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Review

Markers of atherosclerotic development in children with familial hypercholesterolemia: A literature review

Ingunn Narverud [a, c, *], Kjetil Retterstøl [a, c], Per Ole Iversen [a, d], Bente Halvorsen [e, f], Thor Ueland [e, f], Stine M. Ulven [b], Leiv Ose [a, c], Pål Aukrust [e, f, g, h], Marit B. Veierød [a, i], Kirsten B. Holven [a]

[a] Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, P.O. Box 1046, Blindern, 0317 Oslo, Norway
[b] Department of Health, Nutrition and Management, Faculty of Health Sciences, Oslo and Akershus University College of Applied Sciences, P.O. Box 4, St. Olavs Plass, 0130 Oslo, Norway
[c] Lipid Clinic, Oslo University Hospital Rikshospitalet, P.O. Box 4950, Nydalen, 0424 Oslo, Norway
[d] Department of Haematology, Oslo University Hospital, P.O. Box 4950, Nydalen, 0424 Oslo, Norway
[e] Research Institute of Internal Medicine, Oslo University Hospital Rikshospitalet, P.O. Box 4950, Nydalen, 0424 Oslo, Norway
[f] Institute of Clinical Medicine, University of Oslo, P.O. Box 1171, Blindern, 0318 Oslo, Norway
[g] Section of Clinical Immunology and Infectious Diseases, Oslo University Hospital Rikshospitalet, P.O. Box 4950, Nydalen, 0424 Oslo, Norway
[h] K.G. Jebsen Inflammatory Research Center, P.O. Box 1171, Blindern, 0318 Oslo, Norway
[i] Department of Biostatistics, Institute of Basic Medical Sciences, University of Oslo, P.O. Box 1110, Blindern, 0317 Oslo, Norway

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ABSTRACT

Objective: Atherosclerosis is a multi-step process, where lipids, inflammatory and hemostatic mediators orchestrate plaque formation and progression, which subsequently may lead to myocardial infarction and ischemic stroke. Familial hypercholesterolemia (FH) is associated with increased risk of premature atherosclerosis due to the genetically determined elevated low density lipoprotein (LDL)-cholesterol seen in these individuals. Children with FH are suitable to investigate the isolated effect of elevated LDL-cholesterol on early markers of atherosclerosis. The aim of the present paper was to review the literature to summarize the findings of atherosclerotic markers in children with FH to better understand how elevated LDL-cholesterol *per se* promotes atherogenesis.
Methods: We conducted a systematic literature search from the years 1990–2013, resulting in identification of 903 articles. In order to investigate whether intima-media thickness (IMT) is different in children with and without FH, we conducted a meta-analysis of the studies comparing FH children with a control group.
Results: 37 original articles were included. Among these, 24 reported subclinical measurements, whereas other articles reported measurements of atherogenic lipids ($n = 9$), inflammatory markers ($n = 10$), hemostatic markers ($n = 6$) and other surrogate markers of atherosclerosis ($n = 7$). In the meta-analysis ($n = 8$), IMT was significantly thicker in children with FH than in control children (weighted mean difference 0.06, 95% confidence interval [0.01, 0.11]).
Conclusion: Elevated LDL-cholesterol distinguishes children with and without FH, but these groups of children also differ in several other ways. In particular, children with FH display a variety of changes reflecting both the lipid and the inflammatory arm of atherosclerosis. The IMT-meta-analysis result strengthens the evidence of early atherosclerotic development in children with FH. In a clinical perspective, early diagnosing and treatment of children with FH is of high importance to attenuate development of the potential ongoing early atherosclerotic process in these individuals.

* Corresponding author. Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, P.O. Box 1110, Blindern, 0317 Oslo, Norway. Tel.: +47 22851556; fax: +47 22851341.
E-mail addresses: ingunn.narverud@medisin.uio.no (I. Narverud), kjetil.retterstol@medisin.uio.no (K. Retterstøl), p.o.iversen@medisin.uio.no (P.O. Iversen), Bente.Halvorsen@rr-research.no (B. Halvorsen), Thor.Ueland@rr-research.no (T. Ueland), stinemarie.ulven@hioa.no (S.M. Ulven), lose@ous-hf.no (L. Ose), paukrust@ous-hf.no (P. Aukrust), m.b.veierod@medisin.uio.no (M.B. Veierød), Kirsten.holven@medisin.uio.no (K.B. Holven).

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Contents

1. Introduction

The aim of the present paper was to systematically summarize literature findings of atherosclerotic markers in children with FH to better understand how elevated LDL-cholesterol *per se* affects development of atherosclerosis.

Atherosclerosis is a multi-step process, where lipids, inflammatory and hemostatic mediators orchestrate plaque formation and progression with subsequent thrombus formation leading to complications like myocardial infarction (MI) and ischemic stroke [1–3]. Inflammation mediated by numerous cytokines, play a key role during this process, from the initial endothelial dysfunction and subsequent leukocyte recruitment to foam cell formation and plaque progression including development of unstable lesions [4–6].

Atherosclerotic plaque consists of various cell types such as T cells, macrophages, endothelial cells and vascular smooth muscle cells [3,4]. All these cells express, release, and respond to several growth factors, matrix metalloproteinases as well as several chemokines and cytokines such as the inflammatory molecules tumor necrosis factor alpha (TNFα), interleukin (IL)-1β, IL-6, IL-8 and interferon (IFN)-γ causing a chronic inflammation. Such state of chronic or non-resolving inflammation facilitates lipid accumulation in the atheroma, which is a phenotypical hallmark of atherosclerosis that distinguishes this disorder from several other inflammatory disorders [3,4].

Signs of atherosclerosis may be present throughout the lifetime, fatty streaks, representing the earliest sign of this process have been observed in fetal aortas and in children above 3 years of age [7,8]. To characterize the degree of atherosclerosis several surrogate markers have been evolved. Two major characteristics of atherosclerosis, endothelial dysfunction and thickening of the vascular wall, may be assessed by subclinical measurements e.g. flow-mediated dilation (FMD) and intima-media thickness (IMT), respectively [9,10]. Atherogenic lipids, and circulating inflammatory and thrombotic markers, as well as mRNA levels of inflammatory markers in circulating cells, may be unfavorable mediators in atherogenesis and may predict prognosis in patients with established atherosclerosis [11–14].

Familial hypercholesterolemia (FH) is an autosomal dominant disease caused by mutations in the genes encoding the low density lipoprotein (LDL) receptor, proprotein convertase subtilisin/kexin type 9 (PCSK9) or apolipoprotein B (apoB) [15]. Heterozygous FH patients have inherited one mutation from one parent, and are characterized by a 2-fold increase in both plasma total- and LDL-cholesterol levels [15,16]. The elevated serum levels of LDL-cholesterol may in turn give rise to xanthomas, deposits of cholesterol in peripheral tissues like subcutaneous xanthelasms, and accelerated atherosclerosis leading to increased risk of premature coronary artery disease (CAD) [15]. About 5% of the patients suffering an MI below the age of 60 have heterozygous FH [16]. Given that the prevalence of FH in the population is about 0.2%, this suggests almost a 50-fold increase in the risk for MI at an age younger than 60 years.

Compared with adult dyslipidemic patients, children with heterozygous FH are less influenced by lifestyle factors (such as smoking, unfavorable diet, or obesity) and few have initiated cholesterol-lowering medication. Thus, children with FH are suitable to investigate the isolated effect of elevated LDL-cholesterol on early markers of atherosclerotic development. These patients may serve as a human model system to indirectly investigate the impact of elevated LDL-cholesterol levels on the atherosclerotic process, and to characterize possible markers important in the early development of atherosclerosis.

2. Methods

2.1. Literature search

We conducted a systematic literature search in the databases PubMed and OVID/Medline EMBASE in June 2013. The following terms were used: familial hypercholesterolemia (including familial

hypercholesterolemia as all fields, and hyperlipoproteinemia type II as MeSH term) AND arteriosclerosis OR inflammation OR biological markers with the filter child: birth-18 years. The search was limited to publications after 1990. In total 903 articles (without duplicates) were identified. Based on these papers we included all studies that compared subclinical atherosclerotic measurements (e.g. IMT, FMD, post occlusive reactive hyperemia [PORH], measurements of the left ventricular wall), atherogenic lipids, and circulating and mRNA levels of inflammatory and hemostatic markers, between children and young adults below 25 years with and without FH. Exclusion criteria were 1) studies without a healthy control group or a healthy sibling group, 2) studies with a mixed group of children and adults (>25 years) with FH, 3) studies with a mixed group of children with FH and other dyslipidemic/high-risk disorders, 4) intervention studies with effects on atherosclerotic markers, except studies with baseline characteristics which includes the above mentioned measurements, and 5) published abstracts only. All the studies comprise mainly heterozygous FH children if not otherwise stated. Data are shown as mean ± standard deviation or standard error of the mean, or median and interquartile range or minimum–maximum if not otherwise is stated.

2.2. Meta-analysis of IMT

We used a random effects model (DerSimonian and Laird method) to combine estimates from individual studies [17]. This method takes into account within- and between-study variation (weighting was based on the inverse of the variance) in the calculation of the summary mean difference (weighted mean difference [WMD]) between the FH and control groups. The results are presented with 95% confidence intervals (95% CIs). Noto et al. [18] presented their results stratified according to IMT in the FH group, and a weighted average of the means and a pooled standard deviation were used for the FH group in the meta-analysis. Heterogeneity was studied by using the Q and I^2 statistics [19]. I^2 is the amount of total variation that is explained by between study variation, and I^2 values of approximately 25%, 50% and 75% are considered to indicate low, moderate and high heterogeneity, respectively. The meta-analysis was conducted by using Stata, version 13 (StataCorp LP, College Station, Texas).

3. Results and discussion

In total 37 original articles were included, whereof one were cited in an article from the systematic literature search. Among these 37 articles, 24 reported subclinical measurements, nine reported atherogenic lipids, ten reported inflammatory markers, six reported hemostatic markers, and seven reported other surrogate markers of atherosclerosis such as hemorheological changes and urinary isoprostane levels. Main markers of atherosclerotic development are listed in Table 1. Details of the 37 studies included in this review are given in Table 2 and as Supplementary Table 1.

3.1. Subclinical atherosclerotic measurements

3.1.1. IMT and IMT-related measurements

IMT in carotid arteries is a potential predictor of MI, stroke, and aneurysm rupture [10,20,21], and may thus represent a subclinical measurement of the atherosclerotic process. The carotid [18,22–29] and aortic [27] IMT have been reported to be significantly thicker in children with FH compared with control children in 9 studies. In the study by Tonstad and coworkers, carotid artery

Table 1
Overview of main markers of atherosclerotic development.

Name	Abbreviation	Function
Inflammatory markers		
CD40 ligand	CD40L	Involved in interaction between T and B cells, activation of dendritic cells and platelet-mediated inflammation
C-reactive protein	CRP	A stable and reliable marker of up-stream inflammation, in particular IL-6-reacted pathways. Can activate complement
Intercellular adhesion molecule 1	ICAM-1	Reflect interaction between leukocytes and endothelial cells
Interleukin-1 β	IL-1β	Major inflammatory cytokine that is released from activated inflammasomes
Interleukin-10	IL-10	Prototypical anti-inflammatory cytokine
Interleukin-6	IL-6	Induces acute-phase response and differentiaton and anti-body secretion from B cells/plasma cells
Neopterin	N/A	Reflect monocyte/macrophage activation induced by Th1 cells
Regulated on Activation Normally T-cell Expressed and Secreted	RANTES	Inflammatory chemokine that induces activation and attraction of T cells and is involved in platelet-mediated inflammation
Tumour necrosis factor α	TNFα	Prototypical inflammatory cytokines with central role in regulation of inflammation, cell survival/apoptosis/cell death and promotion of oxidative stress.
Atherogenic lipids and related mediators		
Lipoprotein (a)	Lp(a)	Unknown physiological function, pro-atherogenic lipoprotein
Lipoprotein-associated phospholipase A_2	Lp-PLA2	Involved in generation of lipid mediators important in pro-atherogenic processes
Oxidized low density lipoprotein	oxLDL	Pro-inflammatory and -atherogenic lipoprotein particle
Oxidative stress		
Asymmetric dimethylarginine	ADMA	Involved in oxidative stress by inhibiting endothelial nitric oxide synthase
Urine isoprostaglandin $F_{2\alpha}$ type III	N/A	Lipid peroxidation product, marker of oxidative stress
Hemostatic markers		
Fibrinogen	N/A	Precursor to fibrin, necessary for coagulation. Marker of inflammation.
Thrombomodulin	N/A	Inhibits the function of thrombin, by forming a complex with thrombin
Tissue factor/tissue factor pathway inhibitor	TF/TFPI	Main regulators of the initiation of the coagulation process
Tissue plasminogen activator/Plasminogen activator inhibitor-1	tPA/PAI-1	Main regulators of the initiation of fibrinolysis
Von Willebrand Factor	vWF	Marker of endothelial cell activation
Subclinical atherosclerotic measurements		
Intima-media thickness	IMT	Surrogate marker for atherosclerotic thickening of the intima
Flow-mediated dilation	FMD/RH%	Measure of endothelial-dependent dilation, surrogate marker of endothelial dysfunction
Post occlusive reactive hyperemia	PORH	Reflects microvascular endothelial function

Table 2
A review of markers of atherosclerotic development in children with familial hypercholesterolemia.

Author, publication year, (Ref.)	Participants	Markers of atherosclerotic development	Main results
Aggoun Y et al., 2000 [30]	FH ($n = 30$, 11.1 ± 2.0 yrs), ctr ($n = 27$, 11.1 ± 3.0 yrs)	Systolic and diastolic carotid lumen diameters, FMD and GTN-induced dilation of the brachial artery, carotid IMT	Decreased systodiastolic changes in carotid lumen diameter, systodiastolic change in diameter/lumen diastolic diameter ratio, and cross-sectional compliance and distensibility, but increased incremental elastic modulus in FH. Impaired FMD, but not GTN-induced dilation in FH. No difference in IMT.
Barros MR et al., 2006 [67]	FH, parents with early CAD ($n = 8$, 9.8 ± 4.9 yrs), FH, parent without early CAD ($n = 6$, 10.8 ± 5.9 yrs), unaffected children, parents with FH without early CAD ($n = 14$, 10.3 ± 5.1 yrs), ctr children ($n = 12$, 12 ± 3.3 yrs)	Serum levels of LDL(−), anti-LDL(−) auto-antibodies	Lower anti-LDL(−) auto-antibodies in all the FH groups. No difference in LDL(−).
Celermajer DS et al., 1992 [37]	FH ($n = 10$, 8–16 yrs), ctr ($n = 16$, 8–14 yrs),	FMD and GTN-induced dilation	Reduced FMD, but not GTN-induced dilation in FH.
Charakida M et al., 2009 [39]	FH ($n = 38$, 14.8, 7–22 yrs), ctr ($n = 41$, 15.4, 6–22 yrs)	RH% and NH%, circulating inflammatory and hemostatic markers	Reduced RH% and NH% and increased IL-1β, IL-6, PAI-1 in FH. No difference in CRP, fibrinogen vWF or TNFα.
Cracowski JL et al., 2001 [98]	FH ($n = 15$, 14 ± 2.3 yrs), ctrs ($n = 15$, 13.5 ± 1.8 yrs)	Urine isoprostaglandin $F_{2\alpha}$ type III	No difference in urine isoprostaglandin $F_{2\alpha}$ type III
De Groot E et al., 2005 [32]	FH ($n = 44$, 14.8 ± 2.8 yrs), ctr ($n = 44$, 14.9 ± 2.8 yrs)	Carotid and femoral IMT	No differences in IMT.
de Jongh S et al., 2002 [38]	FH ($n = 50$, 9–18 yrs), unaffected siblings ($n = 19$, 9–18 yrs)	FMD	Impaired FMD in FH
de Jongh S et al., 2002 [40]	FH ($n = 50$, 14.6 ± 2.2 yrs), unaffected siblings ($n = 19$, 14.2 ± 3.1 yrs)	FMD	Impaired FMD in FH. Lower FMD in FH with pos. fam. history of CV events vs FH with neg. fam. history of CV events
Di Salvo G et al. 0.2012 [24]	FH ($n = 45$, 11 ± 3 yrs), ctr ($n = 45$, 11 ± 3 yrs)	LV function, IMT	Various abnormalities in LV function and increased IMT in FH
Fabbri-Arrigoni FI et al., 2012 [43]	FH ($n = 29$, 12, 11–13 yrs), unaffected siblings/ctr ($n = 26$, 14, 12–16 yrs)	CFU, CEC, FMD	Lower CFU and higher CEC in FH. No differences in FMD
Guardamagna O et al., 2009 [26]	FH ($n = 25$, 11.6 ± 3.4 yrs), ctr ($n = 44$, 9.8 ± 4.2 yrs	Carotid IMT, sP-selectin and hsCRP	Increased IMT, sP-selectin and hsCRP in FH
Guardamagna O et al., 2009 [27]	FH ($n = 264$, 9.9 ± 4.5 yrs), unaffected siblings ($n = 100$, 9.5 ± 4.7 yrs)	Carotid and aortic IMT	Increased IMT in FH, and in FH with pos. fam. history of premature CAD and LDL receptor neg. mutations vs FH with neg. fam. history of premature CAD and LDL receptor defective mutations, respectively
Holven K et al., 2006 [80]	FH ($n = 33$, 14, 12–16 yrs), ctr ($n = 20$, 14, 13–17 yrs)	Chemokine mRNA in PBMCs, circulating inflammatory markers	Increased RANTES in PBMCs and serum neopterin in FH. No difference in CRP
Jay RH et al., 1991 [88]	FH ($n = 16$, 10.5, 6–16 yrs), ctr ($n = 16$, 12.0, 5–18 yrs)	Hemorheological parameters and fibrinogen	No differences in any of the hemorheological parameters or fibrinogen
Jehlicka P et al., 2009 [42]	FH ($n = 32$, 14.7 ± 2.9 yrs), ctr ($n = 30$, 15.1 ± 1.7 yrs)	Circulating markers: ADMA, oxLDL, hsCRP, and measurement of endothelial function, FMD and DI	Increased ADMA, oxLDL and hsCRP in FH. No differences in FMD and DI
Koivunen-Niemelä T et al., 1994 [60]	FH ($n = 21$, 3–18 yrs), ctr ($n = 68$, 1–25 yrs)	Achilles tendon xanthomata	Thicker Achilles tendons and higher frequency of hypoechoic infiltration of the normal tendon structure
Lehmann ED et al., 1992 [50]	FH ($n = 20$, 15 ± 5.8 yrs), ctr ($n = 20$, 15.0 ± 5.8)	Aortic compliance	Increased aortic compliance in FH
Mietus-Snyder M and Malloy MJ. 1998 [41]	FH ($n = 18$, 12.4 ± 5.0 yrs), Unaffected siblings/ctr ($n = 12$, 11.5 ± 4.8 yrs)	FMD and GTN-induced dilation of the brachial artery	Impaired FMD and GTN-induced dilation in FH
Narverud I et al., 2011 [81]	FH ($n = 102$, 14, 9–20 yrs), ctr ($n = 48$, 14, 8–19 yrs)	Circulating inflammatory markers	Non-significant increased TNFα, reduced sTNFR2, increased TNFα/sTNFRs ratio, reduced IL-10 and increased TNFα/IL-10 ratio in FH. No difference in CRP
Narverud I et al., 2013 [65]	FH ($n = 42$, 12, 11–14 yrs), ctr ($n = 25$, 14, 12–15 yrs)	Serum oxLDL, Lp(a) and CRP, PBMC mRNA levels of TNFSF family members	Increased oxLDL and mRNA levels of OX40L, BAFFR and TRAILR1, but lower mRNA levels of TRAIL and TRAILR3 in FH. No difference in Lp(a), sTNFR1 or CRP
Narverud I et al., 2013 [70]	FH ($n = 9$, 7, 7–10 yrs), ctr ($n = 16$, 8, 7–14 yrs)	Lp(a), circulating hemostatic markers and CRP	Increased PAI-1and TFPI, but lower thrombomodulin in FH. No difference in Lp(a), fibrinogen, vWF, tPA, TF or CRP
Noto N et al., 2011 [18]	FH IMT <0.55 mm (grp 1, $n = 20$, 11.9 ± 3.9 yrs), FH 0.55 mm ≤ IMT ≤ 0.60 mm (grp 2, $n = 17$, 15.3 ± 2.0 yrs), FH IMT >0.60 mm (grp 3, $n = 18$, 14.3 ± 3.2 yrs), ctr ($n = 15$, 13.0 ± 1.4 yrs)	Carotid IMT, textural characteristics of intima-media complex: GSM, skewness, kurtosis, entropy, ASM, contrast, VS	Higher carotid IMT and entropy and lower ASM in FH (grp 2 and 3). Higher contrast in grp 2 and higher VS in grp 3 vs. ctr. No differences in GSM, skewness and kurtosis

Riggio S et al., 2010 [31]	FH ($n = 18$, 11.8 ± 2.8 yrs), ctr ($n = 18$, 9.9 ± 1.9 yrs)	Carotid IMT, arterial stiffness indices: β-index, AC, AIx, PWV, E_p	Higher β-index, AIx, PWV, E_p, but lower AC in FH. No differences in carotid IMT
Rodenburg J et al., 2006 [25]	FH ($n = 178$, 12.9 ± 3 yrs), unaffected siblings ($n = 78$, 14.1 ± 3 yrs)	Oxidized phospholipid markers, carotid IMT, hsCRP	Increased apoB-IC, IgM MDA-LDL, IMT and hsCRP, but lower OxPL/apoB in FH
Ryu SK et al., 2011 [28]	FH ($n = 178$, 12.9 ± 3 yrs), Unaffected siblings ($n = 78$, 12.8 ± 2.8 yrs)	Carotid IMT, hsCRP, Lp(a), Lp-PLA_2 mass and activity	Increased carotid IMT, hsCRP and Lp-PLA2 mass and activity in FH. No difference in Lp(a)
Schlager O et al., 2013 [51]	FH ($n = 16$, 13.2 ± 2.6 yrs), ctr ($n = 91$, 12.7 ± 2.1 yrs)	Microvascular autoregulation by PORH	Higher peak perfusion, longer recovery time and lower biological zero in FH
Sorensen KE et al., 1994 [36]	FH ($n = 30$, 10.9 ± 0.5 yrs), ctr ($n = 30$, 10.9 ± 0.5 yrs)	FMD and GTN-induced dilation of the superficial femoral artery	Impaired FMD and GTN-induced dilation in FH
Stübiger G et al., 2012 [66]	HoFH ($n = 3$, 8 ± 3 yrs), heFH ($n = 3$, 12 ± 8 yrs), ctr ($n = 7$, 15 ± 7 yrs)	Lipid subsets: Lp(a), oxLDL, PC, LPC, SM, oxPC, SOVPC. CRP	Increased SM in hoFH vs. heFH and ctr children. No differences in Lp(a), oxLDL, PC, oxPC, SOVPC or CRP
Tonstad S et al., 1996 [22]	FH ($n = 91$, 10−19 yrs), ctr ($n = 30$, 10−19 yrs)	IMT, fibrinogen	Increased IMT in FH. No differences in fibrinogen
Ueland T et al., 2006 [79]	FH ($n = 207$, 8−18 yrs), unaffected siblings ($n = 85$, 8−18 yrs)	Circulating inflammatory markers	Increased neopterin and CRP in FH. No difference in CD40L
Vaya A et al., 1996 [69]	FH ($n = 40$, 2−17 yrs), ctr ($n = 35$, 2−17 yrs)	Hemorheological parameters, Lp(a) and fibrinogen	Increased EAM_0, EAM_1, PV and Lp(a) in FH. No differences in fibrinogen, $BV230s^{-1}$, $BV23s^{-1}$, hematocrit or leukocyte count
Vaya A et al., 1998 [87]	FH ($n = 29$, 5−16 yrs), ctr ($n = 25$, 5−16 yrs)	Hemorheological parameters, plasma thrombomodulin and fibrinogen	Increased fibrinogen, EAM_0, EAM_1, and plasma viscosity in FH. No differences in plasma thrombomodulin, hematocrit and leukocyte count
Vaya A et al., 1998 [95]	FH ($n = 24$, 5−16 yrs), ctr ($n = 25$, 5−16 yrs)	Hematocrit, erythrocyte indices:, MCV, MCH, MCHC, EEI	No differences in any of the measured markers
Virkola K et al., 1997 [29]	FH ($n = 23$), ctr ($n = 23$), 2−19 yrs	Carotid IMT, arterial distensibility: change of vessel diameter (ΔD), stiffness	Thicker carotid IMT and aterial stiffness, but lower ΔD in FH
Widhalm K and Genser D. 1994 [68]	FH ($n = 20$, 9.7 ± 2.7 yrs), ctr obese ($n = 20$, 12.5 ± 1.5 yrs)	Lp(a)	Non-significant increased Lp(a) in FH
Wiegman A et al., 2004 [23]	FH ($n = 201$, 8−18 yrs), unaffected siblings ($n = 80$, 8−18 yrs)	IMT	Increased IMT in FH
Yang Y et al., 2010 [56]	HoFH ($n = 20$, 13.1 ± 5.4 yrs), ctr ($n = 15$, 15.2 ± 6.9 yrs)	CFVR measured in left anterior descending artery	Lower CFVR, increased diastolic velocities after vasodilator infusion in FH

Data are presented as mean ± standard deviation/standard error of the mean, or median, interquartile range/minimum−maximum. AC, arterial compliance; ADMA, asymmetric dimethylarginine; AIx, augmentation index; apoB-IC, immunoglobulin G and M apolipoprotein B immune complex; ASM, angular second moment ; BAFFR, b-cell-activating factor receptor; $BV23s^{-1}$/$BV230s^{-1}$, whole blood viscosity at different shear stress; CAD, coronary artery disease; CD40L, CD 40 ligand; CEC, circulating endothelial cells; CFU, colony-forming units; CFVR, coronary flow velocity reserve; ctr, controls; CV, cardiovascular; DI, deceleration index; EAM_0, erythrocyte aggregation at stasis; EAM_1, erythrocyte aggregation at low shear rate of 3 s^{-1}; EEI, erythrocyte elongation index; E_p, elastic modulus; fam, family; FH, familial hypercholesterolemia; FMD, flow-mediated dilation; grp, group; GSM, grey scale median; GTN, glyceryl trinitrate; heFH, heterozygote FH; HoFH, homozygote FH; IgM MDA-LDL, immunoglobulin M malondialdehyde LDL; IL, interleukin; IMT, intima-media thickness; LDL(−), electronegative LDL; Lp(a), lipoprotein (a); LPC, lyso-phosphatidylcholine; Lp-PLA2, lipoprotein-associated phospholipase A_2; LV, left ventricular; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; mm, millimeter; mRNA, messenger ribonucleic acid; neg, negative; NH%, nitrate hyperemia; OX40L, OX40 ligand; oxLDL, oxidized LDL; oxPC, oxidized phosphatidylcholine; OxPL, oxidized phospholipid; PAI-1, plasminogen activator inhibitor-1; PBMCs, peripheral blood mononuclear cells; PC, phosphatidylcholine; PORH, post occlusive reactive hyperemia; pos, positive; PV, plasma viscosity; PWV, pulse wave velocity; RANTES, regulated on activation normally T-cell expressed and secreted; ref, reference; RH%, reactive hyperemia; SM, sphingomyelin; SOVPC, s*n*-1-stearoyl-2-oxovaleroyl-phosphatidylcholine; sP-selectin, soluble P-selectin; sTNFR, soluble tumor necrosis factor receptor; TF, tissue factor; TFPI, tissue factor pathway inhibitor; TNFSF, tumor necrosis factor super family; TNFα, tumor necrosis factor alpha; tPA, tissue plasminogen activator; TRAILR, tumor necrosis factor-related apoptosis-inducing ligand receptor; vs, versus; VS, visual scoring; vWF, von Willebrand Factor; yrs, years; Δ*D*, change of vessel diameter.

plaque was found in 10% of the FH children, whereas plaques were not observed in any controls [22]. Regarding atherogenic risk factors and markers, IMT was related to gender [22,23,27], total cholesterol [29], LDL-cholesterol [18,22,23,27,29], triglycerides [29], age [23,27], apoB, fibrinogen and homocysteine [22], and inversely related to the HDL/total cholesterol ratio [29] either in a group of FH children or in an overall group of children. Furthermore, Guardamagna et al. reported that aortic and carotid IMT were thicker in children with a family history of premature CAD and in children with LDL receptor-negative mutations (only carotid IMT), compared with children without a family history of premature CAD and children with receptor-defective and unclassified LDL receptor mutations, respectively [27]. Three studies have reported no differences in IMT between children with and without FH [30–32]. In eight of the studies IMT measurements in children with FH were compared with a healthy independent control group [18,22,24,26,29–32], whereas four articles presented comparisons with healthy siblings [23,25,27,28]. In order to summarize these studies statistically, we conducted a meta-analysis including the studies comparing FH children with an independent control group (Fig. 1). According to the meta-analysis, IMT was significantly thicker in the FH groups than in the control groups (WMD = 0.06, 95% CI [0.01, 0.11], $p = 0.02$). There was significant heterogeneity between the studies ($I^2 = 96.4\%$, $p < 0.001$). Whereas seven studies presented data on IMT measurements from the common carotid artery (for Tonstad et al. [22] and De Groot et al. [32] the value for the common carotid artery was used), one study did not describe in specific detail. The four articles that used healthy siblings as a control group [23,25,27,28] represent three studies (IMT data were similar in two of the articles [25,28]). The mean differences in these studies were 0.05 [27], 0.02 [23] and 0.02 [25], i.e. consistent with the results in Fig. 1. Recently, changes in the textural characteristics of the intima-media complex (i.e. entropy, angular second moment, contrast and visual scoring) have been demonstrated by Noto and coworkers in children with FH as assessed by B-mode ultrasound [18]. Entropy represents the disorder or complexity of the texture of the intima-media complex, and is larger for surfaces that do not have a uniform texture. Angular second moment of the intima-media complex represents the uniformity of texture in the intima-media complex, and is a measure of local homogeneity and the opposite of entropy. Contrast represents the change in density, and is a measure of the amount of local variation in images [18]. They have shown that children with FH have higher entropy, but lower angular second moment than control children. Additionally, children with FH (dependent on IMT) had higher contrast and higher visual scoring compared with controls. No differences were observed in grey-scale median, skewness and kurtosis between children with and without FH. In the overall group of children, IMT correlated with entropy, angular second moment and visual scoring.

3.1.2. *Measurements of endothelial function*

Endothelial dysfunction is crucial in the initiation of atherosclerosis [33], and FMD, a measurement closely related to coronary endothelial function, has been reported as a predictor in the future risk of cardiovascular disease (CVD) [34,35]. Among the 9 studies that investigated the FMD in children with FH, 7 studies have found significantly impaired FMD either in the superficial femoral [36,37] or brachial artery [30,38–41] in children with FH. Among children with FH, FMD has been found to be inversely related to lipoprotein a (Lp[a]) [36]. In FH children with a positive family history of premature CVD, FMD was found to be more impaired than those without family history of premature CVD [40]. Two studies have reported no differences in FMD [42,43] or deceleration index (DI) [42], another measurement for endothelial function, between children with FH and healthy controls. Nitrate-mediated dilation

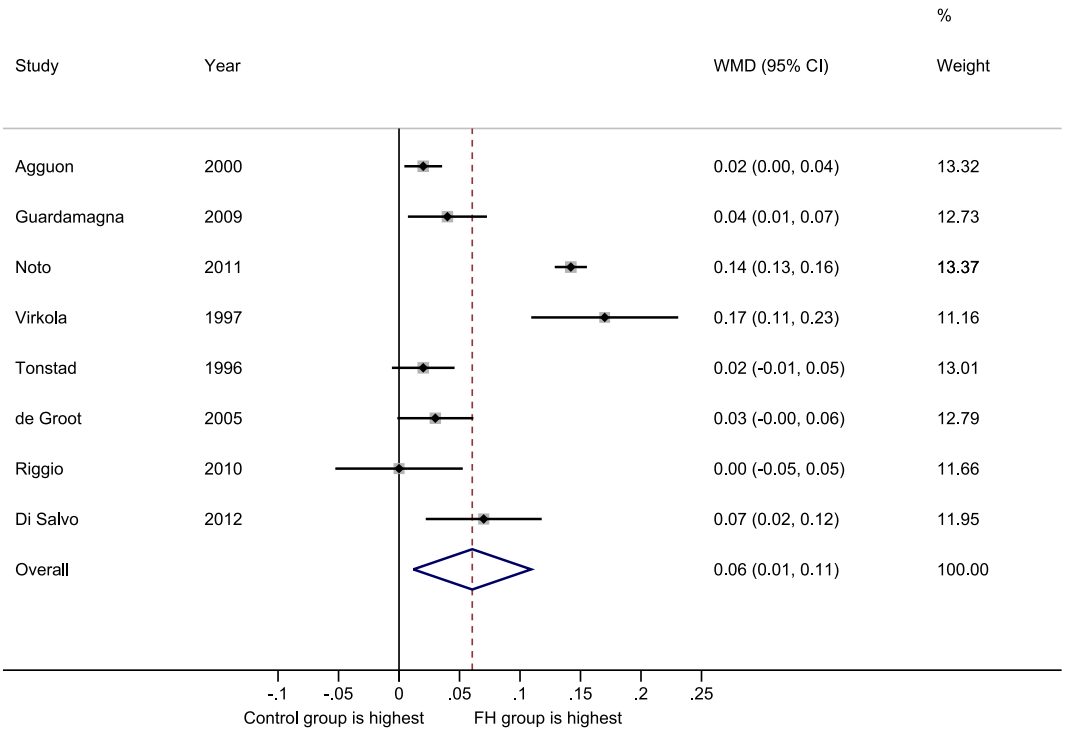

Fig. 1. The result of the meta-analysis including eight identified studies comparing IMT measurements in FH children with a healthy control group using random effects model. The dotted line represents the weighted mean difference (WMD) estimated in the meta-analysis. CI, confidence interval; FH, familial hypercholesterolemia; IMT, intima-media thickness; WMD, weighted mean difference.

(NMD)/glyceryltrinitrate (GTN)-induced dilation may operate as a control test for FMD measurement to ensure that a decreased FMD capacity observed is truly a consequence of endothelial dysfunction and not a reflection of underlying smooth muscle dysfunction [44]. Three studies also demonstrated an impaired NMD or GTN-induced dilation in children with FH compared with healthy controls [36,39,41]. However, children with FH have also been shown to have similar GTN-induced dilation compared with healthy controls [30,37].

Endothelial dysfunction may trigger arterial stiffening resulting in increased pulse pressure in which both are associated with the extent of atherosclerotic disease [45,46]. Arterial stiffness has been considered to be an independent predictor of all-cause and cardiovascular mortality in high-risk patients [47,48] and can be estimated by several indices such as aortic pulse wave velocity (PWV) and augmentation index (AIx) [45,49]. In children with FH all the main indices for arterial stiffness, i.e. PWV, AIx, β-index, and Young elastic modulus (E_p) were significantly higher, however arterial compliance (AC) was significantly lower, compared with healthy controls [31]. In relation to atherosclerotic risk factors, all the above-mentioned indices were positively associated with total and LDL-cholesterol, except for AC which was inversely correlated. Furthermore, Virkola and co-workers found lower change in vessel diameter (ΔD) and higher stiffness in children with FH compared with their healthy counterparts [29]. Conversely, a previous study found higher aortic compliance in FH individuals compared with their healthy counterparts [50]. The authors suggest that this finding may be due to a compensatory enlargement of the aorta as a response to atherosclerosis. Aggoun and coworkers found significant changes in several arterial mechanical features, including lower systodiastolic change in carotid lumen diameter and lumen diameter/lumen diastolic diameter ratio, lower cross-sectional compliance and cross-sectional distensibility, but higher incremental elastic modulus in male children with FH compared with control male children [30]. Taken together, these studies comprising IMT, FMD and arterial mechanical changes may depict early atherosclerosis in the artery tree of children with FH, but does the hypercholesterolemia also affect the microvasculature?

To our knowledge, only one study has revealed an effect of hypercholesterolemia on the microvasculature. Very recently, Schlager and colleagues measured the microvasculature autoregulation in children with FH by PORH using laser Doppler fluxmetry [51]. PORH may reflect microvascular endothelial function and can be used as a sensitive indicator for both macro- and microvascular dysfunction [52–55]. The results by Schlager et al. demonstrated higher peak perfusion and higher recovery time, but lower biological zero, defined as "null flow", in children with FH undergoing PORH as compared with control children [51]. A rationale for these findings is an impairment of the microvasculature's autoregulation, perhaps through altered precapillary vasoconstriction in children with FH.

3.1.3. Miscellaneous measurements of subclinical atherosclerosis

In a group of children with homozygous FH, coronary flow velocity reserve (CFVR) was compared with a group of control children [56]. Mean and peak diastolic velocities in the left anterior descending artery were similar at baseline in the two groups. However, after vasodilator infusion with adenosine, the increase in mean and peak diastolic velocities and CFVR were lower in homozygous FH children compared with control children. Furthermore, early left ventricular abnormalities assessed by echocardiography have been demonstrated in children with FH compared with control children [24]. Taken together, these data suggest a significant different cardiac morphology and function in children with FH.

Xanthomas in the Achilles and other tendons are cardinal signs of FH [15,16,57], and a meta-analysis has demonstrated a 3-fold induction in CVD risk among FH patients with xanthomas compared with FH patients without xanthomas [58]. Moreover, we have previously shown higher inflammatory response in peripheral blood mononuclear cells (PBMCs) from FH patients with xanthomas compared with their counterparts without xanthomas [59], linking the presence of xanthomas to inflammation. Children with FH have been shown to have significantly thicker Achilles tendon xanthomas compared with healthy subjects, whereof 38% of the FH children had hypoechoic infiltration of the normal tendon structure and none of the controls showed any sonographical detectable tendon abnormalities [60].

Taken together, several studies have shown that children with FH have early subclinical signs of atherosclerosis in their vascular wall. In particular, our meta-analysis showed that the difference in IMT was significant between children with FH and healthy controls. These changes are most likely due to lifelong elevated LDL-cholesterol and clearly support an accelerated atherogenesis already at an early age.

3.2. Atherogenic lipids

3.2.1. Modified LDL

Modified and oxLDL particles play a central role in the atherosclerotic process [61,62], and have been shown to exert immunogenic and inflammatory effects [63,64]. Rodenburg and coworkers found higher circulating levels of Immunoglobulin (Ig) G and IgM autoantibodies against apoB and also higher IgM auto-antibodies against modified LDL [25]. However, there was a lower oxidized phospholipids/apoB ratio in children with FH than in unaffected siblings [25]. These findings may be attributed to immunologic responses to the presence of modified LDL in the vessel wall and circulation in children with FH. Indeed, significantly higher circulating levels of oxLDL have recently been demonstrated in children with FH compared with control children [42,65]. However, a small pilot study observed no differences in oxLDL between any of the three groups of homozygous and heterozygous FH children and controls [66]. In accordance with this study, Barros and colleagues found no differences in serum levels of LDL(−) between three groups of FH children and control children [67]. Nevertheless, all the three groups of FH children had lower levels of anti-LDL(−) IgG auto-antibodies compared with their healthy counterparts.

3.2.2. Lp(a) and other lipid-related markers

Two studies have shown that children with FH have higher levels of Lp(a) compared with controls [68,69]. In the studies by Widhalm and colleagues, the frequency distribution of Lp(a) was also different with more FH children in the highest range of Lp(a) compared with controls [68]. In contrast, neither we, Stübiger et al., nor Ryu et al. did not find any difference in Lp(a) between children with and without FH [28,65,66,70]. Importantly, even though different measurement techniques were used among the studies, the comparisons of the Lp(a) concentrations between children with and without FH were based on measurements with the same method. Therefore, even though the Lp(a) concentrations *per se* may not easily be compared between the different studies, the observation of differences within the single studies should be valid. Only two out of six studies, however, showed a significant difference in FH children compared with healthy controls. However, there is no apparent link between FH and Lp(a). Thus, one explanation for higher Lp(a) in some FH populations may be that high levels of Lp(a) may lead to more serious consequences subsequently leading to diagnosing of FH. Despite negative findings regarding the more traditional atherogenic lipids, oxLDL and Lp(a),

Stübiger et al. found that homozygous FH children had higher plasma levels of sphingomyelin compared with heterozygous FH and controls, with no differences between the groups in neither phosphatidylcholine (PC), lyso-PC, oxPC, or *sn*-1-stearoyl-2-oxovaleroyl-PC [66]. Sphingomyelin is a major constituent of cell membranes and lipoprotein particles and is substrate of acid sphingomyelinase [71]. Acid sphingomyelinases are important in lipid raft clustering and redox signaling, processes playing a crucial role in development of endothelial dysfunction [71,72].

The enzyme lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is involved in generation of lipid mediators important in pro-inflammatory and pro-atherogenic processes, and both Lp-PLA_2 mass and activity are associated with increased cardiovascular risk [73–75]. In plasma of normocholesterolemic humans, Lp-PLA_2 is mainly associated with LDL and to a smaller extent HDL [74], however, the enzyme has also been found to be associated with Lp(a) [76]. In comparison with unaffected siblings, children with FH have recently been shown to have higher Lp-PLA_2 mass and activity [28]. This may reflect that Lp-PLA_2 in FH children is carried to a great extent by circulating LDL potentially small dense LDL [28,77] as a natural consequence of their phenotype. Furthermore, Lp-PLA_2 activity was associated with IMT in unadjusted analyses in the total group of children [28].

These findings may be attributed to immunologic responses to the presence of modified LDL or other atherogenic lipids in the vessel wall and circulation in children with FH.

3.3. Inflammatory markers with relevance to atherogenesis

3.3.1. Circulating markers

Atherogenesis is the result of the interacting lipid and inflammatory pathogenic arms, and there is some evidence of enhanced systemic inflammation in children with FH. So far, C-reactive protein (CRP) is the most widely used inflammatory marker in clinical studies [78]. Among the 11 studies investigating CRP in children with FH, five studies found significantly higher circulating CRP levels in FH children compared with a matched control group [25,26,28,42,79]. There were no differences in circulating CRP levels between children with and without FH in the remaining six studies [39,65,66,70,80,81], implying that circulating levels of CRP may be a less sensitive marker of atherosclerotic development in children with FH. Neopterin is an inflammatory mediator derived from activated monocytes/macrophages in response to IFNγ activation thereby also reflecting T helper cell (Th)1 activation [82]. Ueland et al. and Holven et al. have both independently shown significantly higher circulating levels of neopterin among children with FH compared with control subjects, indicating Th1-induced monocyte/macrophage activation may be an early phenomenon in the atherosclerotic process in these individuals [79,80]. The prototypical inflammatory cytokine TNFα is central throughout the atherosclerotic process [5]. Compared with controls, children with FH have a non-significant higher level of TNFα accompanied by lower levels of TNF receptor 2 resulting in a higher ratio between circulating levels of TNFα and its two soluble receptors, (TNF receptor type 1 and 2) which can attenuate TNF activity [83], potentially reflecting higher TNFα activity in children with FH [81]. Moreover, circulating levels of the anti-inflammatory cytokine IL-10 were lower in children with FH, leading to a higher TNFα/IL-10 ratio indicating an imbalance between pro- and anti-inflammatory mediators in these individuals. CD40 ligand is, another central member of the TNF super family (TNFSF), shown to be involved in atherogenesis at least partly reflecting platelet-mediated inflammation [84,85]. However, no differences were found in levels of serum CD40L in children with FH compared with controls [79]. Charakida and coworkers did not observe any difference in TNFα between children with and without FH, but they found higher circulating levels of the inflammatory cytokines IL-1β and IL-6, and also increased levels of intercellular adhesion molecule (ICAM)-1 as a marker of leukocyte and leukocyte-endothelial activation in FH subjects [39]. P-selectin is another adhesion molecule important in recruitment of immune cells to the atherosclerotic lesion [61], and higher circulating levels of P-selectin have been found in children with FH [26]. In the same individuals P-selectin was significantly, positively associated with carotid IMT, supporting a link between inflammation and subclinical measurements of atherosclerosis. As P-selectin is also released from activated platelets, these finding may also suggest that platelet activation could be part of the inflammatory arm that are activated in the atherogenesis in children with FH.

3.3.2. mRNA levels of inflammatory markers in PBMCs

PBMCs comprise monocytes and lymphocytes which are directly involved in atherosclerosis [11]. These cells have been suggested to reflect systemic inflammation, potentially also, at least partly, reflecting plaque inflammation in atherosclerosis [11,12]. Compared with control children, children with FH have higher mRNA levels of the chemokine RANTES (**R**egulated on **A**ctivation **N**ormally **T**-cell **E**xpressed and **S**ecreted) derived from the monocyte compartment in PBMCs [80]. In contrast, there was no difference in circulating levels of RANTES in the two groups of children. Children with FH also display a different mRNA profile of several TNFSF and TNF receptor super family members in PBMCs compared with control children [65].

Altogether these studies indicate the presence of inflammation and inflammatory-related processes in children with FH. Although these markers by no means can be used as markers of atherosclerosis, the raised levels of these markers suggest that these children are characterized by a degree of systemic inflammation linking the high LDL levels in these children to inflammation. Taken together, these studies suggest that inflammation is part of the atherosclerotic process also in the early stages in children with FH.

3.4. Hemostatic markers

Hemostatic markers are expressed and released by immune and endothelial cells, and participate during the development of atherosclerosis [1,86]. In total six studies have investigated hemostatic markers in children with FH [22,39,69,70,87,88]. Some of the most common hemostatic markers measured are fibrinogen and von Willebrand Factor (vWF), but only one study has reported higher levels of fibrinogen in children with FH in comparison with controls [87]. The remaining studies have reported no differences in neither circulating levels of fibrinogen [22,39,69,70,88] nor vWF [39,70] between children with and without FH. In contrast, circulating levels of the anti-fibrinolytic mediator, plasminogen activator inhibitor (PAI)-1 [39,70], but not tissue plasminogen activator (tPA) [70], have been found to be higher in children with FH compared with control children. Moreover, PAI-1 levels were inversely, significantly associated with the endothelial function in both groups [39], suggesting a link between hemostatic markers and subclinical measurement of atherosclerosis. Charakida and coworkers also observed a raised PAI-1/tPA ratio in these individuals [39]. Both tissue factor (TF) and its endogenous inhibitor TF pathway inhibitor (TFPI) are the main regulators of the initiation of the coagulation process [89], and high levels of TFPI have been related to atherosclerosis [90,91]. Very recently we showed in a small study that circulating levels of TFPI, but not TF, were higher and the antithrombotic marker thrombomodulin was lower in children with FH compared with healthy controls [70]. Our findings may together with previous results [90] thus suggest that increased

TFPI could reflect an adaptive response to increased "hemodynamic" stress. In contrast, Vaya and coworkers did not find any difference in thrombomodulin between children with and without FH [87], however if this finding is due to cholesterol-lowering medication in 17 of the 29 children with FH is not known.

Still, few studies have investigated hemostatic markers in children with FH, and these studies show discrepant results for most hemostatic markers. This may potentially be due to a later impact of hemostasis during atherosclerosis partly explaining these discrepancies in childhood and adolescence of FH individuals.

3.5. Other markers

Hemorheological changes are associated with coronary heart disease [92–94]. However, in children with FH neither hematocrit [69,87,88,95], red cell volume [88], cell hemoglobin [88], whole blood viscosity [69,88], erythrocyte deformability [88,95] nor leukocyte count [69,87] were different in comparison with healthy controls. Conflicting results have been shown in respect of other hemorheological measurements. Jay and coworkers found no differences in erythrocyte aggregability and plasma viscosity between children with and without FH [88]. However, increased erythrocyte aggregation at stasis and at low shear stress of 3 s^{-1} (EAM_1), and plasma viscosity were shown in children with FH compared with controls by Vaya and colleagues [69,87]. Furthermore, in relation to hypercholesterolemia, both plasma viscosity [69,87] and EAM_1 [87] were significantly, positively correlated to total cholesterol in children with FH.

Isoprostanes are lipid peroxidation products of arachidonic acid and quantification of these products is an approach to assess oxidative stress *in vivo* [96,97]. However, despite their higher levels of oxLDL and potentially increased risk of oxidative stress, children with familial hypercholesterolemia did not have different levels of urinary isoprostaglandin $F_{2\alpha}$ type III compared with healthy controls [98].

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of endothelial nitric oxide synthase [99,100]. Previously, increased levels of ADMA have been associated with impaired FMD [100], and Jehlicka et al. demonstrated increased circulating levels of ADMA in children with FH [42].

Circulating endothelial cells (CECs) may be regarded as a measurement of endothelial damage [101], while colony-forming units (CFUs) may potentially attenuate atherosclerosis by repairing sites of endothelial injury [102]. Children with FH have been demonstrated to have significantly higher CECs, but lower CFUs compared with healthy children [43].

In conclusion, most hemorheological measurements were not different, however some show conflicting results between children with and without FH, which complicates drawing a conclusion on these measurements. Only three studies have observed isoprostanes, ADMA, or CEC and CFU in children with FH thus more evidence are needed to conclude regarding these and hemorheological markers.

3.6. Clinical perspective

Still, 20 years after the entry of the statins, patients with FH have increased CVD mortality compared with the general population. We have data showing that patients with FH have increased CVD mortality particularly at younger age [Mundal et al. *Unpublished data*] compared with the general population. This implies that the exact phenotypic pattern resulting in their increased CVD risk is still not totally clear and may thus not only be attributed to their high LDL-cholesterol *per se*, but potentially also to other contributors in the atherosclerotic process. Statin treatment has been shown to effectively reduce plasma cholesterol. In a few studies statins even reduced IMT. However, very recently we have shown that despite long-term statin treatment in an adult FH population, they were still characterized by an inflammatory phenotype [103]. This questions whether statin treatment may completely reverse the effects of being exposed to elevated plasma cholesterol in 10–12 years. Therefore more information about early markers of atherosclerotic development in children with FH may help understand the role of high LDL-cholesterol *per se* as well as potentially identify new targets for therapy and more reliable markers of disease progression.

4. Conclusion

In the present review we have summarized the literature on markers of atherosclerotic development in children with FH based on thirty-seven original articles. High LDL-cholesterol is the factor that distinguishes children with and without FH and herein we show that these groups of children also differ in several other ways. In particular, children with FH display a variety of changes reflecting both the lipid and inflammatory arm of atherosclerosis despite young age. The most frequently reported markers of atherosclerotic development in children with FH are undoubtedly early markers of atherosclerosis (i.e., IMT and FMD) and markers of inflammation (i.e., CRP) which were measured in 26 out of the included 37 studies in this review. Several of these can be characterized as high confidence studies including a large number of children with and without FH, thus having a large power. Our meta-analysis on the articles presenting data on IMT revealed significant thicker IMT in children with FH compared with controls thus strengthening the evidence of early atherosclerotic development in children with FH. The conflicting results observed on FMD and CRP may be due to the size of the study populations and different measuring techniques in particularly with regard to FMD. Further, CRP and FMD are considered indirect markers of atherosclerosis as compared with IMT. Other potential and possibly more specific candidates of markers of inflammation than CRP have been pointed out in the present review. In a clinical perspective, early diagnosing and treatment of children with FH is of high importance to attenuate development of the potential ongoing early atherosclerotic process in these individuals. Such treatment may also include anti-inflammatory therapy in the future. To provide more evidence of the mechanisms on how LDL-cholesterol promote atherosclerotic development in FH children, more studies on individual markers of atherosclerotic development must be available to enable future meta-analyses comprising these markers. Forthcoming studies should also include effects of cholesterol-lowering treatment on markers of atherosclerotic development.

Conflict of interest

None declared.

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.atherosclerosis.2014.05.917.

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