc)									
137. Destilados: whisky, vodka, ginebra, coñac (1 copa, 50 cc)									

138. ¿A que edad empezó a beber alcohol (vino, cerveza o licores), incluyendo el que toma con las comidas con regularidad (más de siete "bebidas" a la semana)?

Edad (años)

Decena
Unidad

139. ¿Cuántos años ha bebido alcohol con regularidad (más de siete "bebidas" a la semana)?

Años

Decena
Unidad

119. Otros alimentos de frecuente consumo

119.1 (No marque aquí)

119.2 (No marque aquí)

119.3 (No marque aquí)

Si durante el año pasado tomó vitaminas y/o minerales (incluyendo calcio) o productos dietéticos especiales (salvado, aceite de onagra, leche con ácidos grasos omega-3, flavonoides, etc.), por favor indique la marca y la frecuencia con que los tomó:

Marcas de los suplementos de vitaminas o minerales o de los productos dietéticos	CONSUMO MEDIO DURANTE EL AÑO PASADO								
	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6 +
140.									
140.1									
140.2									

140 (No marque aquí)

140.1 (No marque aquí)

140.2 (No marque aquí)

SUMCO 12637-03-(Rel. 2)

Muchas gracias por su colaboración

2. Informative brochure about lifestyle changes workshops.

Descripció del projecte

El projecte consisteix en la realització de 6 tallers teòrico-pràctics sobre canvis d'estil de vida. Els vostres fills aprendran a menjar de forma més saludable i entendran que és important fer exercici físic habitualment.

Haureu d'acompanyar als vostres fills per millorar l'adherència i augmentar els coneixements de tota la família. A més a més, disposarem d'un "blog", al qual se us convidarà a participar. En el "blog" podreu consultar els diferents tallers, els horaris, i disposareu de material de suport (dibuixos, receptes, etc.).

UNIVERSITAT ROVIRA I VIRGILI
FAMILIAL HYPERCHOLESTEROLEMIA IN CHILDREN: FROM DETECTION AND CHARACTERIZATION TO LIFESTYLE CHANGES
Célia Rodríguez Borjabad

Hàbits saludables per a nens amb nivells elevats de colesterol

Xerrada informativa

Mas Pintat
22 octubre de 2015
a les 18:00

E-mail de contacte:
projectedecopin@gmail.com

Tallers
Canvis Estil de Vida

- ."**Cuinar és divertit**": Taller teòric-pràctic sobre hàbits saludables. Es realitzarà una breu introducció a pares i nens sobre quin menjar és saludable i sobre quines són les millors tècniques culinàries. Seguidament, els nens duran a terme l'elaboració d'un plat saludable amb l'ajuda d'una cuinera amb una llarga trajectòria i experiència en la realització de tallers de cuina.
- ."**Juguem a la OCA"/ "A la captura dels aliments**": Els nens jugaran a jocs de taula tradicionals, com són el trivial (en el cas dels grans) i la Oca (nens 5-7 anys). A partir dels jocs de taula es potenciaran els diferents hàbits saludables.
- ."**Mou-te**": El nens jugaran a jocs tradicionals per tal de potenciar l'activitat física.
- ."**El Superesmorzar**": Se'ls explicarà a nens i familiars com realitzar un esmorzar saludable i es potenciaran els hàbits higiènics.
- ."**El viatge dels sentits**": Els nens experimentaran amb els 5 sentits (gust, textura, so al mastegar, olfacte) diferents aliments.
- ."**Celebracions de colors**": Taller de cuina i manualitats que tractarà de les fruites. Els nens hauran de crear plats suculents amb fruites per promocionar la seva ingesta. En el taller se'ls ensenyarà que les celebracions, o festes, poden anar acompanyades de fruita i no sempre de brioxeria.

Quan es faran els tallers?

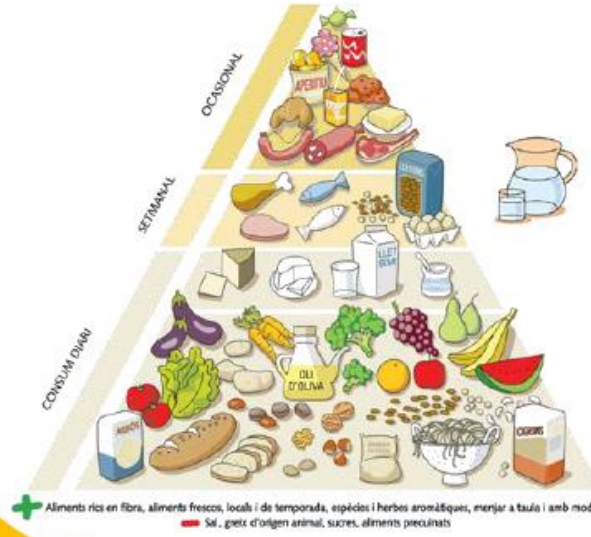
Es realitzarà un taller mensual amb una durada d'una hora. Hi ha un total de 6 tallers, és a dir, que hauran de venir durant 6 mesos un cop al mes.

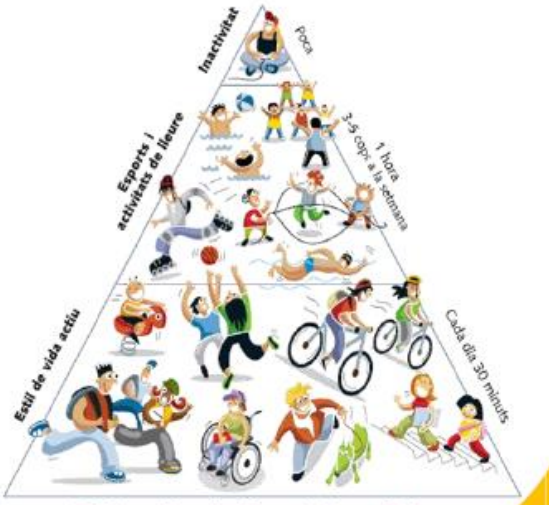
1 HORA

Jugant aprenem a menjar bé...

On es realitzaran?

Mas Pintat. Centre d'Atenció a la Infància i a les Famílies
Carrer de l'Abat Escarré, 11
REUS





Piràmide de l'activitat física
a la infància i a l'adolescència

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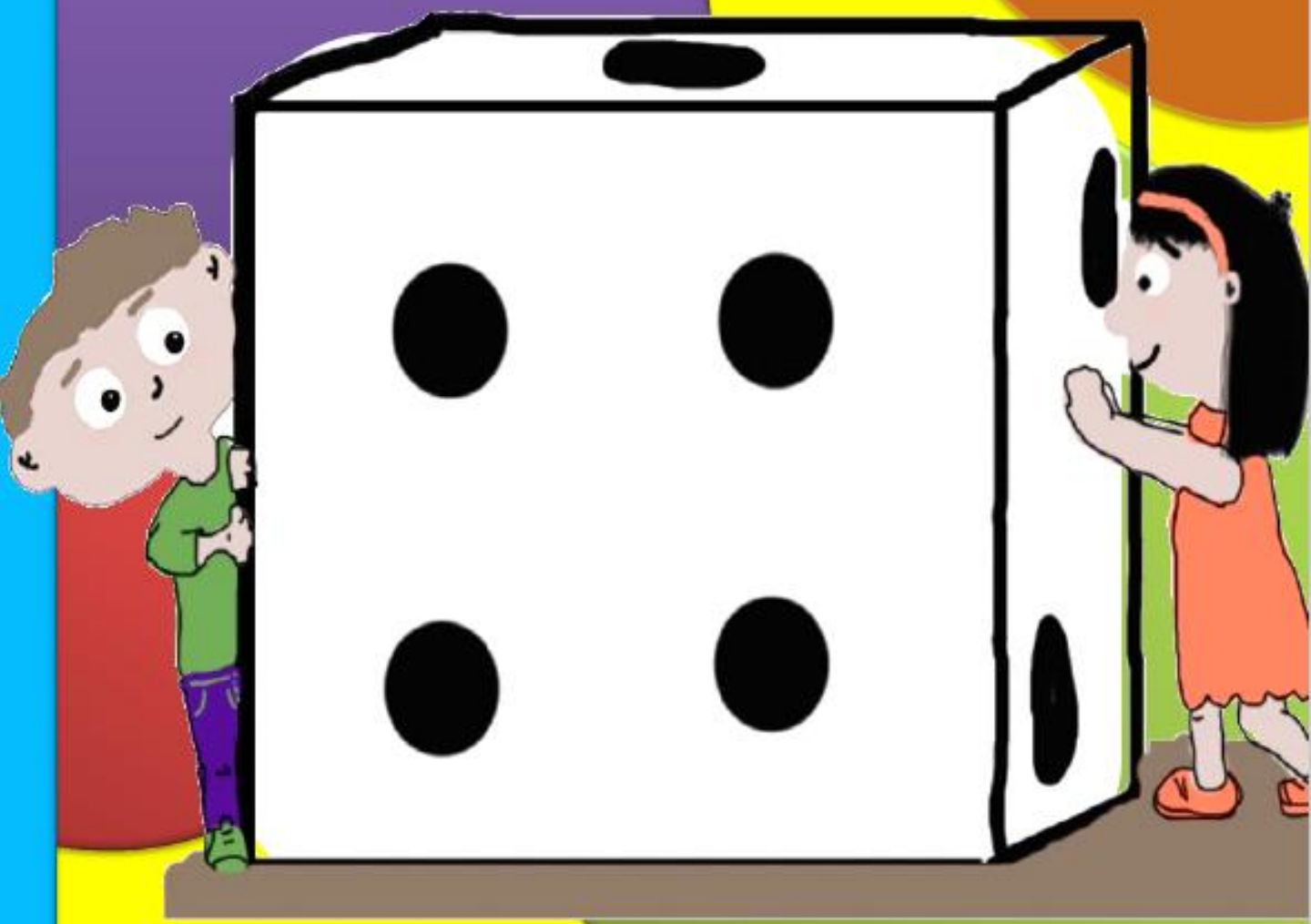
3. Magazines covers used in the lifestyle changes workshops.

Quadern Cardiosaludable

Som a la cuina!

Quadern Cardiosaludable

A la captura dels aliments!



Nom:

Quadern cardiosaludable

Fent esport creixem més sans

Nom:

Quadern Cardiosaludable

El Super Esmorzar

Nom:

El viatge dels sentits

Vull provar coses noves!

Nom:

Celebracions de colors

Una festa saludable!

Nom:

4. Photographs and blog about lifestyle changes workshops.

Cooking is fun

Catching food

Let's move on

Healthy breakfast

The journey of the senses

A celebration of colours

http://projectedecopin.blogspot.com.es/ (PRIVATE BLOG)

5. Diploma delivered at the end of the workshops.

Certificació

DECOPIN

Tallers Cardiosaludables

Projecte DECOPIN

Ha completat el curs correctament
i ja és un expert en COLESTEROL

Firma:

Data:

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"SATISFACTION LIES IN THE EFFORT, NOT IN THE ATTAINMENT, FULL EFFORT IS FULL VICTORY"
(MAHATMA GANDHI)

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