

**Risk Factors for
Cardiovascular
Disease and Death
in Familial
Hypercholesterolemia**

Master Thesis by

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Summary

Background and aims: Familial hypercholesterolemia (FH) is a metabolic autosomal dominant disorder. It is characterised by elevated plasma total cholesterol and low density lipoprotein (LDL) cholesterol levels usually due to a genetic mutation in the LDL receptor gene and have therefore a higher risk of non-fatal or fatal coronary heart disease (CHD). Yet, there is a substantial variation between individuals with FH, even among FH subjects with the same genetic mutations, in susceptibility to CHD in terms of the age of onset and severity. The aim of this thesis was to increase the knowledge about which factors that may affect the onset of CHD in FH subjects. This is an important knowledge in order to determine how intense the treatment the various patients should be offered and how early treatment should be initiated.

Subjects and methods: Two different studies were included in this thesis. The first study, a retrospective data collection study, characterised and compared clinical and biochemical parameters from the medical records of 71 FH subjects with early CHD event and 76 FH subjects with late or no CHD event. In addition, 14 deceased FH subjects with early CHD event and 14 deceased FH subjects with late or no CHD event were compared with each other and with the non-deceased FH groups. In the second study, a case-control study administered by the master student, clinical and biochemical parameters in a smaller group of FH subjects with more strict inclusion criteria than the first study were compared, with special regard to different coagulation factors, CRP and fibrinogen. The case-control study included 19 FH subjects with early CHD event, 15 FH subjects with late or no CHD event and 10 control subjects. All groups except from the control group were subdivided into gender.

Results: FH subjects with early onset of CHD seems to have a more severe risk factor profile compared with FH subjects with late or no CHD event, even though they are more intensively medically treated. Female FH subjects seem to have a more severe risk factor profile in comparison with male FH subjects, suggesting that they are not

well enough medically treated. However, in general, today's lipid-lowering treatment seems to be more effective than the lipid-lowering treatment ten years ago, reducing the risk of fatal CHD in FH subjects. In our study, coagulation markers do not appear to play a determining role in susceptibility to CHD in FH subjects.

Conclusion: Even though the treatment in FH subjects seems to have improved substantially, there is still a potential for improvement concerning reaching the treatment goals for blood lipid levels. Our results may also indicate that female FH subjects may need to be followed up more closely, and to be more extensively treated with lipid-lowering medication and combination medication. The contribution of lipoprotein (a) in particular but also supplementation with omega-3 fatty acids with regard to CHD risk and death in FH subjects should be examined to a greater extent.

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Abbreviations

APC	Activated Protein C
ApoA1	Apolipoprotein A1
ApoB	Apolipoprotein B
ASAT	Aspartate amino transferase
BMI	Body mass index
CAD	Coronary artery disease
CHD	Coronary heart disease
CRP	C-reactive protein
CVD	Cardiovascular disease
FA	Fatty acid
FH	Familial hypercholesterolemia
HMG CoA	3-hydroxy-3-methyl-glutaryl Co-enzyme A
HDL	High density lipoprotein
HDL-C	High density lipoprotein cholesterol
IDL	Intermediate density lipoprotein
IDL-C	Intermediate density lipoprotein cholesterol
LDL	Low density lipoprotein
LDL-C	Low density lipoprotein cholesterol
LDL-R	Low density lipoprotein receptor

Lp(a)	Lipoprotein (a)
MI	Myocardial infarction
NCEP ATP III	The North American National Cholesterol Education Program guidelines
NO	Nitric oxide
OUS	Oslo University Hospital (Oslo Universitetssykehus)
TG	Triglycerides
VLDL	Very low density lipoprotein
vWF	von Willebrand factor
WHO	World Health Organisation

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1. Introduction

1.1 Coronary heart disease

Coronary heart disease (CHD) is one of the leading causes of deaths worldwide (1, 2). In Norway, CHD accounts for approximately 32 % of all deaths in men and 35 % of all deaths in women (3). CHD, also called coronary artery disease (CAD), is a subgroup of cardiovascular disease (CVD). CVD includes all diseases of the heart and circulatory system while CHD primarily affects the coronary arteries and the arteries through the progress of atherosclerosis.

Scientific evidence suggests that the vast majority of coronary events can be explained on the basis of different risk factors for CHD (2). Risk factors are often classified into two groups; modifiable or non-modifiable. Examples of modifiable risk factors are high blood pressure, obesity, diabetes, unhealthy diet, smoking, low physical activity and elevated plasma cholesterol levels. Examples of non-modifiable risk factors are age, gender and genetic susceptibility (4).

The prevalence of CHD increases with increasing age and in the general population clinical manifestations of CHD seldom appear before the fourth decade of life (5). Nevertheless, evidence indicates that atherosclerosis, the major cause of CHD, begins early in life and progresses silently for decades (5, 6).

1.2 Cholesterol metabolism

All cell membranes contain cholesterol (7). Cholesterol is also a precursor of bile acids and steroid hormones. Its distribution is mediated through the blood system. The cholesterol molecule itself is insoluble in blood. Lipoproteins make cholesterol molecules soluble by binding them to form a lipid droplet. Then the insoluble parts point inwards to the core of the lipid droplet and the soluble parts point towards the

blood. There are different types of lipoproteins, some transport mainly fatty acids (FA) in the form of triglycerides (TG), others transport mainly endogenously synthesised cholesterol or absorbed dietary fat (7).

1.2.1 Cholesterol homeostasis

Cholesterol homeostasis in the body is assured through the actions of two major groups of lipoproteins; those containing apolipoprotein B (ApoB) such as chylomicrons, very low density lipoprotein (VLDL) and VLDL remnants, intermediate density lipoprotein (IDL) and low density lipoprotein (LDL), and those containing apolipoprotein A1, principally high density lipoprotein (HDL) (8).

1.2.2 Lipoprotein metabolism

As shown in **figure 1**, dietary cholesterol is absorbed in the gut and transported to the liver in TG-rich chylomicrons (9). Cholesterol and TGs synthesized in the liver are secreted into the circulation in VLDL particles. Chylomicrons and VLDL ensure the distribution of energy in the form of TGs and FAs to peripheral tissues (10). In the circulation, the enzyme lipoprotein lipase releases TGs from VLDL. The VLDL remnants are either removed from the circulation by the liver or converted via IDL into LDL particles by the enzyme hepatic lipase. LDL distributes cholesterol to peripheral cells mediated with the help of LDL receptors (LDL-Rs). HDL transport excess cholesterol back to the liver for degradation and/or and excretion in bile acid. This process is facilitated either directly by a HDL specific receptor in the liver or indirectly through conversion of HDL by the enzyme cholesteryl ester transfer protein to VLDL and LDL followed by uptake in the liver facilitated by LDL-Rs on the liver cells. LDL-C account for 60-70 % of the total serum cholesterol (11).

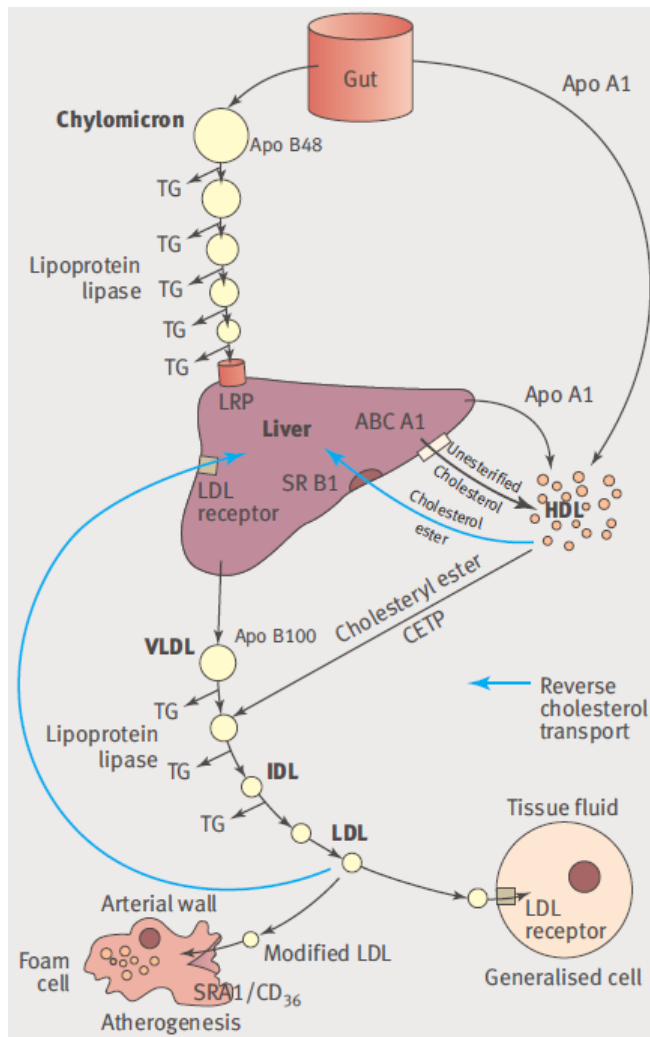


Figure 1. Lipoprotein metabolism. From Bhatnagar et al (9).

1.3 Atherosclerosis

Atherosclerosis can be considered to be a form of multi-step chronic inflammation in the artery walls (12-14). The different risk factors of CHD contribute to cause endothelial injury in the vessels (15). In healthy vessels, the endothelium maintains vascular tone and blood pressure (16). In time, the stress from CHD risk factors leads to endothelial dysfunction and inhibits endothelial production of nitric oxide (NO) (13). NO is a potent vasodilator and anti-inflammatory molecule. The endothelium binds monocytes and T lymphocytes, which migrate into the subendothelial space. There they initiate and maintain the inflammatory process, and the monocytes transform to macrophages (12). LDL particles also bind to endothelial cells and

migrate to the subendothelial space. In the vascular wall the LDL particles are subject to oxidative modifications, and recruit more monocytes from the blood (8, 16). The macrophages take up oxidized LDL in the subendothelium and are transformed into foam cells. This process results in the development of so-called fatty streaks (13).

Macrophages, T lymphocytes and proinflammatory molecules further promote the deposits of cholesterol, cellular waste products of among others smooth muscle cells, calcium and other substances in the arteries (8, 13). The result is fibrous lipid-filled plaques. The plaques may rupture and, if an occluding thrombus is formed, different outcomes such as a heart attack or a stroke may be seen (12, 17).

1.3.1 Cholesterol as a CHD risk factor

While LDL cholesterol (LDL-C) has an essential physiological role in distributing cholesterol to peripheral tissues, increased LDL-C levels are associated with increased CHD risk (11, 12). Increased total cholesterol and LDL-C is considered a CHD risk factor of crucial importance (4). In the dyslipidemic state, subendothelial uptake and oxidation of LDL increases; oxidized lipids stimulate production of adhesion molecules and inflammatory cytokines and may have antigenic properties, promoting a T lymphocyte-mediated immune response and inflammation in the arterial wall (15).

The atheroprotective role of HDL

HDL protects against atherosclerosis. Some of the atheroprotective function is mediated via reverse cholesterol transport (15). However, HDL particles also transport antioxidant enzymes, which can break down oxidized lipids and neutralize their proinflammatory effects (18). Hence, a low HDL cholesterol (HDL-C) level is strongly and inversely associated with CHD risk (11).

1.4 Familial hypercholesterolemia

1.4.1 History and prevalence

Heterozygous familial hypercholesterolemia (FH) is a metabolic autosomal dominant disorder (19).

The condition is caused by defects in one of at least three different genes that code for proteins that affects the normal control of lipoprotein metabolism, and are involved in hepatic clearance of LDL (20). These include, most commonly, different mutations in the LDL-R gene (7, 19, 20). The LDL-R mediates feedback control of cholesterol synthesis (7). Much less common are mutations in the gene coding for ApoB, the major protein of the LDL particle. Rarely, FH also results from mutations in the gene coding for proprotein convertase subtilisin/kexin type 9 (PCSK9), an enzyme involved in LDL-R turnover (20).

Expression of LDL-Rs is subject to feedback control by intracellular cholesterol levels (12). Low levels of intracellular cholesterol activate the sterol regulatory element binding protein-2 (SREBP-2) transcription factors which again stimulate transcription of the LDL-R gene and other genes involved in cholesterol synthesis (12). LDL-R has a dual role in LDL metabolism (7). As shown in **figure 2**, when the LDL-Rs are genetically defective they impede the liver cells from LDL uptake and thereby degradation. At the same time the liver cells produce more LDL through VLDL and IDL. Both processes contribute to increased LDL-C plasma levels.

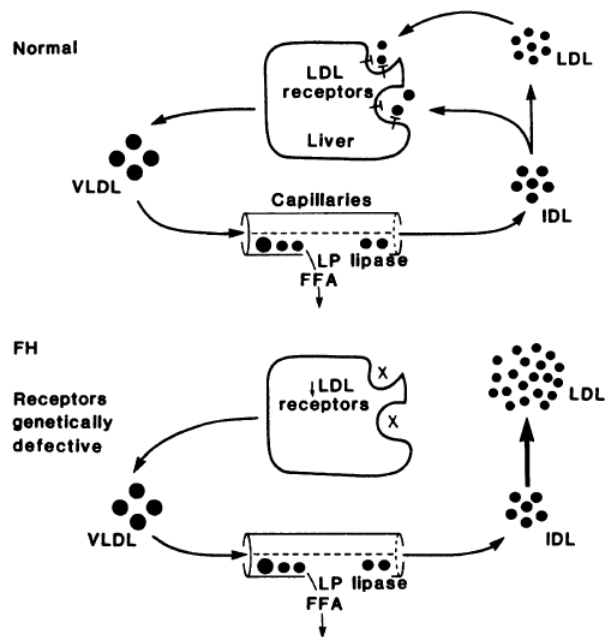


Figure 2. LDL-R controls both production and catabolism of LDL in plasma. From Brown and Goldstein (7).

Hypercholesterolemia in FH subjects is present from birth (21). The mutations result in accumulation of both total cholesterol and LDL-C in the blood plasma and in peripheral tissues and arterial walls which again leads to increased risk of premature CHD (20). Premature CHD is by many defined as CHD event occurring <55 years in men and <65 years in women, respectively (11, 22-24). With a frequency of one in 500, heterozygous FH on a world basis is one of the most common inborn errors of metabolism (19, 25). The prevalence of homozygous FH is one in one million persons. Heterozygous FH affects about one in 300 individuals in the Norwegian population (26), estimated from a study in the county of Østfold, Norway (27).

Worldwide, there have by 25 May 2010 been identified 1,739 allelic variants and 1,120 unique allelic LDL-R mutations (28).

According to Leren et al (26), by May 2007 a total of 3,900 Norwegians had been genetically diagnosed with FH. As of May 2010, unpublished data provided by Leren suggest that the number now has exceeded 4,750 persons. Approximately 130 LDL

receptor mutations were by May 2007 (May 2010: unpublished data suggest 140) identified in Norway (26).

1.4.2 The genetic basis of FH

The LDL-R locus is located on the distal part of the short arm on chromosome 19, on band p13.1 to p13.3 (7, 29). On basis of the LDL-R gene mutations' disturbance in function, the mutations are classified into different groups. There are five classes of mutations that disrupt the structure and function of the LDL-R and cause FH (21), as shown in **figure 3**. Each class of mutations affect different gene sites in the LDL-R metabolism. In class one, so-called receptor-negative or null-allele mutations, no LDL-Rs are synthesized. The receptors are synthesized in class two, but there is a disruption on the transport from the endoplasmic reticulum to the Golgi apparatus. In class three mutations LDL-Rs fail to bind LDL molecules properly even though they reach the liver cell surface. Class four mutations are characterised by the failing of clustering of LDL-Rs into coated pits after binding the LDL molecules. Finally, class five mutations prevent the LDL-Rs from being recycled to the cell surfaces and/or the LDL particles from being released in the endosomes for degradation. In consequence, FH subjects experience few, if any, functional LDL-Rs (30).

In a large cohort study of FH subjects and their unaffected relatives, Umans-Eckenhausen et al (31) found significant variation in LDL-C levels depending on the mutation type. FH persons with receptor-negative mutations appear to have higher LDL-C levels and also elevated risk of CHD (16, 31).

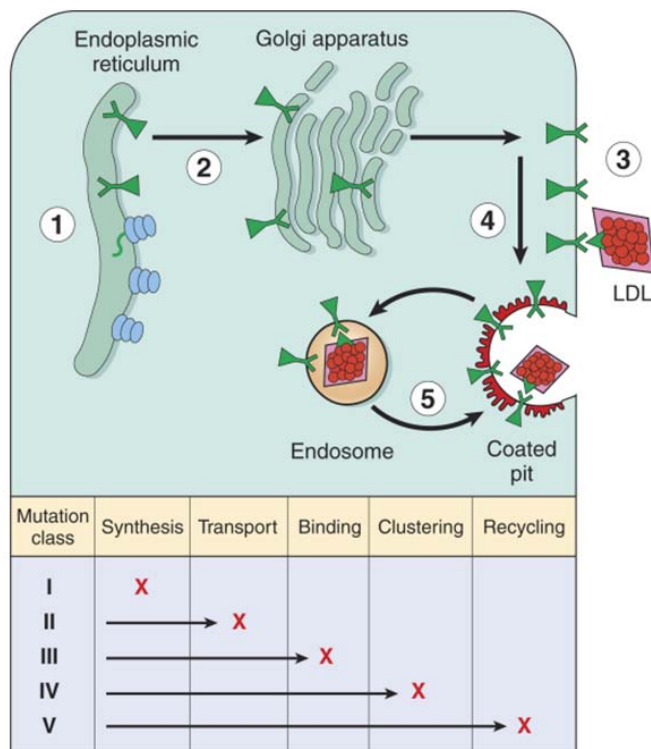


Figure 3. Five classes of LDL-R gene mutations. From Kumar et al (32).

Different LDL-R gene mutations prevail in different populations. A relatively large number of mutations seem to cause FH in most populations. Yet, in a few populations there are founder mutations (33). In Norway, for instance, three mutations account for approximately 40 % of the mutations among FH subjects (34). The most frequent mutations are FH-Elverum, FH-Svartor and C210G, respectively.

Many clinically diagnosed FH subjects do not have a monogenic cause of their hypercholesterolemia and the existence of a LDL-R mutation is not always detected. The detection rate of LDL-R gene mutations vary mainly depending on the clinicians' criteria for using the test and also on the methods used in various laboratories (25). According to this, a higher mutation detection rate has been reported in FH patients with tendon xanthomas than in those without tendon xanthomas. This illustrates that very strong clinical criteria for performing the test naturally will result in a higher detection rate (35). Patients with other types of lipid disorders could have clinical and biochemical characteristics much alike FH, for instance in familial defective

ApoB100 (36), familial combined hyperlipidemia (37) or polygenic hypercholesterolemia (26).

1.4.3 Characteristic clinical features and diagnosis of FH

Characteristics of FH in addition to elevated total cholesterol and LDL-C from birth onwards, is the appearance of tendon xanthomas and premature CHD in the third or fourth decade of life if untreated (21). As shown in **figure 4**, tendon xanthomas typically appear in extensor tendons, such as in the Achilles tendons (most common location) and tendons on the dorsum of the hands. Furthermore, several FH subjects experience xanthelasms on the upper and/or lower eye lid and/or corneal arcus (19, 25). The occurrence of carotid and/or aortic stenosis is frequently seen in persons with FH (21, 38). In comparison, in homozygote FH subjects development of CHD is rapidly progressive and generalized atherosclerosis may develop even before the age of four. This often results in myocardial infarction (MI) and possible death before the age of 30 (19, 30).



Figure 4. Characteristic clinical features of FH. A. Xanthelasm above the eyelid; B. Xanthomata on the dorsum of the hand; C. Corneal arcus; D. Xanthomata on the Achilles tendon; E. Xanthomata on Tuberositas Tibiae. All pictures are used with permission from the owners Leiv Ose (A-D) and Kjetil Retterstøl (E), respectively.

Biochemically, in addition to raised total cholesterol and LDL-C levels, other lipid and non-lipid parameters are often measured in FH subjects. HDL-C levels are generally low in patients with FH (39). Lipoprotein (a) (Lp(a)) levels have been shown to be elevated in FH subjects (40-42). FH subjects often have normal serum TG levels (30).

There are several sets of diagnostic criteria and/or screening recommendations commonly used in order to clinically verify the presence of FH, among others the UK Simon Broome Register Group (25), the USA Make Early Diagnosis to Prevent Early Death (MEDPED) Program (43) and the Dutch Lipid Clinic Network (44). The major difference between the three mentioned here is the use of different cut-offs for premature CHD. The Simon Broome Register group recommends the use of CHD, <60 years in first-degree relatives and, <50 years in second-degree relatives. The MEDPED criteria recommend a cut-off at age 65, while the Dutch Lipid Clinic Network suggest <55 years for men and <65 years for women. At the Lipid Clinic in Norway, the Dutch criteria shown in **table 1** are used.

Table 1. The Dutch Lipid Clinic Network criteria for FH. Adapted from Bhatnagar (21) and World Health Organization (44).

Criteria	Points*
Family history	
First-degree relative with known premature** coronary and vascular disease, or first-degree relative with known LDL-C >95th percentile	1
First-degree relative with tendon xanthomas and/or corneal arcus, or children aged less than 16 years with LDL-C >95th percentile	2
Clinical history	
Patient with premature coronary artery disease	2
Patient with premature cerebral or peripheral vascular disease	1
Physical examination	
Tendon xanthomas	6
Corneal arcus prior to age 45 years	4
Laboratory analysis (mmol/L)**	
LDL-C >8.5	8
LDL-C 6.5 - 8.4	5
LDL-C 5.0 - 6.4	3
LDL-C 4.0 - 4.9	1
DNA analysis	
Functional mutation in the LDL-R gene present	8

* Diagnosis is based on the total numbers of points obtained: a 'definite' FH diagnosis requires more than 8 points, a 'probable' diagnosis requires 6-8 points, a 'possible' diagnosis requires 3-5 points

** Men: <55 years; women: <60 years

*** HDL-C and TGs are normal

There are different ways of screening for FH. One could genetically screen the entire population in a country. Taking into consideration the prevalence of FH (relatively low even though it is reckoned one of the most common inborn errors of metabolism), the different expressions of physical and biochemical characteristics and also the wide variety of mutations, population screening is not cost-effective. One could also screen patients in clinical setting with clinical signs on hypercholesterolemia or persons with premature CHD. However, the most cost-effective way to detect cases across the whole population is so-called cascade screening; to investigate first-degree relatives of already clinically or genetically diagnosed FH subjects (21, 45). Subjects detected by cascade screening have one or more relatives that already have a LDL-R mutation and have a 50 % a priori risk of FH.

In cascade screening FH diagnosis based on genetic verification of a LDL receptor gene mutation has a sensitivity and specificity of 1.0. In clinical or biochemical verification of the disorder, the sensitivity and specificity reaches 0.80-0.85 (26, 43, 46, 47).

Recently, Civeira et al (48), in a cross-sectional study of 825 FH subjects in Spain, suggested that tendon xanthomas and age-adjusted LDL-C cut-off values have the highest value for clinical diagnosis and indication of genetic testing of FH. Recent studies investigating the relevance of genetic testing in FH have found that 52.3 % (49) and 57.7 % (50) of those with clinically defined FH had an LDL-R mutation. As LDL-R gene mutations are not found in all FH families it is important to use combination of LDL-C level and family history to diagnose FH subjects, especially children.

Rees (51) argues in an editorial referring to Neil et al and their cohort study of 3,382 FH subjects in Britain (52) that FH is underdiagnosed and undertreated. Indeed, global estimates suggest 200,000 heterozygous FH subjects die each year of preventable MIs (30).

1.4.4 Treatment

Medical treatment

In contrast to most genetic disorders, efficient therapy is available for FH patients. Before the invention and availability of statins, treatment of FH consisted of resins and dietary advice (25). Some also advocated the use of niacin and fibrates. The therapy only had small effects on plasma cholesterol levels, and the risk of CHD remained high (25).

Most people with FH are diagnosed too late, after their first coronary event. The high prevalence of FH and the associated CHD morbidity and mortality support aggressive screening and treatment (53).

Graham et al argue in the newest version of European guidelines on cardiovascular disease prevention in clinical practice (54, 55) that reduction of plasma cholesterol levels is crucial in reducing CHD risk. **Table 2** shows the optimal cholesterol treatment goals when considering FH an a priori high risk for CHD. In a recent guideline for primary prevention of CHD the Norwegian Directorate of Health recommends LDL-C levels to be lower than 3.0 mmol/L in all individuals independent of CHD risk (23). The Norwegian Directorate of Health follows the European guidelines on cardiovascular disease prevention in clinical practices' recommendations (23, 54, 55).

Table 2. Optimal cholesterol treatment goals in high risk individuals. Adapted from Graham et al in the European guidelines on cardiovascular disease prevention in clinical practice (54, 55).

	Treatment goals	
	Optimal	Optional*
Blood lipid component		
Total cholesterol	<4.5 mmol/L	<4.0 mmol/L
LDL-C	<2.5 mmol/L	<2.0 mmol/L
HDL-C		
Male	>1.0 mmol/L	
Female	>1.2 mmol/L	
TGs	<1.7 mmol/L	

* If feasible

A number of scientists suggest that treatment of FH subjects should start already in childhood (56-58), especially in those with a family history of premature CHD (25). Endothelial cell dysfunction, which is an early reversible stage in the development of atherosclerosis, has been observed in children with FH (56). With cholesterol-lowering statin therapy it is possible to reverse the endothelial cell dysfunction (25, 56). Also, one could reverse the inter-media thickening in the carotid arteries seen in children with FH (59).

According to Neil et al (52), on behalf of the Simon Broome Familial Hyperlipidaemia Register Group, mortality is not significantly higher in FH subjects

who are identified before they develop CHD symptoms than in the general population.

It is not ethical to conduct placebo-controlled randomised trials on FH patients (25, 60). Nevertheless, the safety and efficacy of statin use is well documented in both primary and secondary prevention of patients with hypercholesterolemia of various causes (11, 61-63). Based on evidence from other high risk groups, such as post-MI patients, one could assume similar effects of reduction of total and myocardial mortality of for instance statins and other lipid-lowering drugs to persons with FH (60). Treatment with statins is effective in FH patients (64) and it delays or prevent the onset of CHD (25, 61).

There are five major groups of LDL-C lowering drugs available (65). So-called statins are 3-hydroxy-3-methyl-glutaryl Co-enzyme A (HMG CoA) reductase inhibitors; they inhibit production of cholesterol in the liver by inhibiting the enzyme HMG CoA reductase. Bile acid sequestrants, or commonly called resins, bind bile acid and prevent the body from reabsorbing bile acid containing cholesterol from the gastrointestinal tract. As a result the liver cells produce more LDL-Rs, which again lower the LDL-C levels in serum (23). The cholesterol-lowering function of statins and resins are shown in **figure 5**. The third lipid-lowering drug, ezetimibe, inhibits intestinal uptake of cholesterol from dietary and biliary sources (66). The fourth group of drugs is nicotinic acid (also called niacin), and the fifth group is fibric acids derivatives (also called fibrates) (67). Ezetimibe, resins, niacin and/or fibrates are often used in combination with statins, if the treatment goal is not reached with statins alone. They are less potent than statins; hence statins are a first choice. Combined with lowering of LDL-C, all the groups of LDL-C lowering drugs also contribute on lowering TGs and to increase HDL-C levels (11, 23).

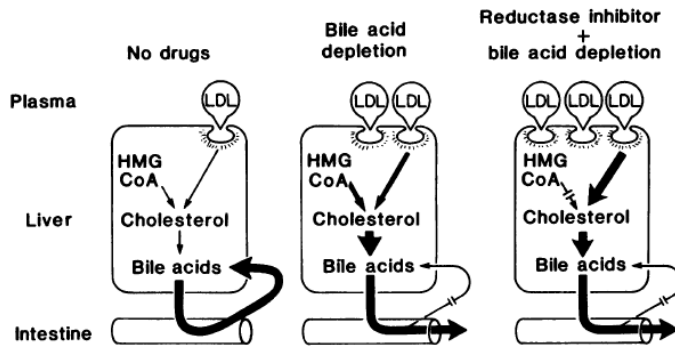


Figure 5. Treatment with statins and resins. From Brown and Goldstein (7).

Some patients are intolerant to statins. An alternative is red yeast rice, which is a product of yeast grown on rice. It naturally contains a small dose of a statin-like substance called monacolin in combination with unsaturated FAs and phytosterols (68, 69), all capable of lowering LDL-C levels to some extent (68, 70). Red yeast rice also alters the TG levels and the total cholesterol levels in a positive direction. However, the HDL-C level is to some extent reduced, and the cholesterol-lowering effect is considered to be less potent than statins (68). Furthermore, monotherapy with ezetimibe is shown to be a good alternative when statins are not suitable (71).

In most FH subjects LDL-C levels need to be reduced about 40-50 % to reach treatment goal (54, 55, 65, 72). A common drug treatment approach is to begin with a moderate dose of statins. If treatment goal is not reached the statin dose should be increased. Resins or ezetimibe can be added if maximal dose of statins is not tolerated. If treatment goal still is not reached the statins should be given in full dose and addition of niacin should be considered in addition (65). At present, however, the combined use of statins, ezetimibe and low dose resins are well tolerated and have been shown to reduce LDL-C levels by almost 70 % in a FH population (73). Tendon xanthomas can regress significantly and even disappear with cholesterol-lowering therapy (21, 74). The lipid-lowering effect of different drugs is shown in **table 3**.

Table 3. Comparison of efficacy of various lipid-lowering drugs in patients with FH (11, 30, 65, 68, 71).

Drug	Influence on blood lipids		
	Decrease in LDL-C	Increase in HDL-C	Decrease in TGs
Statins	18 - 55 %	5 - 15 %	7 - 30 %
Resins	10 - 30 %	3 - 5 %	No change
Ezetimibe*	5 - 25 %	1 - 6 %**	0 - 14 %
Red yeast rice	~ 21 %	No change***	~ 6 %
Niacin/nicotinic acid	5 - 30 %	15 - 35 %	20 - 50 %
Fibric acids	5 - 30 %	10 - 20 %	20 - 50 %

* Ezetimibe effect in combination with statins and not in monotherapy

** A few studies showed a small decrease in HDL-C

*** There was a non-significant (-0,5 %) decrease in HDL-C

Dietary recommendations

Importantly, a genetic cause does not imply that diet and life style habits are without influence (23, 44). Although dietary treatment always is implemented in management of FH, very few randomized controlled trials have been performed on patients with FH and diet (75). In 2010, Shafiq et al (75) in a Cochrane review on 11 randomised trials suggested that no conclusion can be made about the effectiveness of cholesterol-lowering diet in reducing CHD and lowering cholesterol due to lack of adequate data. Nor can their findings support any other dietary intervention considered for FH, like the addition of omega-3 FAs or soya protein (75). However, they found a significant reduction of plasma cholesterol with plant sterols and/or stanols (75).

On the other hand, World Health Organization (WHO) recently proposed that there is convincing evidence that replacing saturated fat with unsaturated fat will contribute to reduce CHD in the general population as well as in high risk subjects (76). Others have suggested that a change in diet to a cholesterol-lowering alternative could reduce plasma LDL-C levels by 15 to 20 % in FH subjects (77).

Nevertheless, diet alone is not a sufficiently effective treatment in FH (30). However, diet is recommended to be the first-line treatment of FH in addition to lifestyle changes like smoking cessation and increased physical activity (5, 30).

In dietary intervention, the main objective is to reduce the LDL-C levels by restriction of the amount and type of fat from the diet (75, 76, 78). In the general population, also including the FH population, the total intake of fat should not exceed 30-35 % of the total energy intake (25, 76, 79). Also, the dietary sources of saturated fat should be limited to a minimum – and substituted by foods containing unsaturated fat (54, 55). As **figure 6** shows, saturated fat reduces hepatic LDL-R expression and increases VLDL synthesis (7, 65). Omega-3 FAs from marine sources are also recommended due to their TG lowering and HDL-increasing effect (80), both through increased dietary intake of seafood and through intake of concentrated omega-3 in capsules. However, the North American National Cholesterol Education Program (NCEP ATP III) guidelines suggest that the use of omega-3 FA supplements is optional and does not recommend a specific amount of omega-3 (11, 81). Other dietary recommendations include a reduction of the dietary cholesterol, an increase of antioxidants through fruits and vegetables and an increase of fibre intake through fruits, vegetables, legumes and whole-grain products. A high amount of soluble fibre in the diet binds bile acid in the colon and retains it from being reabsorbed by the body (82).

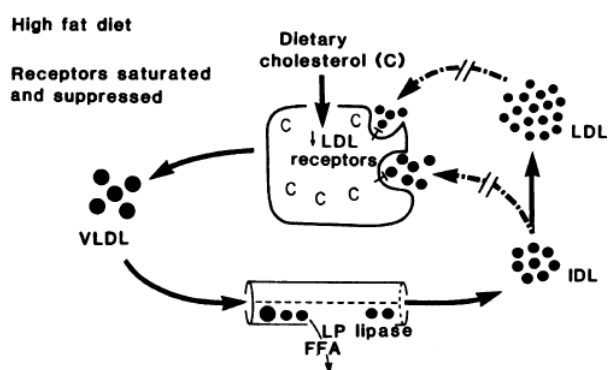


Figure 6. Contribution of high fat diet on LDL-C levels. From Brown and Goldstein (7).

In subjects with FH, as in all people, a high fat diet contributes to elevated LDL-C levels. Since FH patients also have the genetic disposition of elevated LDL-C levels, avoidance of a diet rich in fat, and saturated fat especially, generally is recommended (19). However, effects of dietary changes vary on basis of the initial level of plasma cholesterol and the patients' initial dietary habits.

1.5 Familial hypercholesterolemia and coronary heart disease

According to Oosterveer et al (83) most CVD incidences in FH are CHD. Extensive epidemiologic studies in numerous populations in many countries strongly associates increased plasma total cholesterol and LDL-C levels with CHD (11, 65).

Patients with FH have a higher risk of fatal or non-fatal CHD than persons without FH (25). FH is also associated with premature death (16). The disorder is considered a world public health problem due to the high incidence of premature CHD and reduction of life expectancy (65). Yet, studies of mortality and morbidity of untreated FH subjects are based on register-data and probably over-estimate the CHD risk (84). In men, the mean age of onset of CHD characteristically is between 40 and 45 years, and in female FH subjects approximately ten years later (22). The cumulative risk is more than 50 % by age 50 years in men and at least 30 % in women aged 60 years (25). By the age of 65, approximately 85 % men and 50 % women with FH will experience a coronary event if they are not treated (65).

Yet, there is a substantial variation between individuals with FH, even among FH subjects with the same genetic mutations, in susceptibility to CHD in terms of the age of onset and severity (22, 40, 65, 85). The fact that all phenotype variations cannot be explained only on basis of the LDL-R mutation suggests that other factors may influence the progression and development of atherosclerosis. A large, retrospective cohort study by Jansen et al (86) recently showed that the traditional risk factors may explain less than 20 % of the variation in the prevalence of CVD in FH subjects.

Thus, increased risk of CHD in FH subjects is not solely due to elevated LDL-C levels, but also other risk factors (87). However, the fact that hypercholesterolemia persists from birth in FH is different from other forms of hypercholesterolemia. It should be recognized that polygenetic and life style induced hypercholesterolemia diagnosed in adults may have not persisted that long. Thus, the cholesterol “burden” during years is not as heavy in polygenic hypercholesterolemia as in FH and hence of less importance for the atherosclerotic process than in FH patients. This underlines the need of more specific prognostic tools focused on a FH population exclusively.

In the last ten years, different studies, mainly case-control studies, have analysed the main risk factors associated with CVD and CHD in heterozygous FH in different populations (22, 40, 64, 86-91). Both traditional risk factors for CHD such as smoking, gender, hypertension and diabetes and/or clinical or molecular features specific to FH are analysed (65, 86). Many of the studies show that traditional risk factors play an important role in heterozygous FH, both as risk factors in themselves and on top of the risk factors specific to the condition (16). For instance, risk of premature CHD is much higher in FH subjects than in the general population, but it is also two to five times more frequent in FH men than in FH women (87). In **table 4**, eight major risk factors for CHD in heterozygous FH subjects are presented.

Considering the presence or absence of the listed risk factors Civeira et al (65) argues that one can calculate an absolute risk for a person with FH – and use that as a guiding tool for optimal treatment. According to Jansen et al (86) LDL-C is not reckoned a risk factor in their and many other studies of risk factors within a FH population group. Their argument supporting this theory is that the FH group as a whole are hypercholesterolemic and hence the small differences in the elevated range do not confer any greater risk (86).

Table 4. Major risk factors for CHD in heterozygous FH. Adapted from Civeira et al (65).

CHD risk factors
Age
Men: ≥ 30 years
Women: ≥ 45 or postmenopausal
Cigarette smoking: active smokers
Family history of premature CHD
CHD in male first-degree relative < 55 years
CHD in female first-degree relative < 65 years
Very high LDL-C: > 8.5 mmol/l
HDL-C: < 1.0 mmol/l
High blood pressure ($> 140/90$ mmHg)
Diabetes mellitus
Lp(a): > 600 mg/l

1.5.1 Different manifestation of coronary heart disease in FH subjects

There is inconsistency in the physical and biochemical features in FH patients and not all the characteristic clinical features of FH are shown in all patients (92). Also, the nature and onset of CHD in heterozygote FH persons is variable (93, 94). The literature suggests that environmental, genetic and phenotypic variations could influence on the differences between persons with FH (20, 21, 84). One could especially see this in studies on FH reported from different countries or geographical areas (84, 95), even when controlling for differences in the underlying mutations (16). Different classes of LDL-R defects could be associated with a ‘severe’ or ‘mild’ physical and biochemical characteristics of FH (31). According to de Sauvage Nolting et al (94), even FH subjects with identical mutations and LDL-C levels can have significant differences in clinical outcome. Increased LDL-C levels and premature CHD, the two most typical characteristics of FH, are also significantly influenced by environmental factors (21, 96). For instance, the specificity of family history of CHD as an indicator of a possible genetic risk depends on the prevalence of CHD in the

population being studied (25). Possible causes of different manifestations in FH are shown in **table 5**.

Table 5. Some causes of variability in physical and biochemical characteristics of FH. Adapted from Bhatnagar (21).

Cause	Example
Genetic	Specific mutations leading to FH phenotype Genetic factors that influence lipoprotein metabolism Genetic factors that influence CHD Gender
Environmental	Diet Smoking Prevalence of CHD in community Drugs affecting lipoprotein metabolism used without identifying FH
Metabolic	Hormonal Diet and body weight Lipoproteins and enzymes and apolipoproteins modulating their metabolism, amongst others Lp(a) levels, LDL particle size, HDL-C levels, ApoB/ApoA1 ratio Factors involved in inflammation, clotting and thrombosis, amongst others plasma fibrinogen* and C-reactive protein*

* Are shown to increase risk of CVD in the general/non-FH population, but their role in FH and CHD risk is uncertain

However, solid data addressing the contribution of environmental and genetic factors to the variable physical and biochemical characteristics of FH are scarce.

Lipoprotein(a) as a CHD risk factor in FH

Lp(a) is a LDL-like particle which contains apolipoprotein (a) in addition to ApoB (97, 98). Several epidemiologic studies have suggested that elevated levels of Lp(a) are a strong risk factor for CHD (97, 99), also in FH persons (40, 42, 100). In a recent large-scale prospective data study Bennet et al argue that Lp(a) is not only a CHD risk factor, but an independent risk factor of CHD (97). This finding is supported by others (42, 101, 102). A Lp(a) concentration of >300 mg/L is considered elevated (103-106).

Low HDL concentration in FH

Low HDL-C is a risk factor for CHD in FH (39). Indeed, low HDL-C levels are considered a CHD risk factor at all LDL-C concentrations (107, 108). For plaque regression in atherosclerosis, a minimal elevation of HDL-C in addition to reduction of LDL-C, is by Nicholls et al estimated to be 7.5 % (109). Some statins contribute to such a level of HDL-C increase, but most patients need combination treatment with fibrates and/or niacin to enhance the HDL-C increase (110).

ApoA1, ApoB and ApoB/ApoA1 ratio in FH

Clinical trials have reported that ApoB and ApoA1 are important predictors of CHD risk (2, 111, 112). In the INTERHEART study, with 15,000 cases and controls, ApoA1 and ApoB have been used as surrogate for anti-atherogenic HDL-C and atherogenic LDL-C and VLDL-C, respectively (2). In the Swedish Apolipoprotein Mortality RISK (AMORIS) study measurement of ApoB and ApoA1 improved the prediction of fatal MI at all levels of total cholesterol, LDL-C and TGs (111).

Due to the fact that ApoB and ApoA1 have opposite effects on atherogenic risk, the ratio between the two values appears to be a useful indication of cardiovascular risk (2, 8, 112-114). In an AMORIS follow-up from 2008 (115), ApoB/ApoA1 was found equally predictive with LDL-C on CHD.

Factors involved in inflammation, clotting and thrombosis

C-reactive protein

It is recognized that CHD could be considered an inflammatory process in the body (17, 116). Inflammation is often assessed by measurements of the inflammatory marker C-reactive protein (CRP) (71, 117, 118). Elevated CRP is associated with increased CHD risk (8, 117, 119-121). CRP is an acute phase reactant protein produced primarily in the liver and is released in response to inflammatory processes such as infection or acute injury. NCEP ATI III considers CRP to be an emerging marker for the diagnosis and management of CHD (11, 71). However, CRP was by Drakopoulou et al in a recent review considered more of a nonspecific systemic

marker of infection and tissue damage than a CHD marker (118). Others' findings support those findings (122, 123). Both Ridker et al (124) and Nissen et al (125) suggest that the reduced CHD risk observed after statin treatment correlates with reductions in CRP levels. In the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial, one outcome was that CRP independently predicts vascular events regardless of LDL-C level (126-128). Also, one can see an additive effect on CRP level reduction with a combination of statin and ezetimibe (71). Although there is conflicting information regarding the role of CRP in premature CHD, most of the available evidence is in favour of their use in assessing the prognosis of atherosclerosis and suggest the inclusion of CRP in the standard assessment of cardiovascular risk (8).

A specific range of CRP levels is commonly used to predict CVD. The 2002 joint American Heart Association and Centers for Disease Control Consensus Report (129) suggested that CRP levels <1.0 mg/L indicate a low risk of developing CVD. If CRP levels are between 1.0 and 3.0 mg/L, there is an average risk, whereas CRP levels >3.0 mg/L indicate high risk.

Fibrinogen

Fibrinogen has a pivotal role in haemostasis; it promotes fibrin formation and is a major contributor to platelet aggregation and coagulation (8, 130). Increased fibrinogen levels are associated with CHD (8, 119, 131-134). Fibrinogen is useful in the prediction of atherosclerosis (132, 135), and in patients with manifested atherosclerosis (136). In a recent meta-analysis including 154,211 apparently healthy subjects, the Fibrinogen Studies Collaboration (FSC) registered correlation between fibrinogen and several established CHD risk factors (130). However, it is unclear whether plasma fibrinogen is an independent risk factor for CHD (137), also in FH (21).

Protein C, Activated Protein C resistance and Protein S

The Protein C system is important in anticoagulation by its regulation of the activities of the coagulation factors Factor Va and Factor VIIIa (138). Protein S is a cofactor to

activated Protein C (APC) (139, 140). In addition to its anticoagulant properties, APC has anti-inflammatory functions (138). There are two forms of Protein S, one free and one bound form. The free form acts as a cofactor in anti-coagulation in the inactivation of coagulation factors Factor Va and VIIIa. Protein S also expresses anti-coagulant activity in the absence of APC (139, 141, 142). In inflammatory processes the bound form of Protein S increases and the free form decreases. Consequently, Protein S deficiency – either inherited or as a result of inflammation – could increase the risk of thrombosis and hence CHD (139). A low concentration of Protein C in the form of APC resistance and/or a low concentration of Protein S are clinically interesting; with values below the reference range the risk of thrombosis increases (138).

von Willebrand factor

von Willebrand factor (vWF) plays a central role in haemostasis (143). vWF's primary function is binding to other proteins in the coagulation cascade, in particular Factor VIII (144). It is important in platelet adhesion to wound sites. Deficiency of vWF is associated with increased bleeding tendency. Conversely, increased vWF level and activity could be considered a predictor of cardiovascular disease (144, 145).

2. Aims of the study

2.1 Study rationale

This thesis aims to identify important risk factors for CHD and death in a FH population. It aims to identify which clinical and biochemical factors that could be responsible for the “protection” of a subgroup of heterozygous FH subjects and, on the contrary, to identify the most important risk factors existing in a FH population exclusively. A study by Sijbrands et al (84) which was conducted in a large pedigree without selection for CHD showed that approximately 60 % of untreated persons with FH suffered from a premature death whereas 40 % of the FH subjects had a normal life expectancy. The search for factors that affect the onset of CHD in FH subjects is potentially important for how intense treatment the various patients should be offered and how early treatment should be initiated.

2.2 Study objective

2.2.1 Specific aims of this thesis

Specific aims of this thesis were

- a. to retrospectively characterise and compare clinical and biochemical parameters from the medical records of FH subjects with early CHD event (‘susceptible’) and FH subjects with late or no CHD event (‘resistant’)
- b. to characterise and compare clinical and biochemical parameters from the medical records of deceased FH subjects with premature CHD event or with late or no CHD event with susceptible and resistant FH subjects
- c. to further characterise and compare the differences between a smaller group with more strict inclusion criteria of susceptible and resistant FH subjects and healthy

controls with focus on different coagulation factors, apolipoproteins, CRP and fibrinogen

- d. to characterise and compare female FH subjects and male FH subjects from the group of FH subjects as a whole and divided into subgroups of susceptible and resistant

2.2.2 Hypotheses

Specific hypotheses of this thesis were that

- a. susceptible FH subjects have a more severe CHD risk factor profile in comparison with resistant FH subjects
- b. deceased FH subjects have a more severe CHD risk factor profile compared with non-deceased FH subjects
- c. FH subjects have a more CHD prone coagulation factor profile and higher CRP levels and fibrinogen levels than control subjects, and similarly that susceptible have a more CHD prone coagulation factor profile than resistant
- d. male FH subjects have a more severe CHD risk factor profile in comparison with female FH subjects

3. Subjects and methods

The master thesis was approved by The Regional Committee of Medical Ethics, The Norwegian Directorate of Health and by The Privacy Ombudsman at Rikshospitalet, Oslo University Hospital (OUS). See **appendices 1, 2 and 3**, respectively.

The thesis included two studies; one retrospective data collection study and one case-control study. No intervention or treatment of the participants was involved. There were no immediate anticipated clinical benefits for the study volunteers, other than feedback on the anthropometric and biochemical measurements.

Written informed consent was obtained from all of the participants.

3.1 Subjects

3.1.1 Retrospective data collection study

This part of the thesis was considered a cross-sectional study.

Susceptible and resistant FH subjects

Inclusion and exclusion criteria

Data on FH subjects were available from another ongoing study at the Lipid Clinic. The study was started in 2007, and they are still recruiting participants. The persons with FH classified into the ‘susceptible’ group had had a premature event of CHD and the FH subjects classified into the ‘resistant’ group had had a late event of CHD or no CHD event. It was required that all participants had genetically determined FH. The whole list of inclusion and exclusion criteria is shown in **appendix 4** for susceptible individuals and in **appendix 5** for resistant FH subjects.

The cut-off of the susceptible group and the resistant group were estimated from the total number of individuals available in Norway, based on the FH patient registry at

the Lipid Clinic. By using this approach, one could be sure to find a big enough group of FH subjects to include in the study. First, the PHREG procedure, a tool from SAS/STAT that performs regression analysis of survival data based on the Cox proportional hazards model, was performed. The survival analysis is a so-called ‘time to event analysis’ which is used to observe the time to a certain event of patients, in this case an event of CHD. Estimates were given for the 95 %, 90 %, 85 % and 80 % survival points for four different combinations of gender and smoking status. Then one could find estimates of the age at which for instance 90 % of female non-smokers survive to without suffering from a CHD event. Second, the ‘non-survivors’ were considered all individuals of that age as well as anybody younger who had had a CHD event. Third, the actual choice of survival age and non-survival age depended on the availability of appropriate individuals in the Lipid Clinic FH registry. Different cut-off points were given for FH men and FH women in accordance with the protective property of oestrogen seen in women (11).

Data on a total of 71 subjects with early cardiovascular event and 76 subjects with late or no cardiovascular event had been included in the study and coded when we received the data file. The cut-off of early CHD in FH subjects and the cut-off of late or no CHD for men and women, respectively, are shown in **tables 6 and 7**. For both susceptible and resistant categories, smokers and previous smokers were considered those FH subjects who were a smoker or previous smoker at the time of the study.

Table 6. Retrospective. Cut-off of *early* CHD event in FH patients

	Gender	
	Female	Male
Smoking status		
Smoking and previous smoking	≤47	≤43
Non-smoking	≤50	≤46

Table 7. Retrospective. Cut-off of *late or no* CHD event in FH patients

	Gender	
	Female	Male
Smoking status		
Smoking and previous smoking	≥50	≥46
Non-smoking	≥53	≥48

Anthropometry and biochemical parameters

Except from levels of Lp(a), physical and biochemical parameters from every patient were collected at study-visit. Lp(a) measurement was not a part of the ongoing study at the Lipid Clinic, and was not measured. Thus, the Lp(a) levels used in this analysis was collected from the journals of the participants. Scientific evidence suggest that the Lp(a) level within-person is highly consistent over time (97). Hence, one can find a value measured at a different time and it would still be sufficient to use in the analysis.

Blood samples were undertaken in the same manner in the susceptible and resistant groups. The blood samples were collected by laboratory personnel at the laboratory at the Lipid Clinic. The accredited medical-biochemical laboratory at Rikshospitalet, OUS, analysed the blood samples.

The blood pressure values presented are not comparable due to incomplete information in the data file. We have no information on whether the patients registered with hypertension were medicated for hypertension or not. Hence, the blood pressure could be affected and possibly confounded by medication differences between groups. Hypothetically, all patients in the susceptible group could be medicated for hypertension while nobody in the resistant group was on hypertension drugs. Yet, we chose to present the data to show the values of blood pressure in relation to other parameters.

Missing data

Even though all physical and biochemical parameters theoretically were collected at study-visit, some of the data were missing. One explanation for the missing data could for instance have been that several different doctors carried out the consultations. Despite the fact that the doctors had the same form to fill out at the study-visit consultations, they were not consequent in filling out different parts in the same manner. Other explanations, regarding blood samples, can be that the doctors forgot to requisition all blood samples for all patients, that the laboratory personnel at the Lipid Clinic did not collect all blood samples or that the laboratory personnel analysing the blood samples either forgot to analyse all the samples or did not report all the answers.

*Deceased FH subjects***Inclusion and exclusion criteria**

The medical records from deceased FH subjects were found from storage at the Lipid Clinic. Out of a total 71 journals from deceased patients, 41 were excluded on basis of not fulfilling the FH clinical and/or genetic diagnosis criterion. Many of the deceased FH subjects were not tested for genetic mutation, mainly because they died before their type of mutation had been discovered and added to the mutation registry. In those cases the master student first systematically went through the pedigree of the patients' family to see if any of the first-degree relatives had a verified mutation. If that was not the case, the deceased FH subjects were included or excluded on basis of the Dutch Lipid Clinic Network criteria for FH (44). Out of the remaining 30 deceased FH subjects, 21 were genetically verified and nine were clinically verified on basis of the clinical and genetic diagnosis criterion.

Data assessment

To get the most correct data from the deceased FH subjects, we decided to use the last registered medical record on each subject. The master student assessed, coded and filed information on the deceased subjects' date of birth, their age at the onset of first CHD event, age at time of death, total number of years they had had of lipid-lowering

treatment, type of medication and total number of years on statin treatment. Different clinical information like presence of xanthomas, xanthelasms and corneal arcus, blood pressure, presence of diabetes type II and smoking status was registered too. The pre-treatment plasma total cholesterol level was also collected, together with the last registered plasma levels of lipids.

Non-HDL, ApoB/ApoA1, and total cholesterol/HDL-C were calculated from the assessed blood lipid values.

Division of the group of deceased FH subjects into subgroups

One can expect the group of deceased FH subjects to be as diverse as any other group of FH patients, except from the fact that they are all deceased. Hence, the group was divided into two groups based on the same criteria as the susceptible and resistant groups to perform different analyses of interest.

We found it interesting to split the group of deceased FH subjects into two subgroups, based on the time of their first CHD event. The group then was divided into ‘deceased FH subjects with early CHD’ or ‘deceased FH subjects with late or no CHD’ based on the inclusion criteria in **tables 6 and 7**, from the susceptible group and the resistant group, respectively, used for the retrospective study. Two persons did not match the criteria and were excluded from the deceased FH subject data set in these analyses. The two subgroups each consisted of 14 subjects.

SmartDiet food questionnaire

SmartDiet is a food questionnaire which is developed at the Lipid Clinic. SmartDiet is designed to investigate a patient’s dietary habits and life style. Through the questionnaire, the Lipid Clinic assesses information about the FH subjects’ habitual food composition. It focuses mainly on intake of fat and type of fat, sugar, fibre, fruits and vegetables. Also, SmartDiet contains five questions on life style. The questionnaire is recommended in use for persons with dyslipidemia, FH and in general patients in need of a heart friendly diet. A patient can score a maximum of 36

points. The reproducibility and validity of SmartDiet was tested by Svilaas et al in 2002 (146).

Information on the dietary habits of deceased FH subjects was in most cases collected at their first or second visit to the Lipid Clinic. On basis of detailed medical records on dietary habits, the master student calculated a SmartDiet score for each deceased FH subject. In comparison, in the susceptible and resistant groups the SmartDiet questionnaire was filled out at study-visit. Hence, the SmartDiet scores could not be compared between the groups of non-deceased and deceased FH subjects.

Limitations of the data on deceased FH subjects

We found it important to recognise potential limitations using data from medical records of deceased FH subjects before we performed the analyses and also for interpretation of the results.

Missing values

Not all measurements were found in all subjects, most likely due to the fact that the primary source of data was the patients' medical records. Medical records are primarily intended for patient care and not for research purposes. Different clinicians have different routines and approaches regarding filling out medical records.

Gap between last consultation and time of death

Some patients were followed up by the Lipid Clinic for several years; others had had their first consultation only a short time before they died. Albeit some of the patients had a comprehensive follow-up history at the Lipid Clinic, not all were followed the last couple of years before they died.

In addition to consultations at the Lipid Clinic, most of the patients also were followed up by their family doctor. Therefore, in combination with the severity of their CHD risk profile, the time distance between consultations at the Lipid Clinic varied a lot between the patients. Some had consultations several times a year; others had consultations with years in-between.

We did not have medical records from other hospitals or doctors than from the Lipid Clinic, hence we did not know what medication or plasma lipid values the patients had had between the consultations or during their last years of life.

We do not know with certainty whether the deceased FH subjects who had not been to the Lipid Clinic for the last couple of years before they died had one or more CHD event(s) between their last visit at the Lipid Clinic and time of death. Neither do we have information on the cause of death in every FH subject.

From persons who had been followed for only a few years, the medical reports were not very comprehensive. However, we managed to get useful information from the data at disposal.

Measurements at different points of time

In most of the deceased FH patients, not all analyses were registered at every consultation, and some information was not registered at all. When information of interest was not found in the newest medical record, we found the information from older medical records.

For instance, the ApoA1 and ApoB levels were in many patients not collected every time they visited the Lipid Clinic. Yet, blood lipid values should be measured together since the plasma concentrations of apolipoproteins reflects the plasma cholesterol levels. In all patients where the newest apolipoprotein values and the newest cholesterol values were measured at different consultations, we collected the newest recorded cholesterol values and the cholesterol values measured at the same time of the latest ApoA1 and ApoB values. We then compared the latest registered cholesterol values with the cholesterol values registered at the time of apolipoprotein registration in each subject. If the difference in cholesterol values were not statistically significant different, we decided to use the ApoA1 and ApoB levels in our analysis together with the newest plasma cholesterol levels.

When statin use was not mentioned in the newest medical record, we assumed that the patients were on the same medication as their previous consultation at the Lipid Clinic.

Differences between non-deceased FH subjects and deceased FH subjects

The deceased FH subjects died within a period of 15 years, from the early 1990s to mid-2000. By median, they died around 1998. Hence, there was a difference of about ten years between the journal information on the deceased FH subjects and the non-deceased FH subjects' study-visit. This difference promotes challenges to the comparison of the groups.

First, the last ten years new drugs have become available on the market. For instance, ezetimibe has been used in clinics for about eight years. Consequently, only one of the deceased FH subjects was treated with ezetimibe, compared to approximately three in four of the non-deceased FH subjects.

Second, scientific evidence is produced continually and recommendations change through time. The scientists and doctors know more now than they did ten years ago. The goals of treatment of FH subjects ten years ago are already out of date.

Third, like drug treatment and treatment goals, measuring methods also change over time. New equipment and other reagents contribute to more accurate measurements and results. The outcome in comparison of different parameters between deceased FH subjects and non-deceased FH subjects could be biased on basis of older measuring methods used in the analyses from the deceased FH subjects.

Fourth, treatment of more and more FH subjects begins as a result of genetic verification of FH. In that way, more asymptomatic persons undergo lipid-lowering treatment the more genetic mutations that are revealed. This could be considered a bias when evaluating the results from comparison of clinical features in susceptible and resistant FH patients with deceased FH subjects.

Fifth, the deceased FH subjects with late or no CHD event could have died from virtually anything, especially due to the fact that we do not have data on everyone until their time of death. Nevertheless, we chose to define the group by the same cut-offs as the non-deceased resistant group, since we primarily compared the groups on basis of CHD event and blood lipid parameters.

With these limitations in mind, we still considered it interesting to compare the deceased FH subjects with non-deceased FH subjects. As far as we know, few scientists, if anyone, have systematically according to the time of their first CHD event looked into deceased FH subjects' journals and analysed and compared the data with comparable patients who have not died from similar events. If there are any interesting findings in our study this could be considered a small pilot study, and the implications for further studies are present.

3.1.2 Case-control study

Inclusion criteria

Susceptible and resistant FH subjects

FH patients were identified from the medical journals at the Lipid Clinic. They were included or excluded on basis of similar inclusion and exclusion criteria as the FH patients in the retrospective data collection study, with stricter age cut-offs and with some additional criteria. Inclusion criteria in the first of a total of two inclusion processes, when finding who to invite to participate in the study, considered two elements; a verified genetic mutation and place of residence not more than two-three hours drive from the Lipid Clinic, Oslo. The project was financed solely by scientific funding from the supervisors and could therefore not afford to pay travelling costs for people living far from Oslo; consequently we invited persons living close to the capital area. A total of 76 persons with heterozygous FH were sent an invitation letter. See **figure 7** for flow chart.

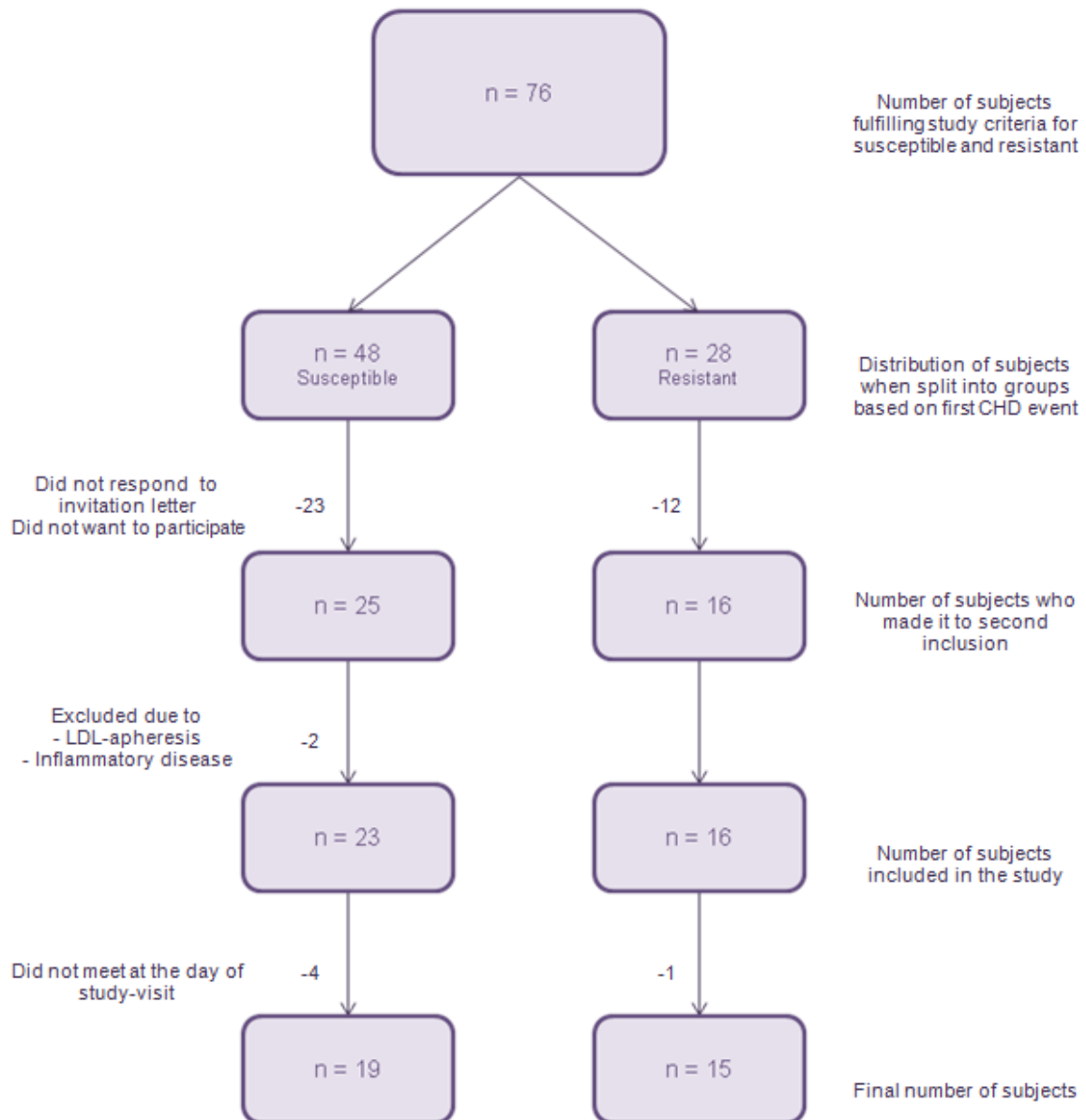


Figure 7. Flow chart showing inclusion and exclusion of susceptible and resistant in case-control study

The 76 FH subjects included in the first round were sorted into two groups according to age at first CHD event and smoking status. Also in the case-control study, the persons with FH classified into the ‘susceptible’ group had had a premature event of CHD and the FH subjects classified into the ‘resistant’ group had had a late event of CHD or no CHD event, see **tables 8 and 9** for cut-off levels of ‘premature’ and ‘late or no’ CHD event. The inclusion criteria in both the susceptible group and the

resistant group were, however, stricter in the case-control study than in the retrospective data collection study.

Table 8. Case-control. Cut-off of *early* CHD event in FH patients

	Gender	
	Female	Male
Smoking status		
Smoking and previous smoking	≤46	≤42
Non-smoking	≤49	≤45

Table 9. Case-control. Cut-off of *late or no* CHD event in FH patients

	Gender	
	Female	Male
Smoking status		
Smoking and previous smoking	≥60	≥55
Non-smoking	≥65	≥60

In the second inclusion process, after including patients by mail and phone follow-up, patients were excluded if they were undergoing regularly LDL aphaeresis or if they had rheumatoid arthritis or other autoimmune diseases. Also, patients were excluded if they recently had had pneumonia, a cold or other infections. One FH subject was undergoing LDL aphaeresis. The patient was excluded because of the variation of blood lipid levels before and after LDL aphaeresis; both a high and a low blood lipid level could affect the median of the group considered the small size of the group. One FH subject was also excluded because of treatment for rheumatoid arthritis. Since we collected blood samples to estimate levels of different inflammation markers in blood serum and white blood cells, there was a risk that a severe autoimmune inflammation could affect the inflammation markers level. Written informed consent, enclosed in **appendix 6**, was provided for all patients in the information letter and obtained from all of them before the consultation at the day of the study-visit.

At the day of the study-visit, some patients did not show up. Consequently, we ended up with 19 and 15 FH subjects in the susceptible and resistant groups, respectively.

In the resistant group, three out of 15 persons had had one or more CHD event(s). Hence, they could be considered a group of FH subjects of relatively good health.

The main reason that the age cut-off for the susceptible group in the case-control study is not more different than one year in comparison with the susceptible group in the retrospective data collection study is that there are not very many patients in the FH registry at the Lipid Clinic fulfilling those strict criteria. In comparison, it was much easier to find older persons to the resistant group in the case-control study than in the retrospective data collection study. Therefore, the difference between the two studies is much bigger concerning the resistant groups.

Controls

Control subjects were ten healthy gender- and age-matched volunteers recruited among friends, family, colleagues and staff at Lipid Clinic and at the University of Oslo, other acquaintances and friends and family of friends. This reference population was included in the study to map potential differences between FH subjects and healthy persons.

Exclusion criteria for controls were identical to those described for cases, with the additional criterion that controls had no previous diagnosis of heart disease or history of exertional chest pain. It was also preferred that the control subjects were not receiving anti-hypertensiva.

Informed consent was provided for all controls and obtained from all of them before the consultation. The letter is shown in **appendix 7**.

Anthropometric measurements

Body weight, height and waist circumference were measured. Measurements were undertaken in the same manner in FH subjects and controls. Body weight was measured on an electronic body weight measurement apparatus of the brand Soehnle

S20_2763. It is calibrated in January every year. The device measures with an accuracy of 0.1 kilograms. The patients were weighed without jackets and shoes. They were also told to empty their pockets.

The patients' height was measured with a manual height measurement scale of the brand Seca. It was last calibrated in September 2008. The scale was attached to the wall. Height was measured without shoes. The patients stood straight against the wall scale, with heels, bottom, back and head touching the wall. Then the height in centimetres was measured.

Waist circumference was measured by the master student using a measuring tape. The measuring tape was placed around the abdomen right above the upper hip bone – or in patients with a wide waist circumference around the widest part. It was then held horizontal around the waist. The reading of waist circumference in centimetres was done when the tape measure was snug but not caused compressions on the patients' skin.

Weight and height were used to calculate body mass index (BMI) with the formula $BMI = \text{weight (kg)}/\text{height}^2 \text{ (m)}$.

Blood pressure and pulse measurement

The blood pressure and pulse was measured by the master student with an electronic blood pressure measurement apparatus of the brand Welch Allyn. Last time it was calibrated was in 2008. Measurements were undertaken in the same manner in FH subjects and controls. The registration was done on the patients' left arm. During the blood pressure and pulse measurement the patients sat still in a relaxed and comfortable position in a chair with both legs touching the floor and both arms in a comfortable position. Three values were measured, with three-four minutes in-between. The averages of the three values, both for blood pressure and pulse, were used in the study. The patients were told not to smoke during the morning before measurement. All patients avoided caffeine and smoking for at least 30 minutes prior to testing, as they contains substances known to increase heart rate.

Hypertension was defined when anti-hypertensive medication was prescribed or if three consecutive measurements of blood pressure were >140 mmHg systolic or >90 mmHg diastolic.

Biochemical parameters

Blood samples were undertaken in the same manner in FH subjects and controls. All participants fasted 12 hours before the blood samples were collected. They were also recommended not to drink alcohol the last 24 hours before the consultation. None of the patients smoked before the blood sample collection. The blood samples were drawn by laboratory personnel at the laboratory at the Lipid Clinic, using a vacutainer system.

Routine laboratory analysis (fibrinogen, aspartate amino transferase (ASAT), alanine amino transferase (ALAT), creatinine, fibrinogen, fasting blood glucose, total cholesterol, LDL-C, HDL-C, TG, Lp(a), ApoA1, ApoB and CRP and coagulation parameters (APC resistance, Protein C, Protein S (total), Protein S (bound), vWF antigen and vWF activity) were analysed at the accredited medical-biochemical laboratory at Rikshospitalet, OUS. Blood samples were centrifuged and analysed within one hour of admission.

Except from one missing Lp(a) value in one FH subject due to a laboratory mistake, all analyses were done in all subjects. Two patients were taking Warfarin, and their blood samples were excluded in the coagulation markers analysis. Also, two patients forgot to fast the entire 12 hours before blood sample collection. However, we used the two patients' data because they had fasted nine and ten hours before the blood sample collection, respectively. One FH subject had a CRP level of 13.0 mg/L. We performed the analysis with and without the individual concerned.

Non-HDL was calculated with the formula: total cholesterol – HDL-C. ApoB/ApoA1 ratio was also calculated for use in statistical analysis.

Since this project is part of an ongoing large research project, together with collection of the above mentioned blood samples, blood samples for estimation of inflammation markers in blood serum and peripheral mononuclear blood cells were collected from the FH subjects and controls. The samples were delivered to the laboratory of Professor Kirsten Holven for subsequent analysis.

SmartDiet food questionnaire

The participants filled out a SmartDiet food questionnaire when they waited for the blood samples to be taken. The questionnaire was updated in 2008-2009 and a new and more detailed version has been used at the Lipid Clinic since July 2009. It has a maximum score of 41 points and not 36 points, which was the maximum score in the 2009 version of SmartDiet. Twenty-seven points or less is considered a low score, and a patient could change diet and lifestyle in several ways to make it more heart-friendly. A score between 28 and 35 points is reckoned a medium score, but a patient could still change diet and lifestyle in many ways to make it healthier. A high score is when a patient scores 36 points or more. Yet, the patients in the latter category receive diet recommendations to “keep up the good work”.

The new version of SmartDiet has also been tested for reproducibility and validity but the data are unpublished. A copy of the latest version of SmartDiet is shown in **appendix 8**.

3.1.3 Medication and influence on blood samples and blood pressure in the case-control study

Participants remained on their usual drug treatment at study-visit, and their currently prescribed drug treatment regimen was registered.

LDL-C concentration

The levels of blood lipids in the susceptible and resistant groups measured in the case-control study were affected of the type and level of drugs they received. Estimation of treatment goals and risk calculation are preferentially based on a person’s LDL-C

concentration without treatment – to get a proper account of the concentration of drugs or the degree of CHD risk.

In order to see what approximately the FH subjects' pre-treatment LDL-C levels were and thereby get an impression of the patients' levels of CHD risk pre-treatment, we decided to estimate the pre-treatment LDL-C level in each FH subject.

To calculate the pre-treatment LDL-C level we had to find the efficacy of the different drugs and different doses of drugs the patients used at the time of study. In this study, three types of statins were registered among the FH subjects; rosuvastatin, atorvastatin and lovastatin. Also, one patient, on basis of allergic reactions to other alternatives, used red yeast rice. Several scientists and study groups have investigated the efficacy of different statins or conducted meta-analysis on other's results (147-151).

Regarding efficacy of statins we decided to use data from the newest meta-analysis on the topic, from 2010, by Nicholls et al (150). The meta-analysis included data on changes in LDL-C with increasing statin dose on rosuvastatin and atorvastatin. Similar data on lovastatin were found from Helfand et al (151). An overview over the different doses of statins and the reduction in LDL-C levels is shown in **table 10**.

Table 10. Changes in LDL-C level with increasing statin dose. Adapted from Nicholls et al (150) and Helfand et al (151).

	Percent reduction in LDL-C level		
	Rosuvastatin	Atorvastatin	Lovastatin
Daily statin dose			
5 mg	38.8	NA	NA
10 mg	44.1	35.5	21.0
20 mg	49.5	41.4	27.0
40 mg	54.7	46.2	31.0
80 mg	NA*	50.2	42.0

* NA = Not applicable

Furthermore, we took into consideration the lipid-lowering effects of other medication as well; resins, ezetimibe and nicotinic acid. No one in the FH study group used fibric acids. Ezetimibe is usually given in a 10 mg dose in addition to a statin (71). Bays et al showed that a combination drug of the statin simvastatin and ezetimibe gives an additional reduction in LDL-C of 4.2 – 14.0 % compared to only statin (71). The middle value is an LDL-C reduction of approximately 9 %, of which we used in further estimations of pre-treatment LDL-C levels. Concerning the nicotinic acid, we used a recent study by Fazio et al (152), who estimated the added effect of nicotinic acid in combination with ezetimibe and statin. They showed that the added effect of nicotinic acid was approximately 5 %. We did not take into consideration different doses of resins or nicotinic acid shown in the patients using this drug. We estimated the efficacy of resins when combined with statin to be approximately 11 %, from a meta-analysis of Backes et al (153). In general, when the effect in percent of LDL-C lowering was given in a range, we decided to choose the middle value of the min and max values.

With red yeast rice we based our estimations on 21 % reduction, as estimated from a newly published study of Venero et al (68).

When the pre-treatment LDL-C levels were estimated we calculated the difference from pre-treatment LDL-C levels with study-visit LDL-C levels in mmol/L and percentage difference.

In the retrospective data collection study pre-treatment total cholesterol levels and study-visit total cholesterol levels were available in the data file. Hence, we calculated the difference from pre-treatment total cholesterol with study-visit total cholesterol levels in the same manner.

Blood pressure

Although blood pressure was recorded both in FH subjects and controls at study-visit, the levels in FH subjects both in comparison with controls and when comparing subgroups of FH subjects with each others, would be systematically affected by the

medication they received, such as blood pressure medication, β -blockers, nitrates and angiotensin-converting-enzyme (ACE) inhibitors. Since hypertension in FH subjects was defined as blood pressure above 140/90 mm Hg on two or more separate measurements or the use of antihypertensive drugs, it could be inappropriate to compare blood pressure between FH subjects and controls. Yet, if controls still had lower blood pressure than patients with FH, one could suggest that the FH subjects were not well-treated. And if there were approximately the same number of persons receiving each of the drugs in the susceptible and resistant group, they could be compared to each other. However, blood pressure also naturally increases with age (154). With a difference between the susceptible and resistant groups regarding median age at study-visit, age would be a possible confounding factor.

3.2 Statistical analysis

All statistical analyses were performed using SPSS version 16.0 statistical package for Windows (SPSS, Inc., Chicago, Illinois, USA).

A $P < 0.05$ was considered statistically significant. For all analyses, the upper limit for a *tendency of difference* was put at $P < 0.1$.

3.2.1 Continuous variables

Histograms and Normal Q-Q plots were used to evaluate whether the data material was normal distributed. Most distributions were not normal. Besides, an $n < 30$ in study-groups usually indicates that one should consider using non-parametric tests (155). In that way, one would not miss the importance of the outliers in the material. Since our data material was quite small, we decided to use non-parametric tests.

When there were three or more groups to compare, the non-parametric Kruskal-Wallis test was used both in the retrospective data collection study and in the case-control study to determine differences in lipid concentrations, dietary and anthropometric parameters and other continuous variables between the independent groups. The

significance level was considered at $P < 0.05$. However, one does not know which of the groups are statistically significantly different from one another when one obtains a statistical significant result from Kruskal-Wallis test (155). We then conducted a post-hoc Mann-Whitney U test between pairs of groups. In order to adjust for the inflation of type I errors due to multiple comparisons, we applied a Bonferroni correction to the P -value level of 0.05 when performing post-hoc Mann-Whitney U test (155). With three and four comparisons the adjusted P -value was considered significant at $P < 0.017$ and $P < 0.013$, respectively.

When we compared only two groups, for instance female susceptible and female resistant in the retrospective data collection study or in the case-control study, the Mann-Whitney U test was used with a significance level of $P < 0.05$.

In more or less normal distributions where the P -value was significant or between 0.051 and 1.00, we decided to run an Independent-samples t -test to verify the tendency of statistically significant difference.

Continuous data were presented as median values with range (min-max).

3.2.2 Categorical variables

Differences in categorical variables, such as appearance of tendon xanthomas and smoking status between the groups were assessed by Chi-square test for independence in some cases. In most cases, when the assumptions for chi-square were violated, Fisher's exact two-tail probability test was used. The significance level was considered at $P < 0.05$.

Categorical variables were presented as count with percentage.

Some values were missing in the retrospective data collection study. We decided to exclude cases pairwise and not listwise since we had a quite small data material.

Except from Bonferroni correction we have performed no adjustment for multiple testing.

4. Results

4.1 Retrospective data collection study

In addition to characterisation and comparison of the susceptible and resistant groups, we performed comparisons based on gender and on subgroups.

4.1.1 Susceptible and resistant groups

Characterisation of the subjects and comparison of the groups is shown in **table 11**. As expected from our inclusion criteria in the susceptible and resistant groups, age at first CHD event was statistically significant lower in susceptible than resistant. The same was the case with age at study-visit.

The susceptible group had in general a less beneficial lipid profile than the resistant group, with statistically significant higher median Lp(a) level, TG level and pre-treatment total cholesterol level. However, the individuals in the susceptible group were significantly supplementary medically treated; 33.8 % and 12.7 % of the susceptible compared to 15.8 % and 2.6 % of the resistant received resin and niacin treatment, respectively. The TG levels in both groups were within reference range.

The statistically significant differences between the two groups in mmol/L reduction of total cholesterol level, total cholesterol/HDL ratio, ApoB level and ApoB/ApoA1 ratio disappeared when performing Bonferroni correction on the *P*-values.

Medianwise, the resistant group had smoked for six years longer than the susceptible group – but this association also disappeared with Bonferroni correction.

Table 11. Characterisation and comparison of susceptible and resistant

	Susceptible		Resistant		<i>P</i> ^{2,3}
	<i>n</i> ¹		<i>n</i>		
Demographics					
Total	71		76		
Female	71	23 (32.4%)	76	37 (48.7%)	0.066
Age at study-visit	71	53 (39-73)	76	59 (47-76)	0.001
Age at first CHD event	52	30 (22-36)	14	57 (47-68)	<0.001
Early CHD in first-degree relative	71	54 (76.1%)	72	41 (56.9%)	0.025
Classical CHD risk factors					
Smoker (both pre and now)	71	47 (66.2%)	76	51 (67.1%)	1.00
Yrs smoking total	46	18 (2-50)	51	24 (2-47)	0.017*
Yrs since quit smoking	34	17 (3-40)	22	18 (1-45)	0.89
Hypertension	71	16 (22.5%)	74	17 (23.0%)	0.95
Diabetes type II	71	7 (9.9%)	75	7 (9.3%)	1.00
Drug treatment					
Statin	71	69 (97.2%)	76	73 (96.1%)	1.00
Resin	71	24 (33.8%)	76	12 (15.8%)	0.019
Niacin	71	9 (12.7%)	76	2 (2.6%)	0.027
Ezetimibe	71	57 (80.3%)	76	57 (75.0%)	0.57
Total yrs lipid-lowering treatment	52	18.0 (3.0-27.0)	60	17.0 (1.0-36.0)	0.61
Total yrs statin treatment	56	18.0 (3.0-24.0)	63	16.0 (1.0-23.0)	0.40
Diet parameters					
Omega-3 supplement	35	27 (77.1%)	56	42 (75.0%)	1.00
SmartDiet score (out of total 36)	52	30 (22-36)	61	30 (21-36)	0.88
Physical examination					
Xanthomas at study-visit	70	46 (65.7%)	75	57 (76.0%)	0.24
Xanthelasms at study-visit	70	5 (7.1%)	75	1 (1.3%)	0.11
Corneal arcus at study-visit	70	32 (45.7%)	75	38 (50.7%)	0.67
BMI (kg/m ²)	71	27.0 (18.0-38.6)	76	27.6 (18.4-40.2)	0.55
Systolic BP (mmHg)	71	124 (88-176)	76	128 (96-183)	-**
Diastolic BP (mmHg)	71	77 (56-112)	76	80 (60-96)	-**
Laboratory parameters					
TC pre-treatment (mmol/L)	68	11.7 (6.7-17.0)	75	10.4 (7.5-16.0)	0.011
TC at study-visit (mmol/L)	71	4.8 (3.1-9.2)	75	4.7 (2.8-14.9)	0.27
Difference LDL-C (mmol/L)	68	-6.8 (-11.3- -0.4)	74	-5.9 (-11.5 - +2.8)	0.036*
Difference LDL-C (%)	68	-57.3 (-74.4 - -5.7)	74	-57.2 (-77.9 - +23.1)	0.56
HDL-C at study-visit (mmol/L)	71	1.2 (0.7-3.1)	75	1.3 (0.5-3.1)	0.15
LDL-C at study-visit (mmol/L)	71	3.2 (1.7-6.6)	75	2.9 (1.4-10.9)	0.091
TG at study-visit (mmol/L)	70	0.9 (0.4-2.4)	74	0.8 (0.3-2.7)	0.008
Non-HDL (TC - HDL-C) (mmol/L)	71	3.7 (2.1-7.4)	75	3.3 (1.6-13.3)	0.067
TC/HDL-C	71	4.0 (2.3-7.0)	75	3.6 (2.2-9.3)	0.016*
ApoA1 level at study-visit (g/L)	70	1.4 (0.8-2.5)	64	1.5 (0.9-2.3)	0.47
ApoB level at study-visit (g/L)	70	0.9 (0.6-1.6)	64	0.9 (0.5-2.6)	0.031*
ApoB/ApoA1	70	0.66 (0.36-1.25)	64	0.61 (0.33-1.44)	0.033*
Lp(a) (mg/L)	58	518 (60-3130)	54	170 (60-1890)	<0.001

Data are given as n (%) or median (min-max)

¹ n indicates number of individuals

² Chi-square test for independence or Fisher's exact two-tail probability test, statistically significant when $P < 0.05$

³ Post-hoc Mann Whitney U test, statistically significant when $P < 0.05$

* Statistic significant P -value disappears when performing Bonferroni adjustment

** Cannot compare groups on basis of incomplete information in data file

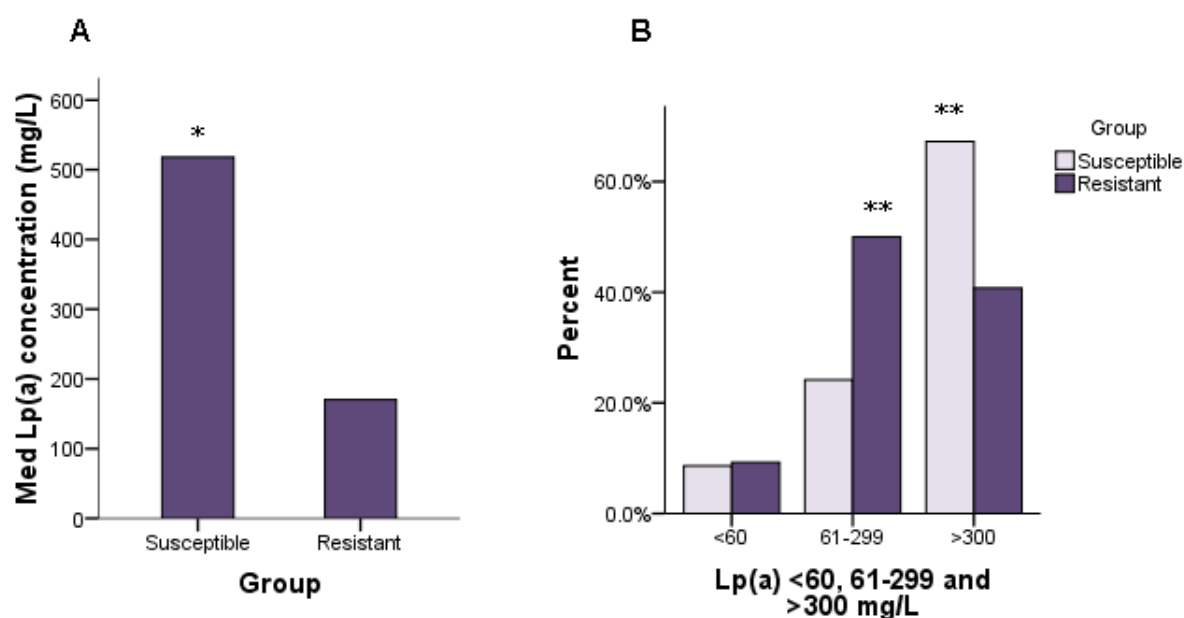
CHD = coronary heart disease; BMI = body mass index; BP = blood pressure; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol;

TG = triglycerides; ApoA1 = apolipoprotein A1; ApoB = apolipoprotein B; Lp(a) = lipoprotein(a)

Categorisation of Lp(a) values

In **figure 8a**, the median level of Lp(a) of 518 and 170 mg/L in the susceptible and resistant groups, respectively, are shown, $P < 0.001$. In **figure 8b**, the value of Lp(a) is divided into three categories. The lowest category represents the smallest value measurable at the medical-biochemical laboratory at Rikshospitalet, OUS. A Lp(a) value below 300 mg/L represents the 75-percentile in the general population, and values above 300 mg/L are considered to increase CHD risk (104, 106).

Fifty percent of resistant FH subjects had a Lp(a) level in the 61-299 mg/L range, which was a significantly greater number than that of susceptible FH subjects (24.1%). Conversely, the number of susceptible FH subjects with a Lp(a) level above 300 mg/L was statistically significant greater in comparison with the number of resistant FH subjects, with a number of 67.2 % and 40.7 %, respectively. Both comparisons were statistically significant at a P -value of 0.011. There was no difference between the two groups regarding Lp(a) level <60 mg/L.



Figures 8a and 8b. Categorisation of Lp(a) values. A. The median Lp(a) value of the susceptible and resistant groups shown in bar chart. The two groups have a median value of 518 (60-3130) and 170 (60-1890) mg/L, respectively, * $P < 0.001$. B. Distribution of subjects when Lp(a) is divided into three groups, ** $P = 0.011$.

4.1.2 Female and male

When splitting the whole material of FH subjects into groups based on gender, one could potentially reveal differences that were not seen in the comparison of susceptible and resistant groups.

As shown in **table 12**, there was a statistically significant difference between male and female FH subjects in the medication treatment, where more male subjects were treated with statins as well as resins and niacin.

Furthermore, FH men had significantly lower median total cholesterol level at study-visit than what FH women had. Also, male FH subjects tended towards having a bigger percent reduction of total cholesterol. However, not surprisingly, the women had significantly higher median HDL-C level and higher ApoA1 level and hence lower ApoB/ApoA1 ratio. The FH women also had significantly lower total cholesterol/HDL ratio in comparison with the FH men.

Female FH subjects had a tendency of lower median TG concentration than male FH subjects, with $P = 0.082$.

The age at study-visit was significantly different between the male group and female group; women were by median four years older than men. Also, a trend showed that the FH men were younger at their first CHD event in comparison with FH women. However, those findings were as expected, with oestrogen's protective role in female before menopause (11).

Table 12. Characterisation and comparison of female and male

	Female		Male		P ^{2,3}
	n ¹		n		
Demographics					
Total	60		87		
Age at study-visit	60	58 (39-76)	87	54 (40-75)	0.035
Age at first CHD event	25	45 (30-63)	60	39 (25-68)	0.076
Early CHD in first-degree relative	57	42 (73.7%)	86	53 (61.6%)	0.19
Classical CHD risk factors					
Smoker (both pre and now)	60	40 (66.7%)	87	58 (66.7%)	1.00
Yrs smoking total	39	19 (2-40)	58	19 (2-50)	0.79
Yrs since quit smoking	24	16 (1-45)	32	19 (2-40)	0.91
Hypertension	58	14 (24.1%)	87	19 (21.8%)	0.90
Diabetes type II	59	6 (10.2%)	87	8 (9.2%)	1.00
Drug treatment					
Statin	60	55 (91.7%)	87	87 (100.0%)	0.010
Resin	60	8 (13.3%)	87	28 (32.2%)	0.016
Niacin	60	1 (1.7%)	87	10 (11.5%)	0.028
Ezetimibe	60	45 (75.0%)	87	69 (79.3%)	0.68
Total yrs lipid-lowering treatment	48	18 (1-36)	64	18 (3-23)	0.63
Total yrs statin treatment	51	17 (1-24)	68	17 (3-23)	0.95
Diet parameters					
Omega-3 supplement	41	30 (73.2%)	50	39 (78.0%)	0.63
SmartDiet score (out of total 36)	48	30 (21-35)	65	31 (22-36)	0.12
Physical examination					
Xanthomas at study-visit	59	40 (67.8%)	86	63 (73.3%)	0.60
Xanthelasms at study-visit	59	2 (3.4 %)	86	4 (4.7%)	1.00
Corneal arcus at study-visit	59	26 (44.1%)	86	44 (51.2%)	0.50
BMI (kg/m ²)	60	27.8 (18.0-40.2)	87	26.9 (18.9-38.6)	0.80
Systolic BP (mmHg)	60	128 (88-183)	87	126 (96-176)	-*
Diastolic BP (mmHg)	60	77 (56-98)	87	78 (57-112)	-*
Laboratory parameters					
TC pre-treatment (mmol/L)	59	11.0 (7.0-17.0)	84	11.3 (6.7-16.0)	0.74
TC at study-visit (mmol/L)	60	5.0 (3.3-14.9)	86	4.7 (2.8-7.9)	0.042
Difference TC (mmol/L)	59	-5.9 (-11.3 - +2.8)	59	-6.6 (-11.5 - -0.7)	0.11
Difference TC (%)	83	-54.6 (-71.3 - +23.1)	83	-57.6 (-77.9 - -10.4)	0.082
HDL-C at study-visit (mmol/L)	60	1.4 (0.8-3.1)	86	1.2 (0.5-2.2)	<0.001
LDL-C at study-visit (mmol/L)	60	3.2 (1.6-10.9)	86	3.0 (1.4-5.5)	0.16
TG at study-visit (mmol/L)	59	0.8 (0.3-2.4)	85	0.9 (0.4-2.7)	0.061
Non-HDL (TC - HDL-C) (mmol/L)	60	3.6 (2.0-13.3)	86	3.5 (1.6-6.5)	0.61
TC/HDL-C	60	3.5 (2.2-9.3)	86	4.0 (2.3-7.6)	0.004
ApoA1 level at study-visit (g/L)	54	1.6 (1.0-2.5)	80	1.3 (0.8-2.0)	<0.001
ApoB level at study-visit (g/L)	54	0.9 (0.5-2.6)	80	0.9 (0.6-1.5)	0.57
ApoB/ApoA1	54	0.58 (0.33-1.44)	80	0.68 (0.35-1.27)	0.014
Lp(a) (mg/L)	44	276 (60-3130)	68	435 (60-2460)	0.13

Data are given as n (%) or median (min-max)

¹ n indicates number of individuals

² Chi-square test for independence or Fisher's exact two-tail probability test, statistically significant when $P < 0.05$

³ Post-hoc Mann Whitney U test, statistically significant when $P < 0.05$

* Cannot compare groups on basis of incomplete information in data file

CHD = coronary heart disease; BMI = body mass index; BP = blood pressure; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol;

TG = triglycerides; ApoA1 = apolipoprotein A1; ApoB = apolipoprotein B; Lp(a) = lipoprotein(a)

4.1.3 Susceptible female and resistant female

By splitting the susceptible and resistant groups into groups based on gender, one could somewhat meet the challenge of gender as a confounding factor.

Characterisation of the subjects and comparison of the groups of susceptible female and resistant female is shown in **tables 13a and 13b**, respectively.

In general, the susceptible female group had a more atherogenic blood lipid profile than the group of resistant female. First of all, the susceptible women had a statistically significant higher median Lp(a) level. In addition, the susceptible women had significantly higher median total cholesterol pre-treatment level, LDL-C level, TG level, non-HDL level, ApoB level, ApoB/ApoA1 ratio and total cholesterol/HDL-C ratio.

Interestingly, the susceptible women also had medianwise significantly bigger reduction in total cholesterol measured in mmol/L. However, the only medication that differed between the two groups was resin treatment, a significantly higher number of persons in the susceptible women group received resins in combination with statins.

Moreover, the amount of years of both lipid-lowering treatment and statin treatment were greater in the susceptible female group compared with the resistant female group.

Significantly more susceptible women had early CHD in first-degree relatives than the resistant women.

As expected, the susceptible women were younger at first CHD event than the resistant women. Furthermore, there were only two women in the resistant group who had had a CHD event.

4.1.4 Susceptible male and resistant male

As shown in **tables 13a and 13b**, respectively, there were unexpectedly few differences between the group of susceptible male and the group of resistant male compared to the findings between the female groups.

The only statistically significant finding was that more men in the susceptible male group medianwise received niacin treatment in comparison with the men in the resistant male group.

Not surprisingly, the susceptible men were significantly younger at their first CHD event than the resistant men – and they also were younger at study-visit.

Interestingly, there was a tendency of difference between the two groups considering the number of years they had received lipid-lowering treatment, with a median of 16.0 years in susceptible men and 18.5 years in resistant men, $P = 0.071$. At the same time, there was a trend towards resistant men having been smoking medianwise for four more years than susceptible men.

Table 13a. Characteristics and comparison of female and male subdivided into groups of susceptible and resistant

	Female				<i>P</i> ^{2,3}	Male				<i>P</i> ⁴	<i>P</i> ⁵	<i>P</i> ⁶
	Susceptible		Resistant			Susceptible		Resistant				
	<i>n</i> ¹		<i>n</i>			<i>n</i>		<i>n</i>				
Demographics												
Total	23		37			48		39				
Age at study-visit	23	57 (39-73)	37	58 (50-76)	0.84	48	51 (40-73)	39	60 (47-75)	<0.001	0.023	0.67
Age at first CHD event	23	44 (30-48)	2	61 (58-63)	0.020	48	37 (25-45)	12	56 (47-68)	<0.001	0.001	0.32
Early CHD in first degree relative	23	21 (91.3%)	34	21 (61.8%)	0.015	48	33 (68.8%)	38	20 (52.6%)	0.19	0.042	0.59
Classical CHD risk factors												
Smoker (both pre and now)	23	14 (60.9%)	37	26 (70.3%)	0.58	48	33 (68.8%)	39	25 (64.1%)	0.82	0.60	0.74
Yrs smoking total	13	17 (3-30)	26	25 (2-40)	0.092	33	18 (2-50)	25	22 (3-47)	0.082	0.34	0.81
Yrs since quit smoking	11	11 (4-35)	13	17 (1-45)	0.73	23	19 (3-40)	9	18 (2-32)	0.85	0.67	0.95
Hypertension	23	8 (34.8%)	35	6 (17.1%)	0.21	48	8 (16.7%)	39	28 (71.8%)	0.30	0.13	0.28
Diabetes type II	23	4 (17.4%)	36	2 (5.6%)	0.20	48	3 (6.2%)	39	5 (12.8%)	0.46	0.20	0.43
Drug treatment												
Statin	23	21 (91.3%)	37	34 (91.9%)	1.00	48	48 (100.0%)	39	39 (100.0%)	1.00	0.10	0.11
Resin	23	6 (26.1%)	37	2 (5.4%)	0.045	48	18 (37.5%)	39	10 (25.6%)	0.34	0.43	0.025
Niacin	23	0 (0.0%)	37	1 (2.7%)	1.00	48	9 (18.8%)	39	1 (2.6%)	0.021	0.027	1.00
Ezetimibe	23	19 (82.6%)	37	26 (70.3%)	0.37	48	38 (79.2%)	39	31 (79.5%)	1.00	1.00	0.43
Total yrs lipid-lowering treatment	16	20.0 (4.0-27.0)	32	15.5 (1.0-36.0)	0.009	36	16.0 (3.0-23.0)	28	18.5 (10.0-23.0)	0.071	0.003	0.063
Total yrs statin treatment	17	19.0 (4.0-24.0)	34	15.5 (1.0-23.0)	0.004	39	16.0 (3.0-23.0)	29	17.0 (8.0-22.0)	0.18	0.029	0.057
Diet parameters												
Omega-3 supplement	13	9 (69.2%)	28	21 (75.0%)	0.72	22	18 (81.8%)	28	21 (75.0%)	0.73	0.43	1.00
SmartDiet score (out of total 36)	16	30 (25-35)	32	30 (21-35)	0.92	36	31 (22-36)	29	31 (26-36)	0.61	0.40	0.18

Data are given as n (%) or median (min-max)

¹ n indicates number of individuals

² Mann-Whitney U test and C hi-square test for independence or Fisher's exact two-tail probability test, statistically significant when *P* < 0.05 were used

³ between susceptible female and resistant female

⁴ between susceptible male and resistant male

⁵ between susceptible female and susceptible male

⁶ between resistant female and resistant male

CHD = coronary heart disease

Table 13b. Characteristics and comparison of female and male subdivided into susceptible and resistant group

	Female					Male						
	Susceptible		Resistant		<i>P</i> ^{2,3}	Susceptible		Resistant		<i>P</i> ⁴	<i>P</i> ⁵	<i>P</i> ⁶
	<i>n</i> ¹		<i>n</i>			<i>n</i>		<i>n</i>				
Physical examination												
Xanthomas at study-visit	22	15 (68.2%)	37	25 (67.6%)	1.00	48	31 (64.6%)	38	32 (84.2%)	0.072	1.00	0.11
Xanthelasma at study-visit	22	1 (4.5%)	37	1 (2.7%)	1.00	48	4 (8.3%)	38	0 (0.0%)	0.13	1.00	0.49
Corneal arcus at study-visit	22	11 (50.0%)	37	15 (40.5%)	0.59	48	21 (43.8%)	38	23 (60.5%)	0.18	0.82	0.13
BMI (kg/m ²)	23	28.6 (18.0-35.6)	37	27.7 (18.4-40.2)	0.83	48	26.4 (18.9-38.6)	39	27.5 (21.6-38.0)	0.25	0.46	0.63
Systolic BP (mmHg)	23	132 (88-165)	37	125 (99-183)	.*	48	123 (100-176)	39	137 (96-168)	.**	.*	.**
Diastolic BP (mmHg)	23	74 (56-98)	37	80 (60-92)	.*	48	77 (57-112)	39	80 (60-96)	.**	.*	.**
Laboratory parameters												
TC pre-treatment (mmol/l)	22	12.0 (7.0-17.0)	37	10.4 (7.5-15.7)	0.006	46	11.6 (6.7-16.0)	38	10.5 (8.5-16.0)	0.32	0.25	0.58
TC at study-visit (mmol/l)	23	5.5 (3.7-9.2)	37	4.8 (3.3-14.9)	0.084	48	4.7 (3.1-7.9)	38	4.6 (2.8-7.4)	0.65	0.013	0.41
Difference LDL-C (mmol/l)	22	-7.3 (-11.3 - -0.4)	37	-5.7 (-10.1 - +2.8)	0.036	46	-6.8 (-11.0 - -0.7)	37	-6.0 (-11.5 - -2.1)	0.55	0.78	0.093
Difference LDL-C (%)	22	-56.9 (-71.3 - -5.7)	37	-54.4 (-70.8 - +23.1)	0.62	46	-57.3 (-74.4 - -10.4)	37	-58.9 (-77.9 - -23.3)	0.86	0.47	0.13
HDL-C at study-visit (mmol/l)	23	1.3 (0.8-3.1)	37	1.5 (0.9-3.1)	0.25	48	1.2 (0.7-2.2)	38	1.2 (0.5-2.0)	0.97	0.025	<0.001
LDL-C at study-visit (mmol/l)	23	3.4 (1.9-6.6)	37	2.9 (1.6-10.9)	0.031	48	3.1 (1.7-5.5)	38	2.9 (1.4-5.1)	0.49	0.023	0.78
TG at study-visit (mmol/l)	23	0.9 (0.4-2.4)	36	0.7 (0.3-2.4)	0.030	47	0.9 (0.5-2.4)	38	0.9 (0.4-2.7)	0.21	0.75	0.12
Non-HDL (TC - HDL-C) (mmol/l)	23	4.1 (2.5-7.4)	37	3.2 (2.0-13.3)	0.013	48	3.6 (2.1-6.5)	38	3.3 (1.6-5.7)	0.72	0.039	0.49
TC/HDL-C	23	4.4 (2.3-6.5)	37	3.3 (2.2-9.3)	0.006	48	4.0 (2.5-7.0)	38	4.0 (2.3-7.6)	0.97	0.98	0.001
ApoA1 level at study-visit (g/l)	22	1.6 (1.1-2.5)	32	1.6 (1.0-2.3)	0.74	48	1.4 (0.8-2.0)	32	1.3 (0.9-2.0)	0.84	0.006	0.005
ApoB level at study-visit (g/l)	22	1.1 (0.7-1.6)	32	0.9 (0.6-2.6)	0.005	48	0.9 (0.6-1.5)	32	0.9 (0.6-1.4)	0.58	0.019	0.49
ApoB/ApoA1	22	0.66 (0.36-1.07)	32	0.53 (0.33-1.44)	0.018	48	0.66 (0.45-1.25)	32	0.69 (0.35-1.27)	0.97	0.89	0.006
LDL-C/ApoB	22	3.6 (2.3-4.3)	32	3.4 (2.8-4.2)	0.59	48	3.4 (2.4-4.6)	32	3.3 (2.2-4.3)	0.40	0.095	0.019
Lp(a) (mg/l)	19	953 (60-3130)	25	140 (60-606)	<0.001	39	458 (60-2460)	29	411 (60-1890)	0.25	0.32	0.014

Data are given as n (%) or median (min-max)

¹ n indicates number of individuals

² Mann Whitney U test and Chi-square test for independence or Fisher's exact two-tail probability test, statistically significant when *P* < 0.05 were used

³ between susceptible female and resistant female

⁴ between susceptible male and resistant male

⁵ between susceptible female and susceptible male

⁶ between resistant female and resistant male

* Cannot compare groups on basis of incomplete information in data file

BMI = body mass index; BP = blood pressure; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; TG = triglycerides; ApoA1 = apolipoprotein A1;

ApoB = apolipoprotein B; Lp(a) = lipoprotein(a)

4.1.5 Susceptible female and susceptible male

In **tables 13a and 13b**, respectively, the characterisation of the subjects and comparison of the groups of susceptible female and susceptible male is shown.

Although the susceptible women had been treated with lipid-lowering drugs and statins significantly longer in comparison with the susceptible men, they had higher median total cholesterol level at study-visit, LDL-C level, non-HDL level and ApoB level. Furthermore, the only lipid-lowering drug significantly more used by men than women was niacin.

As anticipated, median HDL-C level was significantly higher in susceptible female compared to susceptible male.

Significantly more susceptible women than susceptible men had early CHD in first-degree relatives.

The median age at study-visit and the median age at first CHD event were, not unexpectedly, significantly higher in susceptible female in comparison with susceptible male.

4.1.6 Resistant female and resistant male

Characterisation of the subjects and comparison of the groups is shown in **tables 13a and 13b**, respectively. The resistant male group had statistically significant higher median Lp(a) level than the resistant female group.

As expected, the women had medianwise higher level of HDL-C and ApoA1, and accordingly higher median ApoB/ApoA1 ratio level in comparison with the men. The total cholesterol /HDL-C ratio was lower in resistant women than resistant men.

Significantly more resistant men than resistant women received resin treatment. The resistant male group also tended to have received lipid-lowering treatment and statin treatment for a greater number of years than the resistant female group.

The group of resistant women tended to have lower mmol/L difference in LDL-C levels in comparison with the group of resistant men.

4.1.7 Deceased FH subjects with early CHD event and late or no CHD event

As shown in **table 14**, median ApoA1 level was significantly lower in the group of deceased FH subjects with early CHD event compared with the group of deceased FH subjects with late or no CHD event. The same situation was seen for the ApoB/A1 ratio, but the difference between the groups disappeared with Bonferroni correction. The other blood lipid values were not significantly different between the two groups. However, median total cholesterol/HDL-C ratio tended to be higher in the group of deceased FH subjects with early CHD event.

In the group with early CHD event, a statistically significant lower percent of individuals were taking omega-3 supplements compared with the group with late or no CHD event.

Interestingly, the deceased FH subjects with early CHD event died at a median age of 45, suggesting that they did not only have a CHD incidence early; they also died from it. The two groups were classified into groups from the subjects' age at first CHD event; yet, the deceased FH subjects with early CHD event died at a significantly younger median age than what the subjects in the group of FH subjects with late or no CHD event did.

As expected, median age at first CHD event was significantly lower in the group of deceased FH subjects with early CHD event.

Table 14. Characterisation and comparison of deceased FH subjects with early CHD event and deceased FH subjects with late or no CHD event

	Deceased FH subjects				<i>P</i> ^{2,3}
	Early CHD		Late/no CHD		
	<i>n</i> ¹		<i>n</i>		
Demographics					
Total	14		14		
Female	14	5 (35.7%)	14	8 (57.1%)	0.45
Age at time of death	14	45 (33-62)	14	66 (49-85)	<0.001
Age at first CHD event	14	34 (26-46)	8	50 (46-55)	<0.001
Early CHD in first-degree relative	13	9 (69.2%)	13	8 (61.5%)	1.00
Classical CHD risk factors					
Smoker (both pre and now)	13	8 (61.5%)	14	7 (50.0%)	1.00
Hypertension	13	5 (38.5%)	13	7 (53.8%)	0.69
Diabetes type II	3	0 (0.0%)	4	2 (50.0%)	0.43
Drug treatment					
Statin	14	14 (100.0%)	14	13 (92.9%)	1.00
Resin	14	7 (50.0%)	14	6 (42.9%)	1.00
Niacin	14	1 (7.1%)	14	2 (14.3%)	1.00
Ezetimibe	14	1 (7.1%)	14	0 (0.0%)	1.00
Total yrs lipid-lowering treatment	14	12.0 (2.0-44.0)	13	8.5 (1.0-20.0)	0.17
Total yrs statin treatment	14	9.5 (1.5-18.0)	14	7.5 (0.0-15.0)	0.31
Diet parameters					
Omega-3 supplement	13	2 (15.4%)	14	9 (64.3%)	0.028
SmartDiet score (out of total 36)	12	25 (21-29)	12	27 (21-34)	0.28
Physical examination					
Xanthomas at study-visit	13	9 (69.2%)	14	8 (57.1%)	0.70
Xanthelasms at study-visit	13	7 (53.8%)	14	7 (50.0%)	1.00
Corneal arcus at study-visit	13	7 (53.8%)	13	9 (69.2%)	0.69
BMI (kg/m ²)	12	23.9 (16.6-40.4)	9	28.7 (20.9-40.0)	0.20
Systolic BP (mmHg)	10	129 (107-160)	10	124 (95-177)	-**
Diastolic BP (mmHg)	10	80 (64-90)	10	75 (60-90)	-**
Laboratory parameters					
TC pre-treatment (mmol/L)	14	13.3 (7.9-19.0)	14	13.0 (9.7-24.0)	0.98
TC at study-visit (mmol/L)	14	7.8 (4.3-11.2)	14	6.9 (4.9-12.1)	0.33
Difference LDL-C (mmol/L)	14	-4.1 (-14.7 - +0.5)	14	-5.4 (-16.4 - -1.9)	0.25
Difference LDL-C (%)	14	-35.2 (-77.4 - +5.1)	14	-46.9 (-68.3 - -13.6)	0.25
HDL-C at study-visit (mmol/L)	14	1.0 (0.6-2.5)	14	1.2 (0.8-2.9)	0.26
LDL-C at study-visit (mmol/L)	14	5.4 (3.2-9.4)	14	4.7 (3.1-9.9)	0.22
TG at study-visit (mmol/L)	14	1.2 (0.6-5.0)	14	1.4 (0.4-2.6)	0.58
Non-HDL (TC - HDL-C) (mmol/L)	14	6.6 (3.7-10.2)	14	5.3 (3.9-11.1)	0.22
TC/HDL-C	14	7.2 (2.8-11.2)	14	5.2 (2.7-12.1)	0.081
ApoA1 level at study-visit (g/L)	10	1.0 (0.9-1.7)	11	1.5 (1.2-2.2)	0.010
ApoB level at study-visit (g/L)	10	1.8 (1.1-2.8)	11	1.2 (1.0-2.8)	0.11
ApoB/ApoA1	10	1.72 (0.73-2.91)	11	0.92 (0.55-2.12)	0.035*
Lp(a) (mg/L)	11	436 (17-1119)	8	309 (24-1075)	0.74

Data are given as n (%) or median (min-max)

¹ n indicates number of individuals

² Chi-square test for independence or Fisher's exact two-tail probability test, statistically significant when $P < 0.05$

³ Post-hoc Mann Whitney U test, statistically significant when $P < 0.05$

* Statistic significant P -value disappears when performing Bonferroni adjustment

** Cannot compare groups on basis of incomplete information in data file

CHD = coronary heart disease; BMI = body mass index; BP = blood pressure; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol;

TG = triglycerides; ApoA1 = apolipoprotein A1; ApoB = apolipoprotein B; Lp(a) = lipoprotein(a)

4.1.8 Susceptible and deceased FH subjects with early CHD event

Characterisation of the subjects and comparison of the two groups is shown in **table 15**. In general, the non-deceased susceptible group had a more favourable blood lipid composition than that of the deceased FH subjects with early CHD event.

Median total cholesterol levels at study-visit, percent reduction in total cholesterol, LDL-C level, non-HDL level, total cholesterol/HDL-C ratio, ApoA1 level, ApoB level and ApoB/ApoA1 ratio were significantly lower in the non-deceased susceptible group. The group of non-deceased susceptible FH subjects also had lower median TG level, but with Bonferroni correction the significant *P*-value disappeared. Lp(a) levels and HDL-C levels were not significantly different between the two groups.

A significantly greater number of non-deceased susceptible had reported a regularly intake of omega-3 supplement in comparison to what was found in the deceased patients' medical reports.

The number of non-deceased susceptible FH subjects with xanthelasms was significantly lower than the number of deceased FH subjects with early CHD event with xanthelasms; the percentage of individuals were 7.1 % and 53.8 %, respectively.

Median total number of years of statin treatment was significantly higher in non-deceased susceptible compared to the deceased FH subjects with early CHD event. The same was the case with lipid-lowering treatment. Considering the latter, the significant difference between the groups disappeared after Bonferroni correction.

Not surprisingly, taking into consideration the time difference between the two groups, a significantly greater number of susceptible FH subjects received ezetimibe treatment in comparison with the deceased FH subjects with early CHD event.

Table 15. Characterisation and comparison of susceptible and deceased FH subjects with early CHD event

	Early CHD				<i>P</i> ^{2,3}
	Susceptible		Deceased FH subjects		
	<i>n</i> ¹		<i>n</i>		
Demographics					
Total	71		14		
Female	71	23 (32.4%)	14	5 (35.7%)	1.00
Age at study-visit	71	53 (39-73)	-	-	-
Age at time of death	-	-	14	45 (33-62)	-
Age at first CHD event	52	30 (22-36)	14	34 (26-46)	0.13
Early CHD in first-degree relative	71	54 (76.1%)	13	9 (69.2%)	0.73
Classical CHD risk factors					
Smoker (both pre and now)	71	47 (66.2%)	13	8 (61.5%)	0.76
Hypertension	71	16 (22.5%)	13	5 (38.5%)	0.30
Diabetes type II	71	7 (9.9%)	3	0 (0.0%)	1.00
Drug treatment					
Statin	71	69 (97.2%)	14	14 (100.0%)	1.00
Resin	71	24 (33.8%)	14	7 (50.0%)	0.36
Niacin	71	9 (12.7%)	14	1 (7.1%)	1.00
Ezetimibe	71	57 (80.3%)	14	1 (7.1%)	<0.001
Total yrs lipid-lowering treatment	52	18.0 (3.0-27.0)	14	12.0 (2.0-44.0)	0.021*
Total yrs statin treatment	56	18.0 (3.0-24.0)	14	9.5 (1.5-18.0)	<0.001
Diet parameters					
Omega-3 supplement	35	27 (77.1%)	13	2 (15.4%)	<0.001
Physical examination					
Xanthomas at study-visit	70	46 (65.7%)	13	9 (69.2%)	1.00
Xanthelasms at study-visit	70	5 (7.1%)	13	7 (53.8%)	<0.001
Corneal arcus at study-visit	70	32 (45.7%)	13	7 (53.8%)	0.76
BMI (kg/m ²)	71	27.0 (18.0-38.6)	12	23.9 (16.6-40.4)	0.12
Systolic BP (mmHg)	71	124 (88-176)	10	129 (107-160)	-**
Diastolic BP (mmHg)	71	77 (56-112)	10	80 (64-90)	-**
Laboratory parameters					
TC pre-treatment (mmol/L)	68	11.7 (6.7-17.0)	14	13.3 (7.9-19.0)	0.13
TC at study-visit (mmol/L)	71	4.8 (3.1-9.2)	14	7.8 (4.3-11.2)	<0.001
Difference LDL-C (mmol/L)	68	-6.8 (-11.3- -0.4)	14	-4.1 (-14.7 - +0.5)	0.12
Difference LDL-C (%)	68	-57.3 (-74.4 - -5.7)	14	-35.2 (-77.4 - +5.1)	0.001
HDL-C at study-visit (mmol/L)	71	1.2 (0.7-3.1)	14	1.0 (0.6-2.5)	0.076
LDL-C at study-visit (mmol/L)	71	3.2 (1.7-6.6)	14	5.4 (3.2-9.4)	<0.001
TG at study-visit (mmol/L)	70	0.90 (0.40-2.40)	14	1.17 (0.60-5.01)	0.041*
Non-HDL (TC - HDL-C) (mmol/L)	71	3.7 (2.1-7.4)	14	6.6 (3.7-10.2)	<0.001
TC/HDL-C	71	4.00 (2.32-7.00)	14	7.17 (2.80-11.20)	<0.001
ApoA1 level at study-visit (g/L)	70	1.40 (0.80-2.50)	10	1.04 (0.90-1.70)	0.002
ApoB level at study-visit (g/L)	70	0.90 (0.60-1.60)	10	1.83 (1.10-2.80)	<0.001
ApoB/ApoA1	70	0.655 (0.360-1.250)	10	1.717 (0.725-2.913)	<0.001
Lp(a) (mg/L)	58	518 (60-3130)	11	436 (17-1119)	0.14

Data are given as n (%) or median (min-max)

¹ n indicates number of individuals

² Chi-square test for independence or Fisher's exact two-tail probability test, statistically significant when $P < 0.05$

³ Post-hoc Mann Whitney U test, statistically significant when $P < 0.05$

* Statistic significant P -value disappears when performing Bonferroni adjustment

** Cannot compare groups on basis of incomplete information in data file

CHD = coronary heart disease; BMI = body mass index; BP = blood pressure; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol;

TG = triglycerides; ApoA1 = apolipoprotein A1; ApoB = apolipoprotein B; Lp(a) = lipoprotein(a)

4.1.9 Resistant and deceased FH subjects with late or no CHD event

In **table 16** the characterisation of the subjects and comparison of the non-deceased resistant group and the group of deceased FH subjects with late or no CHD event is shown.

Median total cholesterol level pre-treatment and at study-visit, LDL-C level, non-HDL level, total cholesterol/HDL-C ratio, ApoB level and ApoB/ApoA1 ratio were significantly lower in the group of non-deceased resistant FH subjects in comparison with the group of deceased FH subjects with late or no CHD event. Also, median TG level was significantly lower in non-deceased FH subjects, but Bonferroni correction eliminated the significance.

A lower number of individuals in the non-deceased resistant group had hypertension in comparison with the deceased FH subjects group.

Total number of years of lipid-lowering therapy and total number of years of statin treatment were significantly higher in the non-deceased resistant FH subjects compared to the deceased FH subjects with late or no CHD event. The number of resistant subjects that received resins and ezetimibe treatment was significantly higher than that of deceased FH subjects with late or no CHD event.

Noteworthy, eight out of 14 deceased FH subjects had experienced a CHD event in the deceased FH subjects with late or no CHD event group compared with 14 out of 76 subjects in the resistant group. The deceased FH subjects had their first CHD event at an earlier age than that of the resistant. However, the significance disappeared when performing Bonferroni correction on the *P*-value.

Xanthelasms was found in a significantly lower number of the non-deceased resistant individuals compared to the deceased FH subjects.

Table 16. Characterisation of resistant and deceased FH subjects with late or no CHD event

	Late/no CHD				<i>P</i> ^{2,3}
	Resistant		Deceased FH subjects		
	<i>n</i> ¹		<i>n</i>		
Demographics					
Total	76		14		
Female	76	37 (48.7%)	14	8 (57.1%)	0.77
Age at study-visit	76	59 (47-76)	-	-	-
Age at time of death	-	-	14	66 (49-85)	-
Age at CHD first event	14	57 (47-68)	8	50 (46-55)	0.015*
Early CHD in first-degree relative	72	41 (56.9%)	13	8 (61.5%)	1.00
Classical CHD risk factors					
Smoker (both pre and now)	76	51 (67.1%)	14	7 (50.0%)	1.00
Hypertension	74	17 (23.0%)	13	7 (53.8%)	0.039
Diabetes type II	75	7 (9.3%)	4	2 (50.0%)	0.062
Drug treatment					
Statin	76	73 (96.1%)	14	13 (92.9%)	0.50
Resin	76	12 (15.8%)	14	6 (42.9%)	0.031
Niacin	76	2 (2.6%)	14	2 (14.3%)	0.11
Ezetimibe	76	57 (75.0%)	14	0 (0.0%)	<0.001
Total yrs lipid-lowering treatment	60	17.0 (1.0-36.0)	13	8.5 (1.0-20.0)	0.001
Total yrs statin treatment	63	16.0 (1.0-23.0)	14	7.5 (0.0-15.0)	<0.001
Diet parameters					
Omega-3 supplement	56	42 (75.0%)	14	9 (64.3%)	0.51
Physical examination					
Xanthomas at study-visit	75	57 (76.0%)	14	8 (57.1%)	0.19
Xanthelasms at study-visit	75	1 (1.3%)	14	7 (50.0%)	<0.001
Corneal arcus at study-visit	75	38 (50.7%)	13	9 (69.2%)	0.25
BMI (kg/m ²)	76	27.6 (18.4-40.2)	9	28.7 (20.9-40.0)	0.83
Systolic BP (mmHg)	76	128 (96-183)	10	124 (95-177)	-**
Diastolic BP (mmHg)	76	80 (60-96)	10	75 (60-90)	-**
Laboratory parameters					
TC pre-treatment (mmol/L)	75	10.4 (7.5-16.0)	14	13.0 (9.7-24.0)	0.003
TC at study-visit (mmol/L)	75	4.7 (2.8-14.9)	14	6.9 (4.9-12.1)	<0.001
Difference LDL-C (mmol/L)	74	-5.9 (-11.5 - +2.8)	14	-5.4 (-16.4 - -1.9)	0.97
Difference LDL-C (%)	74	-57.2 (-77.9 - +23.1)	14	-46.9 (-68.3 - -13.6)	0.061
HDL-C at study-visit (mmol/L)	75	1.3 (0.5-3.1)	14	1.2 (0.8-2.9)	0.67
LDL-C at study-visit (mmol/L)	75	2.9 (1.4-10.9)	14	4.7 (3.1-9.9)	<0.001
TG at study-visit (mmol/L)	74	0.8 (0.3-2.7)	14	1.4 (0.4-2.6)	0.017*
Non-HDL (TC - HDL-C) (mmol/L)	75	3.3 (1.6-13.3)	14	5.3 (3.9-11.1)	<0.001
TC/HDL-C	75	3.6 (2.2-9.3)	14	5.2 (2.7-12.1)	0.005
ApoA1 level at study-visit (g/L)	64	1.5 (0.9-2.3)	11	1.5 (1.2-2.2)	0.99
ApoB level at study-visit (g/L)	64	0.9 (0.5-2.6)	11	1.2 (1.0-2.8)	<0.001
ApoB/ApoA1	64	0.61 (0.33-1.44)	11	0.92 (0.55-2.12)	0.002
Lp(a) (mg/L)	54	170 (60-1890)	8	309 (24-1075)	0.77

Data are given as n (%) or median (min-max)

¹ n indicates number of individuals

² Chi-square test for independence or Fisher's exact two-tail probability test, statistically significant when *P* < 0.05

³ Post-hoc Mann Whitney U test, statistically significant when *P* < 0.05

* Statistic significant *P*-value disappears when performing Bonferroni adjustment

** Cannot compare groups on basis of incomplete information in data file

CHD = coronary heart disease; BMI = body mass index; BP = blood pressure; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol;

TG = triglycerides; ApoA1 = apolipoprotein A1; ApoB = apolipoprotein B; Lp(a) = lipoprotein(a)

4.2 Case-control Study

4.2.1 Susceptible, resistant and controls

Characterisation of the subjects and comparison of the groups is shown in **tables 17a and 17b**, respectively.

FH subjects and controls

First, we compared the group of FH subjects with the group of controls. Median Lp(a) level was significantly higher in FH subjects compared with controls. The group of FH subjects also tended to have higher median ApoB level and ApoB/ApoA1 ratio. Interestingly, the FH subjects also had a higher median percent of both vWF activity and vWF antigen than that of the controls. The same was the case with Protein S level, both free and total, Protein C level and fibrinogen level. However, all coagulation parameter values and the fibrinogen level were within reference range.

Furthermore, in comparison with the control group, the group of FH subjects had significantly higher median blood glucose level, median BMI and median waist circumference. In addition, a greater number of individuals in the FH group had a regularly intake of omega-3 supplement. Furthermore, in comparison to controls a significantly greater number of FH subjects had hypertension.

Three in four FH subjects had experienced early CHD in first-degree relatives, which was a significantly larger number of people than that of the control group – where no one had early CHD in their closest family.

Not surprisingly, median ASAT level and ALAT level were statistically significant higher in the group of FH subjects, most probably due to the lipid-lowering medication.

Since the individual with CRP level of 13.00 mg/L potentially could affect the result, the CRP *P*-value was estimated without this individual. The difference was still non-significant, with $P = 0.25$ (not shown in table).

Susceptible and resistant groups

Second, we compared the groups of susceptible FH subjects with resistant FH subjects. There were unexpectedly few differences between the group of susceptible and the group of resistant individuals. The susceptible group tended to have higher median Lp(a) value than the resistant group.

A greater number of individuals in the susceptible group were medicated with β -blockers compared to the resistant group.

There was a trend towards lower BMI in susceptible FH subjects in comparison with resistant FH subjects.

As expected, the median age at first CHD event in the susceptible group was lower than that of the resistant group. Furthermore, the susceptible group had a lower median age at study-visit. However, the resistant group in the latter comparison only consisted of three individuals.

Susceptible and controls

Third, we compared the group of susceptible with the control group. Susceptible FH subjects had statistically significant higher median Lp(a) level and tended to have higher median ApoB level compared to controls.

Furthermore, the susceptible group had significantly higher median percentage of vWF antigen and vWF activity and median level of free and total Protein S in comparison with the control group. However, all parameters were within the range of reference and the vWF antigen difference disappeared with Bonferroni correction.

A significantly greater number of individuals in the susceptible group had early CHD in first-degree relatives in comparison with the controls. What is more, a greater number of susceptible individuals had hypertension.

Not surprisingly, median level of ASAT and ALAT were significantly higher in susceptible patients.

Significantly more susceptible FH subjects in comparison with controls had a regularly intake of omega-3 supplement. In addition, the median waist circumference of susceptible was greater than that of controls, however when performing Bonferroni correction the statistically significant *P*-value disappeared. The susceptible also tended to have higher median fibrinogen level, median blood glucose level and median BMI.

Resistant and controls

Fourth, a comparison of the resistant group and the control group was accomplished. As registered in susceptible compared with controls, resistant also had significantly higher median percentage of vWF antigen, vWF activity and median level of free and total Protein S. The statistically significant difference between the resistant group and the control group disappeared with Bonferroni correction.

Furthermore, resistant FH subjects had significantly higher median BMI and waist circumference in comparison with control subjects, in addition to higher median blood glucose level. In addition, a greater number of resistant individuals had omega-3 intake on a regularly basis. However, with Bonferroni correction the significant difference in blood glucose disappeared.

The resistant group had significantly higher median fibrinogen level compared to the control group, but the difference disappeared when performing Bonferroni correction on the *P*-value.

A significantly greater number of individuals in the resistant group had early CHD in first-degree relatives in comparison with the controls.

As expected, the group of resistant FH subjects had higher median ASAT and ALAT levels. With Bonferroni correction, the significance of the difference between the groups in ASAT disappeared.

There were found no other differences in blood lipid levels.

Table 17a. Characterisation and comparison of susceptible, resistant and controls

			FH subjects					
	FH subjects	Controls	<i>P</i> ¹	Susceptible	Resistant	<i>P</i> ²	<i>P</i> ³	<i>P</i> ⁴
	(n = 34)	(n = 10)		(n = 19)	(n = 15)			
Demographics								
Female	12 (35.3%)	4 (40.0%)	1.00	7 (36.8%)	5 (33.3%)	1.00	1.00	1.00
Age at study-visit	59 (42-77)	60 (42-74)	0.77	56 (72-43)	62 (55-77)	0.033*	0.63	0.24
Age at first CHD event	40 (28-63)**	-	-	39 (28-48)	62 (59-63)**	0.006	-	-
Early CHD in first-degree relative	25 (73.5%)	0 (0.0%)	<0.001	14 (73.7%)	11 (73.3%)	1.00	<0.001	0.001
Classical risk factors								
Smoker (now)	3 (8.8%)	1 (10.0%)	1.00	1 (5.3%)	2 (13.3%)	0.57	1.00	1.00
Smoker (pre and/or now)	23 (67.6%)	4 (40.0%)	0.15	12 (63.2%)	11 (73.3%)	0.71	0.27	0.12
Hypertension	16 (47.1%)	0 (0.0%)	0.007	10 (52.6%)	6 (40.0%)	0.51	0.005	0.051
Diabetes type II	2 (5.9%)	0 (0.0%)	1.00	0 (0.0%)	2 (13.3%)	0.19	1.00	0.50
Drug treatment								
Statin	33 (97.1%)	-	-	19 (100.0%)	14 (93.3%)	0.44	-	-
Resin	13 (38.2%)	-	-	8 (42.1%)	5 (33.3%)	0.73	-	-
Niacin	4 (11.8%)	-	-	4 (21.1%)	0 (0.0%)	0.11	-	-
Ezetimibe	32 (94.1%)	-	-	18 (94.7%)	14 (93.3%)	1.00	-	-
Albyl E	27 (79.4%)	-	-	17 (89.5%)	10 (66.7%)	0.20	-	-
B-blockers	12 (35.3%)	-	-	11 (57.9%)	1 (6.7%)	0.003	-	-
Diet parameters								
Omega-3 supplement	29 (85.3%)	4 (40.0%)	0.008	16 (84.2%)	13 (86.7%)	1.00	0.032	0.028
SmartDiet score (out of total 41)	35 (29-41)	35 (26-37)	0.35	33 (29-41)	36 (29-40)	0.26	0.60	0.23
Physical examination								
BMI (kg/m ²)	27.1 (18.2-37.8)	23.3 (20.3-25.8)	0.003	26.6 (18.2-36.9)	27.9 (22.8-37.8)	0.069	0.054	<0.001
Waist circumference (cm)	98 (65-117)	85 (70-95)	0.001	95 (65-117)	98 (88-116)	0.22	0.022*	<0.001
Systolic BP (mmHg)	135 (106-136)	125 (113-150)	0.077	132 (106-164)	138 (109-176)	0.41	0.12	0.10
Diastolic BP (mmHg)	86 (56-109)	81 (62-95)	0.45	85 (56-104)	87 (71-109)	0.45	0.63	0.36

Data are given as n (%) or median (min-max), n indicates number of individuals

Post-hoc Mann Whitney U test, Chi-square test for independence or Fisher's exact two-tail probability test, statistically significant when $P < 0.05$, were used

¹ between FH subjects and controls

² between susceptible and resistant

³ between susceptible and controls

⁴ between resistant and controls

* Statistic significant P -value disappears when performing Bonferroni adjustment

** n = 22 among FH subjects / n = 3 among resistant due to only 3 incidents of CHD

CHD = coronary heart disease; BP = blood pressure; BMI = body mass index

Table 17b. Characterisation and comparison of susceptible, resistant and controls

	FH subjects							
	FH subjects	Controls	<i>P</i> ¹	Susceptible	Resistant	<i>P</i> ²	<i>P</i> ³	<i>P</i> ⁴
	(n = 34)	(n = 10)		(n = 19)	(n = 15)			
Laboratory parameters								
Blood lipid parameters								
TC at study-visit (mmol/L)	4.9 (3.0-11.5)	4.9 (4.1-7.0)	0.81	5.3 (3.0-8.4)	4.5 (3.8-11.5)	0.97	0.96	0.60
HDL-C at study-visit (mmol/L)	1.4 (0.7-3.2)	1.7 (0.9-2.7)	0.35	1.3 (0.7-3.2)	1.5 (0.9-2.0)	0.20	0.29	0.56
LDL-C at study-visit (mmol/L)	3.2 (1.5-9.0)	2.9 (1.1-5.4)	0.79	3.2 (1.7-5.2)	3.2 (1.5-9.0)	0.59	0.80	0.82
Estimated LDL-C pre-treatment (mmol/L)	9.0 (3.7-16.7)	-	-	9.1 (4.3-16.7)	8.6 (3.7-14.6)	0.40	-	-
Difference LDL-C (mmol/L)	-6.4 (-14.1 - +0.2)	-	-	-6.4 (-14.1 - +0.2)	-4.0 (-10.0 - -0.5)	0.40	-	-
Difference LDL-C (%)	-67.2 (-84.4 - +4.7)	-	-	-68.6 (-84.4 - +4.7)	-61.1 (82.6 - -11.1)	0.32	-	-
TG at study-visit (mmol/L)	0.8 (0.3-3.7)	0.9 (0.5-2.5)	0.90	0.8 (0.4-3.7)	0.8 (0.3-3.4)	0.77	0.80	0.96
Non-HDL (TC-HDL-C) (mmol/L)	3.5 (1.8-10.5)	3.3 (1.4-6.1)	0.71	4.0 (1.8-6.1)	3.4 (2.2-10.5)	0.51	0.51	0.96
TC/HDL-C	3.4 (2.2-11.5)	3.0 (1.5-7.8)	0.41	3.7 (2.3-6.5)	3.3 (2.2-11.5)	0.44	0.27	0.76
ApoA1 level at study-visit (g/L)	1.5 (0.9-2.4)	1.6 (1.0-2.2)	0.66	1.5 (0.9-2.4)	1.6 (1.0-2.1)	0.47	0.55	0.89
ApoB level at study-visit (g/L)	1.0 (0.5-2.4)	0.8 (0.3-2.4)	0.066	1.0 (0.5-1.4)	1.0 (0.6-2.4)	0.79	0.082	0.12
ApoB/ApoA1	0.63 (0.37-2.00)	0.48 (0.15-1.17)	0.085	0.65 (0.37-1.08)	0.63 (0.41-2.00)	0.70	0.10	0.15
Lp(a) (mg/L)	585 (60-2460)**	172 (85-913)	0.013	1210 (60-2460)**	434 (60-1890)	0.096	0.005	0.13
Coagulation parameters								
APC resistance	1.06 (0.63-1.15)***	1.07 (0.99-1.11)	0.23	1.06 (0.89-1.10)***	1.05 (0.63-1.15)	0.37	0.10	0.64
Protein S (free) (%)	88 (62-105)***	75 (63-83)	0.003	89 (65-105)***	87 (62-105)	0.68	0.011	0.005
Protein S (total) (%)	99 (85-105)***	86 (73-105)	0.001	93 (85-105)***	103 (86-105)	0.091	0.009	0.002
Protein C (%)	128 (87-154)***	107 (82-134)	0.049	128 (87-154)***	128 (87-144)	0.84	0.92	0.067
vWF antigen (%)	155 (61-243)***	117 (65-151)	0.011	156 (81-243)***	153 (61-236)	0.91	0.025*	0.021*
vWF activity (%)	131 (61-266)***	95 (61-120)	0.001	157 (81-243)***	129 (61-211)	0.64	0.003	0.006
Other laboratory parameters								
Fasting blood glucose (mmol/L)	5.5 (4.5-8.2)	5.0 (4.4-6.3)	0.031	5.5 (4.5-6.3)	5.8 (4.7-8.2)	0.36	0.069	0.037*
Creatinine (µmol/L)	74 (48-99)	65 (54-89)	0.21	74 (55-99)	74 (48-94)	0.96	0.22	0.33
ASAT (U/L)	34 (19-85)	24 (16-44)	0.002	37 (24-85)	32 (19-58)	0.18	0.001	0.019*
ALAT (U/L)	37 (13-136)	22 (13-36)	0.001	43 (13-136)	33 (20-67)	0.092	0.001	0.007
Fibrinogen (g/L)	3.4 (2.4-5.7)	3.1 (2.6-3.5)	0.026	3.4 (2.4-4.4)	3.5 (2.5-5.7)	0.18	0.089	0.017*
CRP (mg/L)	0.6 (0.6-13.0)	0.6 (0.6-1.8)	0.20	0.60 (0.60-2.00)	0.77 (0.60-13.00)	0.15	0.42	0.11

Data are given as n (%) or median (min-max), n indicates number of individuals

Post-hoc Mann Whitney U test, Chi-square test for independence or Fisher's exact two-tail probability test, statistically significant when $P < 0.05$, were used

¹ between FH subjects and controls

² between susceptible and resistant

³ between susceptible and controls

⁴ between resistant and controls

* Statistic significant P -value disappears when performing Bonferroni adjustment

** n = 33 among FH subjects / n = 18 among susceptible due to one missing value

*** n = 32 among FH subjects / n = 17 among susceptible due to exclusion of two patients on Warfarin treatment

TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; TG = triglycerides; ApoA1/B = apolipoprotein A1/B;

Lp(a) = lipoprotein(a); APC resistance = Activated protein C-resistance; vWF = von Willebrand Factor; ASAT/ALAT = aspartate/alanine aminotransferase; CRP = C-reactive protein

4.2.2 Female and male

As shown in **table 18** there were unexpectedly few differences between the group of female FH subjects and the group of male FH subjects in the case-control study.

The female group had significantly higher estimated median pre-treatment LDL-C level than what was estimated in the male group. However, there were no differences between the groups on other blood lipid parameters.

A lower median creatinine concentration was registered in the group of FH women in comparison with the group of FH men.

The number of female FH subjects with hypertension was significantly greater in comparison with then number of hypertensive male FH subjects. However, there was no difference in use of anti-hypertensive drugs.

A lower number of female FH subjects tended towards using Albyl E compared to the male FH subjects.

Table 18. Characterisation and comparison of female and male

	Female	Male	<i>P</i> ¹
	(n = 12)	(n = 22)	
Demographics			
Age at study-visit	64 (42-73)	58 (44-77)	0.083
Age at first CHD event	39 (30-48)*	40 (28-63)**	0.92
Early CHD in first-degree relative	10 (83.3%)	15 (68.2%)	0.44
Classical CHD risk factors			
Smoker (now)	0 (0.0%)	3 (13.3%)	0.54
Smoker (pre and/or now)	7 (58.3%)	16 (72.7%)	0.46
Hypertension	9 (75.0%)	7 (31.8%)	0.030
Diabetes type II	0 (0.0%)	2 (9.1%)	0.53
Drug treatment			
Statin	11 (91.7%)	22 (100.0%)	0.35
Resin	3 (25.0%)	10 (45.5%)	0.29
Niacin	0 (0.0%)	4 (18.2%)	0.27
Ezetimibe	11 (91.7%)	21 (95.5%)	1.00
Albyl E	7 (58.3%)	20 (90.9%)	0.070
B-blockers	4 (33.3%)	8 (36.4%)	1.00
Diet parameters			
Omega-3 supplement	10 (83.3%)	19 (86.4%)	1.00
SmartDiet score (out of total 41)	36 (29-39)	34 (29-41)	0.59
Physical examination			
BMI (kg/m ²)	27.9 (18.2-37.8)	27.0 (21.9-36.9)	0.68
Waist circumference (cm)	97 (65-116)	99 (81-117)	0.46
Systolic BP (mmHg)	141 (106-168)	132 (109-176)	0.35
Diastolic BP (mmHg)	79 (56-105)	87 (71-109)	0.73
Laboratory parameters			
Blood lipid parameters			
TC at study-visit (mmol/L)	5.1 (3.0-11.5)	4.7 (3.1-8.0)	0.97
HDL-C at study-visit (mmol/L)	1.5 (0.7-3.2)	1.4 (0.9-2.2)	0.91
LDL-C at study-visit (mmol/L)	3.0 (1.9-9.0)	3.3 (1.5-4.9)	0.93
Estimated LDL-C pre-treatment (mmol/L)	10.8 (5.9-16.7)	7.9 (3.7-15.8)	0.042
Difference LDL-C (mmol/L)	-6.7 (-14.1 - -2.5)	-5.1 (-12.1 - +0.2)	0.21
Difference LDL-C (%)	-69.8 (-84.4 - -21.2)	-61.9 (-82.6 - +4.7)	0.33
TG at study-visit (mmol/L)	0.8 (0.3-3.2)	0.8 (0.3-3.7)	0.61
Non-HDL (TC - HDL-C) (mmol/L)	3.5 (1.8-10.5)	3.5 (1.9-6.4)	0.90
TC/HDL-C	3.6 (2.3-11.5)	3.4 (2.2-6.2)	0.90
ApoA1 level at study-visit (g/L)	1.5 (0.9-2.4)	1.5 (1.0-2.1)	0.86
ApoB level at study-visit (g/L)	1.0 (0.7-2.4)	1.0 (0.5-1.6)	0.65
ApoB/ApoA1	0.63 (0.46-2.00)	0.62 (0.37-0.92)	0.72
Lp(a) (mg/L)	429 (60-1770)***	786 (60-2460)	0.35
Coagulation parameters			
APC-resistance	1.04 (0.96-1.15)	1.06 (0.63-1.15)	0.27
Protein S (free) (%)	82 (65-105)	90 (62-105)	0.11
Protein S (total) (%)	98 (85-105)	100 (86-105)	0.45
Protein C (%)	128 (94-144)	128 (87-154)	0.37
vWF antigen (%)	155 (93-243)	154 (63-232)	0.53
vWF activity (%)	145 (93-266)	125 (61-237)	0.51
Other laboratory parameters			
Fasting blood glucose (mmol/L)	5.3 (4.5-6.5)	5.6 (4.5-8.2)	0.22
Creatinine (µmol/L)	64 (48-90)	77 (63-99)	0.017
ASAT (U/L)	35 (13-136)	43 (20-81)	0.090
ALAT (U/L)	30 (24-85)	37 (19-58)	0.43
Fibrinogen (g/L)	3.4 (2.4-4.7)	3.4 (2.5-5.7)	0.80
CRP (mg/L)	0.60 (0.60-13.00)	0.60 (0.60-3.7)	0.83

Data are given as n (%) or median (min-max), n indicates number of individuals

¹ Mann Whitney U test, Chi-square test for independence or Fisher's exact two-tail probability test, significant when $P < 0.05$

* n = 7 among female, ** n = 15 among male, *** n = 11 among female

CHD = coronary heart disease; BMI = body mass index; BP = blood pressure; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; TG = triglycerides; ApoA1 = apolipoprotein A1; ApoB = apolipoprotein B; Lp(a) = lipoprotein(a); APC-resistance = Activated protein C-resistance; vWF = von Willebrand Factor; ASAT/ALAT = aspartate/alanine aminotransferase; CRP = C-reactive protein

4.2.3 Female and male subdivided into groups of susceptible and resistant

Susceptible female and resistant female

Characterisation of the subjects and comparison of the susceptible female group and the resistant female group is shown in **tables 19a and 19b**, respectively.

The group of susceptible women had significantly lower median Protein S level, both free and total, in comparison with the group of resistant women. However, all values were within the range of reference.

The susceptible female group had statistically significant lower median CRP level in comparison with the resistant group. However, the median values both were within reference range. Since there was one individual with a CRP level of 13.00 mg/L, which was considerably higher than the others in the resistant group, we also performed the analysis without the individual. The median value then was 0.88 mg/L and the range was 0.60 – 1.80 mmol/L, and there still was a trend towards the resistant female group having higher CRP median level than the susceptible female group with a $P = 0.080$.

A greater number of susceptible women were medicated with Albyl E in comparison with resistant women, and a greater number of susceptible women also tended to use β -blockers.

The group of resistant women had a median BMI of 30.3 kg/m², which was statistically significant higher than the median BMI in the group of susceptible women, which was 25.3 kg/m².

Interestingly, there were found no differences in blood lipid levels.

Susceptible male and resistant male

As shown in **tables 19a and 19b**, respectively, there were few differences between the group of susceptible men and the group of resistant men.

Susceptible men had significantly higher median level of free Protein S in comparison with resistant men. However, the median level in both groups is within reference range.

A statistically significant greater number of susceptible men received β -blocker medication in comparison with the number of resistant men. In addition, there was a trend towards a greater number of susceptible men receiving niacin compared to resistant men.

Not surprisingly, the susceptible male group were significantly younger than the resistant male group considering median age at first CHD event.

No differences in blood lipid levels were found.

Table 19a. Characterisation and comparison of female susceptible and resistant and male susceptible and resistant

	Female		<i>P</i> ¹	Male		<i>P</i> ²
	Susceptible	Resistant		Susceptible	Resistant	
	(n = 7)	(n = 5)		(n = 12)	(n = 10)	
Demographics						
Age at study-visit	57 (42-73)	67 (62-69)	0.29	53 (44-70)	59 (55-77)	0.074
Age at first CHD event	39 (30-48)	-*	-	39 (28-43)	62 (59-63)**	0.009
Early CHD in first-degree relative	7 (100.0%)	3 (60.0%)	0.15	7 (58.3%)	8 (80.0%)	0.38
Classical risk factors						
Smoker (now)	0 (0.0%)	0 (0.0%)	1.00	1 (8.3%)	2 (20.0%)	0.57
Smoker (pre and/or now)	3 (42.9%)	4 (80.0%)	0.29	9 (75.0%)	7 (70.0%)	1.00
Hypertension	6 (85.7%)	3 (60.0%)	0.52	4 (33.3%)	3 (30.0%)	1.00
Diabetes type II	0 (0.0%)	0 (0.0%)	1.00	0 (0.0%)	2 (20.0%)	0.20
Drug treatment						
Statin	7 (100.0%)	4 (80.0%)	0.42	12 (100.0%)	10 (100.0%)	1.00
Resin	2 (28.6%)	1 (20.0%)	1.00	6 (50.0%)	4 (40.0%)	0.69
Niacin	0 (0.0%)	0 (0.0%)	1.00	4 (33.3%)	0 (0.0%)	0.096
Ezetimibe	7 (100.0%)	4 (80.0%)	0.42	11 (91.7%)	10 (100.0%)	1.00
Albyl E	7 (100.0%)	0 (0.0%)	0.001	10 (83.3%)	10 (100.0%)	0.48
B-blockers	4 (57.1%)	0 (0.0%)	0.081	7 (58.3%)	1 (10.0%)	0.031
Diet parameters						
Omega-3 supplement	6 (85.7%)	4 (80.0%)	1.00	10 (83.3%)	9 (90.0%)	1.00
SmartDiet score (out of total 41)	35 (29-38)	36 (33-39)	0.51	33 (29-41)	36 (29-40)	0.45
Physical examination						
BMI (kg/m ²)	25.3 (18.2-31.8)	30.3 (27.7-37.8)	0.028	27.1 (21.9-36.9)	26.5 (22.8-31.6)	0.82
Waist circumference (cm)	93 (65-101)	101 (94-116)	0.051	100 (81-117)	98 (88-110)	0.97
Systolic BP (mmHg)	139 (106-164)	150 (122-168)	0.29	132 (120-146)	135 (109-176)	0.84
Diastolic BP (mmHg)	75 (56-104)	93 (75-105)	0.16	87 (75-91)	84 (71-109)	0.67

Data are given as n (%) or median (min-max), n indicates number of individuals

Mann Whitney U test, Chi-square test for independence or Fisher's exact two-tail probability test, statistically significant when $P < 0.05$, were used

¹ between female susceptible and female resistant

² between male susceptible and male resistant

* n = 0 in the female resistant group due to 0 CHD incidents

** n = 3 among male resistant due to only 3 incidents of CHD

CHD = coronary heart disease; BP = blood pressure; BMI = body mass index

Table 19b. Characterisation and comparison of female susceptible and resistant and male susceptible and resistant

	Female		<i>P</i> ¹	Male		<i>P</i> ²
	Susceptible	Resistant		Susceptible	Resistant	
	(n = 7)	(n = 5)		(n = 12)	(n = 10)	
Laboratory parameters						
Blood lipid parameters						
TC at study-visit (mmol/L)	4.8 (3.0-8.4)	5.7 (4.0-11.5)	0.37	5.6 (3.1-6.9)	4.5 (3.8-8.0)	0.43
HDL-C at study-visit (mmol/L)	1.3 (0.7-3.2)	1.6 (1.0-2.0)	0.19	1.3 (0.9-2.2)	1.5 (0.9-1.8)	0.55
LDL-C at study-visit (mmol/L)	2.8 (1.9-5.2)	3.5 (1.9-9.0)	0.87	3.6 (1.7-4.5)	3.0 (1.5-4.9)	0.51
Estimated LDL-C pre-treatment (mmol/L)	10.2 (7.4-16.7)	12.4 (5.9-14.6)	0.69	9.0 (4.3-15.8)	6.0 (3.7-12.6)	0.22
Difference LDL-C (mmol/L)	-6.4 (-14.5 - -2.1)	-7.0 (-10.0 - -3.1)	0.87	-6.4 (-12.1 - +0.2)	-3.2 (-8.2 - -0.5)	0.39
Difference LDL-C (%)	-68.6 (-84.4 - -32.5)	-71.0 (-76.9 - -21.2)	0.69	-69.1 (-80.7 - +4.7)	-53.3 (-82.6 - -11.1)	0.47
TG at study-visit (mmol/L)	0.7 (0.5-1.4)	1.2 (0.3-3.2)	0.46	1.0 (0.4-3.7)	0.8 (0.3-3.4)	0.29
Non-HDL (TC-HDL-C) (mmol/L)	3.3 (1.8-6.1)	3.7 (2.4-10.5)	0.69	4.3 (1.9-5.5)	3.2 (2.2-6.4)	0.28
TC/HDL-C	4.1 (2.3-6.5)	2.9 (2.5-11.5)	0.57	3.5 (2.5-6.2)	3.4 (2.2-5.0)	0.62
ApoA1 level at study-visit (g/L)	1.5 (0.9-2.4)	1.6 (1.2-1.9)	0.37	1.5 (1.1-2.0)	1.6 (1.0-2.1)	0.87
ApoB level at study-visit (g/L)	0.9 (0.7-1.4)	1.0 (0.7-2.4)	0.74	1.1 (0.5-1.3)	0.9 (0.6-1.6)	0.57
ApoB/ApoA1	0.67 (0.46-1.08)	0.63 (0.47-2.00)	0.69	0.62 (0.37-0.92)	0.65 (0.41-0.91)	0.92
Lp(a) (mg/L)	878 (204-1770)*	370 (60-606)	0.14	1260 (60-2460)	569 (60-1890)	0.28
Coagulation parameters						
APC-resistance	1.04 (0.97-1.09)	1.04 (0.96-1.15)	0.87	1.06 (0.89-1.10)**	1.07 (0.63-1.15)	0.32
Protein S (free) (%)	72 (65-89)	94 (74-105)	0.023	95 (79-105)**	85 (62-95)	0.007
Protein S (total) (%)	88 (85-99)	105 (98-105)	0.008	100 (86-105)**	100 (86-105)	0.81
Protein C (%)	117 (94-144)	137 (120-144)	0.10	129 (87-154)**	117 (87-141)	0.52
vWF antigen (%)	146 (93-243)	169 (137-236)	0.37	157 (81-232)**	142 (61-220)	0.35
vWF activity (%)	121 (93-266)	149 (110-211)	0.25	148 (89-237)**	121 (61-172)	0.15
Other laboratory parameters						
Fasting blood glucose (mmol/L)	5.2 (4.5-5.7)	5.8 (4.7-6.5)	0.33	5.6 (4.5-6.3)	5.8 (4.8-8.2)	0.87
Creatinine (µmol/L)	64 (55-90)	63 (48-83)	0.69	77 (63-99)	76 (63-94)	0.84
ASAT (U/L)	30 (24-85)	30 (25-33)	0.56	38 (29-47)	35 (19-58)	0.45
ALAT (U/L)	42 (13-136)	31 (26-38)	0.19	43 (23-81)	38 (20-67)	0.25
Fibrinogen (g/L)	3.4 (2.4-4.2)	3.5 (3.3-4.7)	0.28	3.4 (2.7-4.4)	3.6 (2.5-5.7)	0.47
CRP (mg/L)	0.60 (0.60-1.40)	0.99 (0.60-13.00)	0.037	0.62 (0.60-2.00)	0.60 (0.60-3.70)	0.83

Data are given as n (%) or median (min-max), n indicates number of individuals

Mann Whitney U test, Chi-square test for independence or Fisher's exact two-tail probability test, statistically significant when $P < 0.05$, were used

¹ between female susceptible and female resistant

² between male susceptible and male resistant

* n = 6 among female susceptible due to one missing value, ** n = 17 among male susceptible due to exclusion of two patients on Warfarin treatment

TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; TG = triglycerides; ApoA1/B = apolipoprotein A1/B;

Lp(a) = lipoprotein(a); APC-resistance = Activated protein C-resistance; vWF = von Willebrand Factor; ASAT/ALAT = aspartate/alanine aminotransferase; CRP = C-reactive protein

5. Discussion

In this thesis, two studies have been conducted; one cross-sectional study and one case-control study. Both of the studies have advantages and potential limitations, which are discussed below.

Subsequently, the principal results will be discussed and put into context of the findings in other scientific studies.

5.1 Discussion of subjects and methods

5.1.1 Retrospective data collection study

Study design

This part of the thesis was considered a cross-sectional study.

Other studies have also investigated groups of FH subjects based on CHD event, with a focus on risk factors for CHD. However, no one, as far as we know, has split the groups of interest into 'susceptible' and 'resistant' as we have. Hopkins et al (90) compared in their case-control study subjects with and without CAD and included FH subjects in the early CAD event group on basis of CAD event occurring <55 years in men and <65 years in women, respectively. FH subjects with later onset of CAD were not included in the study. In a retrospective cohort study to investigate the contribution of classical risk factors to CVD in FH, Jansen et al compared FH subjects with and without CVD, independent of when the FH subjects had their first CVD event (86). In a third similar study, a cross-sectional study by de Sauvage Nolting et al, FH subjects were not recruited on basis of CVD event. Instead, the FH subjects were split into two groups after they were recruited; one group with present or earlier CVD event and one group without any symptoms of CVD (94).

Advantages

Cross-sectional studies can be used to study several associations at once (156). All of the variables were measured at one point in time. In this study, for instance, we examined age, gender and a great number of anthropometric and biochemical measurements. The aim of our study was to compare these parameters in a group of FH subjects with early CHD event versus a group of FH subjects with late or no CHD event.

The study was relatively inexpensive in comparison to for instance cohort studies, and could be conducted over a short period of time. The participants only had to come on one study-visit and there was no follow-up period. The FH subjects were continually included as they came to polyclinic follow-up at the Lipid Clinic.

Limitations

According to Lewington et al (157), on behalf of the Prospective Studies Collaboration, the results from retrospective studies of CHD can be distorted by reverse causality. Since exposure and disease are measured at the same point of time, one cannot be sure whether an exposure preceded or followed a health outcome. Blood cholesterol can affect blood pressure; however, the influence also goes in the opposite direction. On the other hand, prospective studies in people with no previous CHD history have to be very large to assess reliability concerning which one risk factor affects the relevance of other risk factors (157). To manage the challenge of this potential bias we have included both a group with early CHD event and a group with late or no CHD event in this study.

There is a risk of response bias, where those individuals who wanted to participate in the study were systematically different from those who did not want participate. The individuals who wanted to participate could for instance have been more motivated on lifestyle changes, more concerned about health matters or more likely to follow up their drug regimen. Consequently, there was a potential risk that our results would not be representative for the entire population of FH subjects. However, to minimise this

risk the FH subjects were recruited in different ways; by invitation letters, by telephone calls or when they were at the Lipid Clinic at their regular follow-up.

This type of study does not yield incidence or relative risk. However, in this study we were not looking for other findings except differences in certain parameters between the groups.

Data assessment

Susceptible and resistant groups

One weakness of this retrospective data collection study concerning susceptible and resistant FH subjects was the missing data of variables due to incomplete assessment of data at the study-visit, for example registration of smoking history. As pointed out earlier there are several potential underlying causes for the missing data. However, the missing data was not considered a problem since we in most parameters only missed data from one to three persons in each group, which was fewer than 5 % of the total number of participants.

Deceased FH subjects

Limitations of the data on deceased FH subjects were presented in the 'subjects and methods' section.

5.1.2 Case-control study

Study design

This part of the thesis was considered a case-control study. At the same time it had similarities with the retrospective data collection study, and many of the points discussed in the retrospective data collection study were also applicable for this case-control study. With that in mind, we mainly emphasise the advantages and limitations with case-control studies in the following.

Advantages

Case-control studies also have advantages (156, 158). First, they are rapid and cost-effective to conduct, especially compared to cohort studies (156). The case-control studies require less time, money and size than cohort studies (156). In a clinical master thesis, a study must be planned, accomplished and finished in one year. Thus, if a master project is not part of a bigger study or project, the most efficient study form to choose is a case-control study or a cross-sectional study.

Second, case-control studies usually investigate less common diseases (158), in this case FH. It is generally less important to devote extraordinary resources toward confirming that control subjects are free of FH because the disorder is genetic conditioned. So, even though the controls were not randomly picked out in this study, the majority of potential controls would have been likely to be free of the disorder anyway, based on family history and incidence of CHD.

Case-control studies can be useful for evaluating multiple risk factors for a disease (158).

Limitations

Our case-control study had several potential limitations (156, 158). First, a case-control design does not provide incidence, relative risk or natural history of a disease (158) or, in this circumstance, different risk factors. We looked at a number of existing cases at a point of time. We compared two groups of cases, FH subjects with an early onset of CHD and FH subjects with late or no onset of CHD, to the same extent as we compared FH subjects with controls. Primarily, the healthy population of controls provided information on a reference level of the different parameters measured. In other words, both of the FH groups could have a higher or lower level than what was expected and also a higher or lower level than healthy controls even though the FH groups and subgroups between themselves did not differ.

Second, there is a potential risk of recall bias (156, 158). Information on among other parameters such as physical activity, food intake, alcohol intake, smoking habits and

dietary supplements like omega-3 intake, was self-reported through the SmartDiet food questionnaire. We could not control whether the participants reported what their actual intake of different foods or beverages was. When one fills out a questionnaire, it may be difficult to remember what one usually eats or drinks. The FH-subjects had through their follow-up at the Lipid Clinic received dietary advice several times before. In comparison, no one of the controls had ever received dietary advice. Hence, the SmartDiet questionnaire was familiar to the FH subjects since it is a tool the Lipid Clinic frequently uses in treatment and follow-up. The scheme was new to the controls. Also, some of the patients knew how to summarize points from the SmartDiet questionnaire to get a good score. However, in a recent thesis by Fæhn at the University of Oslo (159) it was suggested that FH subjects have a healthier diet compared with patients with multifactorial hypercholesterolemia – suggesting that the FH subjects actually may be more concerned about their health – minimising the risk of bias concerning the FH subjects' knowledge to SmartDiet.

Another example is that many of the participants reported that they had a daily intake of cod liver oil or omega-3 FAs. Since this was a “yes or no”-question in the SmartDiet questionnaire, we could not see whether they had an intake of omega-3 FAs on a regular basis or every now and then. In Norway, the tradition is to take a fish-oil or omega-3 supplement during the winter, but not necessarily during the summer months. If the study had been committed in June the frequency of omega-3 intake might have been different from what we registered in January and February. However, some of these issues could be expected to affect all participants, independent of group, to the same extent. Nevertheless, to minimise the risk of recall bias the master student read through the SmartDiet questionnaire together with the participants.

Most of the control subjects were working in a hospital or were colleagues of the master student or of persons working at the Lipid Clinic. They might have been biased concerning their level of knowledge and hence more conscious about their health than other potential controls because of their work or knowledge to our work.

When comparing controls with FH subjects, the group of susceptible FH subjects could also be more concerned about their health than controls or resistant FH subjects as a result of their early CHD history. The resistant FH subjects could as well be more concerned about their health because of their genetically increased risk of CHD.

Third, there is always a risk of interviewer bias. We minimised this factor by using standardised methods for data collection in both FH subjects and controls, with the master student collecting all the anthropometric information on the participants. The inclusion of the participants was based on questions on a form to be sure all participants were asked the same questions. On all subjects we used the same scale, the same blood pressure measurement apparatus, the same height measurement scale and the same measuring tape. The same laboratory personnel at the Lipid Clinic collected all the blood samples. At the accredited medical-biochemical laboratory at Rikshospitalet, OUS, strict routines were followed during blood sample analysis.

Fourth, inclusion restrictions of FH subjects may limit generalisation (156). In our study we found it necessary to use restriction of the two groups of FH subjects in order to reduce potential biases and to increase feasibility. We found restriction essential to ensure a valid study.

Fifth, the control subjects were selected amongst colleagues, and are not representative for the population as a whole. Sampling of controls from a general population is expensive (156) and was not a realistic alternative in this small-scale study. Furthermore, they might have been more likely to cooperate than people in the general population since they were colleagues and acquaintances. Ideally, we could have selected more than one control group to see whether the selection of controls influenced the measured associations between FH subjects and controls. However, controls were recruited from the same geographical area as the FH subjects and were age- and gender matched to the FH subjects. It was never a precondition that they were supposed to be representative on basis of a population. Intentionally, the most important feature of the control group was to have a reference population to put the characteristics of the two FH groups into perspective. All routine blood parameters

were within the normal range in the control group, suggesting it was an appropriate reference group. What applies to the recruitment of all FH subjects is that most of them contacted us after receiving an invitation letter; consequently the FH subjects probably were as much likely to cooperate as the controls.

Data assessment

We used standardised methods for data collection in all participants. Whereas some of the data were assessed or measured with high accuracy (for instance blood lipid values), others (for instance smoking history, intake of different types of food and physical activity) were based on self-reporting and therefore potentially biased. Still, to standardise the patients' answers as much as possible, one scheme with questions were used on all patients. All anthropometric measurements were done with the same scale, the same blood pressure measurement apparatus, the same height measurement scale and the same measuring tape.

The blood lipid values in FH subjects were affected by the drugs they were on, compared to controls who did not report use of any lipid-lowering medication. When comparing FH subjects with early CHD event and FH subjects with late or no CHD event, they were all on medication; hence it did not matter whether the blood lipid values were without medication or not. To get a picture of the blood lipid concentrations' influence of CHD risk we estimated the pre-treatment values of LDL-C, since LDL-C is considered one of the most potent CHD risk factor (11).

A possible bias regarding blood pressure registration was that some participants rested for a shorter time before blood pressure measurement than others. According to a report on CHD prevention from The Norwegian Directorate of Health, a person should sit still for several minutes before the blood pressure registration (23). This could result in somewhat higher blood pressure levels in some of the patients compared to their actual blood pressure level. The background for this difference was that the blood tests had to be delivered for analysis to the medical-biochemical laboratory at Rikshospitalet, OUS, within one hour after assessment. When there were

two participants on one day we had to follow a strict time management plan to deliver the blood samples within one hour. To cope with this potential bias we measured blood pressure three times and calculated the mean value for further analysis.

5.1.3 Statistics

We chose to use the non-parametric techniques Kruskal-Wallis test and Mann-Whitney U test to compare the different groups. Non-parametric techniques take into consideration outliers in a small data set using median as the middle value of the data. Hence, the outliers did not affect the analyses as much as they potentially would with parametric techniques and comparison of means. However, non-parametric tests are less powerful than the parametric tests (160). We could risk missing statistically significant P -values. To approach this potential bias Independent-samples t -test was ran in addition to the non-parametric tests to verify the tendency of statistically significant difference.

We could also have manipulated the non-parametric data into parametric data by log transforming them. There is some controversy concerning log transformation of data; the data is not raw data anymore when one uses them in further statistical analysis.

LDL-C values are usually calculated with the Friedewald formula; total cholesterol level – HDL-C level – $0.45 \cdot$ TG level (161). The formula assumes that the patients are fasting when the blood sample are retrieved. However, it is known to be less reliable as the TG concentration increases. Ideally, the TG level should not exceed 4.5 mmol/L. In the group of deceased FH subjects we do not know whether the patients fasted or not before the blood tests were assessed. On the other hand, the Lipid Clinic follows strict routines in all their analyses, and one could expect that most patients had fasted. In a recent analysis of 300,000 people with initial vascular disease, the Emerging Risk Factor Collaboration suggested that blood lipids except from TGs may be measured without the need to fast (162).

The number of participants in both the retrospective data collection study and the case-control study was acceptable in comparison to other published studies carried out on FH subjects (38, 56, 163, 164). Yet, limitations were connected to the number of participants in several of our statistical analyses. In analyses where the group of non-deceased FH subjects or group of deceased FH subjects have been subdivided according to gender or incidence of CHD event, the number of participants was too small to be representative. Particularly, this is pronounced among the deceased FH subjects where the number of subjects at some analyses, for instance Lp(a) level, included only eight individuals. Also, in the case-control study, when the groups were divided into gender, the female susceptible group and female resistant group consisted of seven and five individuals, respectively. The control group consisted of ten individuals in the first place; hence it was unsuitable for being subdivided into smaller groups. On basis of the small number of subjects in many of the analyses, potential significant results may have disappeared. However, even in some of the comparisons with a small number of persons significant results appeared which may suggest strong associations between the tested parameters and subjects.

5.2 Discussion of results

5.2.1 Blood lipid parameters

Lipoprotein (a)

In the retrospective data collection study, the median Lp(a) concentration in the susceptible group was considerably higher than the median level in the resistant group, with a level of 518 and 170 mg/L, respectively. In the case-control study there was a trend towards the susceptible FH subjects having higher median levels of Lp(a) in comparison with resistant FH subjects with a median of 1210 and 434 mg/L, respectively – however it was not statistically significant probably due to the small number of participants. In neither of our studies a significant difference was seen

when dividing the group of FH subjects into groups of female and male. However, the subgroup of susceptible women had significantly higher median Lp(a) level compared with resistant women, suggesting that the level of Lp(a) in susceptible women account for the difference registered between the susceptible and the resistant groups when not divided by gender.

Our results on the differences between the susceptible and resistant groups are consistent with other findings. In a study of 115 FH subjects with and without CHD by Seed et al (40), higher Lp(a) levels were reported in patients with FH with CHD in comparison with those with no visible complications, with Lp(a) levels of 570 and 180 mg/L, respectively. The same association was also seen in the study by Jansen et al (86). However, no difference was seen between FH subjects with and without CHD in two other studies (90, 94). de Sauvage Nolting et al (94) found a non-significantly higher Lp(a) concentration in men in comparison with women, which also is consistent with our findings. In a recent cross-sectional study of 811 FH subjects, Alonso et al (24) found no differences between female and male FH subjects. However, neither of the studies compared FH men and FH women divided into subgroups of susceptible and resistant FH subjects.

In the case-control study, the FH subjects had significantly higher median level of Lp(a) in comparison with controls, with a Lp(a) level of 585 and 172 mg/L, respectively. In a Greek case-control study of 82 FH subjects and 82 controls, Elisaf et al found a similar association (164).

According to a recent review by Chapman et al (110), niacin is the only commercially available drug known to specifically reduce circulating Lp(a) levels; it can lower Lp(a) by up to 30 %. Other studies have found similar associations (97, 165, 166). In our retrospective data collection study, a significantly higher number of susceptible FH subjects received niacin treatment in comparison to resistant FH subjects, and the same was the case with FH men compared to FH women. The niacin medication may have contributed to a lower median level than what was really the case in susceptible

and male FH subjects, contributing to a non-significantly higher Lp(a) in the latter in comparison with female FH subjects.

In a recent meta-analysis of 36 studies by Insull et al (166), associations of Lp(a) with CHD was found independent of level of non-HDL-C. Accordingly, when Lp(a) is elevated it is even more important to reduce other potential or manifested CHD risk factors.

Our results are supported by a current study examining genetic data from three different trials from Kamstrup and coworkers who suggested that patients must systematically be screened for their Lp(a) levels (167). They further suggested that every time the concentration of Lp(a) is doubled the cardiovascular risk is increased by approximately 20 % (167).

Total cholesterol, LDL, HDL and triglycerides

Total cholesterol

When dividing the group of FH subjects in the retrospective data collection study into groups based on gender, the FH women had significantly higher median level of total cholesterol compared to FH men, with 5.0 mmol/L and 4.7 mmol/L, respectively. The same tendency was seen in female susceptible versus female resistant and in female susceptible versus male susceptible. In the case-control study, the susceptible group and the resistant group had a median total cholesterol level of 5.3 and 4.5 mmol/L, respectively. The deceased FH subjects, both in the group of deceased FH subjects with early CHD and the group of deceased FH subjects with late or no CHD, the median total cholesterol level was significantly higher than in the non-deceased FH subjects, with levels of 7.8 mmol/L and 6.9 mmol/L in the two groups of deceased FH subjects, respectively. There was not a statistically significant difference between-group in the deceased FH subjects or between the susceptible and resistant groups. According to the European guidelines on cardiovascular disease prevention in clinical practice and as shown in **table 2**, the recommendations for subjects at high risk for CHD, including FH subjects, is <4.5 mmol/L (54, 55). An interpretation of the results

from our studies in comparison with the recommendations is that many of the subjects in our study do not reach the total cholesterol treatment goals. The deceased FH subjects had significantly higher cholesterol levels than the non-deceased FH subjects, suggesting that the medical treatment nowadays lowers cholesterol levels more effectively than the treatment that was offered ten years ago. Hopkins et al conducted their case-control study of 262 FH subjects in 2001, and, interestingly, the mean total cholesterol level in cases (FH subjects with premature CAD) and controls (FH subjects without CAD) were 6.7 and 7.7 mmol/L, respectively (90), which is in accordance with our results from the deceased population. The total cholesterol levels in Hopkins' study were measured at the approximately same time as the median time of deceased FH subjects' death, confirming our theory on improvement of total cholesterol levels with newer medication. In other studies similar to ours, FH subjects had had a six weeks wash-out period before their cholesterol levels were measured; hence, the total cholesterol values are not comparable (86, 94).

Interestingly, in the retrospective data collection study, the susceptible group had significantly higher median pre-treatment total cholesterol than that of the resistant group, with 11.7 and 10.4 mmol/L, respectively. However, there was not a pronounced difference between FH men and FH women nor between deceased FH subjects and non-deceased FH subjects. In Jansen's cohort study of 2,400 FH subjects and in de Sauvage Nolting's cross-sectional study on 526 FH subjects where all participants had had wash-out periods (86, 94), the total cholesterol levels are in line with our findings.

LDL cholesterol

Regarding median LDL-C level, the same pattern as observed for total cholesterol was seen in both the retrospective data collection study and the case-control study. This is consistent with similar studies (86, 94, 168). One of the most interesting findings in our two studies was that the median estimated pre-treatment LDL-C level in the case-control study was significantly different between female FH subjects and male FH subjects; a median level of 10.8 mmol/L and 7.9 mmol/L was registered in

women and men, respectively. Since LDL-C is considered a powerful atherogenic lipoprotein (11) and treatment goals often consider LDL-C levels in preference to total cholesterol levels (23, 54, 55), we estimated the FH subjects' pre-treatment LDL-C levels on basis of their dose of statins and the presence of other lipid-lowering drugs. The LDL-C lowering effects of the different statins were found from several trials and meta-analyses (147-151). However, the estimation of pre-treatment LDL-C levels did not take into consideration between-subjects differences. Some persons could react better to statin and/or combination treatment than others (35, 169).

In the other trials studying pre-treatment LDL-C, no significant difference between FH subjects with and without CHD has been found (86, 94, 168).

Triglycerides and HDL cholesterol

According to the European guidelines on cardiovascular disease prevention in clinical practice, the recommended treatment goal for TG levels is <1.7 mmol/L (54, 55). The FH subjects in both our studies had a median TG level at study-visit below 1.0 mmol/L, except from the deceased FH subjects where the median TG level was 1.2 mmol/L and 1.4 mmol/L in the groups of deceased FH subjects with early CHD event and deceased FH subjects with late or no CHD event, respectively.

The observed levels of TGs in FH subjects were higher than what was observed in the study by Hopkins et al (90), with 1.9 mmol/L and 1.8 mmol/L in FH subjects with early onset of CAD and with no clinical history of CAD, respectively. However, our results are consistent with theirs regarding the observation that there was no differences between the groups. In a cohort study of 1,185 FH patients, the Scientific Steering Committee on behalf of the Simon Broome Register Group (64) registered TG levels in men and women more in line with ours, with a mean TG level of 1.4 and 1.2 mmol/L, respectively; however, no comparison test was run. On the other hand, both de Sauvage Nolting et al (94) and Jansen et al (86) found significantly higher TG levels in FH with CVD compared with FH without CVD. However, the differences seen in de Sauvage Nolting and Jansen's studies are based on pre-treatment values.

Niacin and resins are more potent drugs than statins concerning lowering TGs (170, 171), and more susceptible FH subjects than resistant FH subjects and more male FH subjects than female FH subjects received niacin in our study. Nevertheless, TG levels were reported to be normal in FH subjects (30, 44).

The European guidelines on cardiovascular disease prevention in clinical practice recommends the HDL-C levels in high-risk individuals to be >1.0 mmol/L and >1.2 mmol/L in men and women, respectively (54, 55). Our findings are in line with these recommendations and the FH subjects disregarding of subgroups both in the retrospective data collection study and in the case-control study had a higher median HDL-C level than the minimum recommended values. However, the median HDL-C level in deceased FH subjects was lower than in the non-deceased FH subjects, with a median concentration of 1.0 mmol/L and 1.2 mmol/L in the groups of deceased FH subjects with early CHD event and deceased FH subjects with late or no CHD event, respectively. As expected, FH women had significantly higher median HDL-C level in comparison with FH men in our retrospective data collection study; however, there was no significant difference in our case-control study. A possible interpretation of the results from our study could be that there are differences between the groups of deceased and non-deceased FH subjects due to the HDL-C increasing effect of supplemental medication (71, 170, 171).

The three other studies comparing subjects with and without CHD found a significantly higher mean level of HDL-C in FH subjects with no CHD in comparison with FH subjects with CHD (86, 90, 94). Our findings are inconsistent with those findings, perhaps due to the small number of subjects in our studies.

Other blood lipid parameters

ApoA1, ApoB and ApoB/ApoA1 ratio

The same pattern as observed for LDL-C and HDL-C was seen in both the retrospective data collection study and the case-control study. However, even though FH women tended to have higher total cholesterol and LDL-C levels in the

retrospective data collection study, their median ApoB/ApoA1 ratio was significantly higher than that of FH men, probably due to their increased HDL-C levels. A recent prospective case-control study by van der Steeg et al suggested that the ApoB/ApoA1 ratio adds little to the existing measures for CHD risk (172). On the contrary, Rasouli et al suggested in a recent case-control study that the ApoB/ApoA1 ratio was suitable for use in clinical practices (173). However, as of today little is known regarding the efficacy of using the ratio in FH subjects.

Non-HDL and total cholesterol/HDL-C ratio

For treatment of patients with, or at risk of CHD, some guidelines focus primarily on total cholesterol levels and/or LDL-C levels (170). However, in both European guidelines on cardiovascular disease prevention in clinical practice and NCEP ATP III other parameters also are taken into consideration, among others non-HDL-C, respectively (11, 54, 55). Non-HDL-C and total cholesterol/HDL-C ratio are useful tools in predicting the dyslipidemic state of individuals and persons at a high risk for development of CHD (11, 174). As NCEP ATP III suggests, the non-HDL is recommended as a secondary target for persons with TG levels ≥ 2.3 mmol/L, and is hence not applicable for our studies since most of the FH subjects had TGs below the cut-off (11). A 2002 review by Hirsch et al suggested that non-HDL in high-risk subjects should be < 3.4 mmol/L (175). Most of our findings are close to that value. The Norwegian Directorate of Health suggests total cholesterol/HDL-C should be ≤ 5.0 (23). In all groups and subgroups in both our studies the median was below the cut-off, except from the deceased FH subjects. Our findings suggest that, even though the ratios have been advocated by many (174-178), it may not be the best predictor of risk in FH subjects.

5.2.2 Lipid-lowering treatment treatment

Plasma lipid levels are a major modifiable risk factor for CHD (78, 179). Several primary and secondary prevention trials have demonstrated that lowering cholesterol levels contribute to reduce the risk of CHD events (11, 52, 61, 65, 109, 180-183).

Lipid-lowering treatment in our two studies

Nearly all of the patients in the studies of this thesis received statin treatment, both in the retrospective data collection study and in the case-control study. However, there were several differences between different subgroups considering the duration of lipid-lowering treatment and statin treatment and supplemental medication with other drugs in addition to statins.

In the retrospective data collection study a significantly greater number of susceptible FH subjects received resin and niacin treatment in comparison with resistant FH subjects. This difference was not pronounced in the case-control study. Yet, that could be due to the low number of participants. However, when the retrospective data collection study group of FH subjects was split into groups of FH men and FH women, several differences appeared. A significantly smaller number of FH women received statins, resins and niacin compared to FH men. At the same time, the median duration of lipid-lowering treatment and statin treatment was longer in the susceptible female group in comparison with both the resistant female group and the susceptible male group. Consequently, there is a tendency towards that FH women, and especially women with early CHD event, do not reach target levels on lipid parameters even though they are under treatment for a long time. In addition, it seems that men are more aggressively treated with medication than women – maybe due to the inequality in focus on CHD between men and women. Nonetheless, an interpretation of our findings could be that women do not receive optimal lipid-lowering treatment compared to men.

The fact that female FH subjects in general had a less beneficial lipid profile than the male FH subjects is noteworthy, especially as it is known that oestrogen upregulates the LDL-R which should contribute to lower LDL-C levels (184) in addition to prescribed medication.

In the retrospective data collection study, there was no difference in medical treatment between the deceased FH subjects with early CHD event and the deceased FH subjects with late or no CHD event. Furthermore, there was no difference in number

of persons receiving statin treatment between deceased FH subjects and non-deceased FH subjects. However, in comparison with the non-deceased FH subjects, a significantly smaller number of deceased FH subjects with early CHD event received ezetimibe than susceptible FH subjects and a smaller number of deceased FH subjects with late or no CHD event received ezetimibe and resins than resistant FH subjects. This difference in medication between the groups may suggest that supplemental medication treatment in fact contribute to less fatal events among FH subjects.

The median duration of lipid-lowering treatment and statin treatment was significantly longer in the susceptible and resistant groups (18.0 and 16.0 years, respectively) in comparison with the groups of deceased FH subjects with early CHD and deceased FH subjects with late or no CHD (9.5 and 7.5 years, respectively), respectively. When taking into consideration the time difference between the median time of death in the deceased FH subjects and the median time of study-visit in the non-deceased FH subjects, the time difference of ten years is reflected in the difference in duration of medication. This indicates that most FH subjects received statin treatment as soon as it became available on the market. However, we do not know if there was a difference in health and CHD risk between deceased and non-deceased FH subjects when they started on statin treatment.

Statins are shown to reduce both xanthomas (21, 74) and xanthelasms (185). A significantly higher number of deceased FH subjects had xanthomas compared to the non-deceased FH subjects. Due to the difference between the two groups regarding dose and duration of statin treatment, the difference in the number of subjects with xanthelasms in the two groups was not a surprise.

According to Nicholls et al (150) in an individual patient data pooled analysis of 32,258 subjects at CHD risk, the more elevated the baseline lipid levels were the fewer patients reached their treatment goals with statins. In our retrospective study, both the susceptible and resistant groups were quite similar treated with drugs, with the differences mentioned above, but the susceptible group did not reach the same cholesterol level that the resistant group did, even though the susceptible FH subjects

were more intensively treated with supplementary medication than the resistant FH subjects. Interestingly, the susceptible group had significantly higher median total cholesterol level in comparison with the resistant group – in accordance with Nicholls' findings.

Does the treatment of FH patients reach treatment goals?

As discussed above, the medical treatment in our studies' FH subjects does not seem to cause the FH subjects to reach the treatment goals. Nevertheless, the combination treatment today lowers blood lipids more effectively than what the available medication did ten years ago.

According to Ballantyne (53) there are two major factors that prevent effective treatment of FH. The first obstacle is insufficient screening for FH in people who may be at increased risk. The other is the failure of common lipid-lowering therapies to achieve treatment goals like adequate total cholesterol or LDL-C levels in accordance with CHD ten-year risk level calculated according to the presence of CHD event(s) and/or clinical or subclinical atherosclerosis (11, 53, 65). Additionally, Frolkis et al (186) suggest that the use of statins in clinical practices lead to observed reductions in LDL-C levels that are significantly less than those projected in other clinical trials. The difference between clinical trials in controlled situations and clinical practices can be due to reduced patient compliance in the latter (186).

The British National Institute for Health and Clinical Excellence (NICE) guidelines suggest that treatment should start with a high intensity statin (such as simvastatin 80 mg or appropriate doses of atorvastatin and rosuvastatin) to achieve a >50% reduction in LDL-C concentrations, increasing to the maximum tolerated dose if necessary (79).

In our case-control study, the median level of LDL-C at study-visit in the group of FH subjects was 3.2 mmol/L. Thirty out of 34 FH subjects received a maximum dose of rosuvastatin or atorvastatin. In addition, use of ezetimibe was registered in 91.4 % of the persons with FH. Resins and niacin was registered in 38.2 % and 11.8 % of the FH subjects, respectively. The estimated median level of pre-treatment LDL-C in the

group of FH subjects was 9.0 mmol/L. That resulted in an estimated median reduction of LDL-C of 67.2 %. Yet, with both statin treatment and supplemental medication, the group of FH subjects still did not reach the treatment goal recommended by the of European guidelines on cardiovascular disease prevention in clinical practice and the Norwegian Directorate of Health, with a LDL-C level below 2.5 mmol/L – or if feasible below 2.0 mmol/L (23, 54, 55). Our results are in accordance with the results in a study from the Netherlands by Huijgen et al, where mean treated LDL-C levels in 781 FH subjects was 3.2 mmol/L (187).

A recent meta-analysis by Delahoy et al (188) including 25 trials investigated the relationship between reduction in LDL-C by statins and reduction in risk of CVD outcomes. They found that there was a significant positive relationship between LDL-C level reduction and reduction in CVD risk (188). The Heart Protection Study (HPS) support these findings in a randomised placebo-controlled trial in 20,536 high-risk individuals; they demonstrated that LDL-C reduction to levels as low as 1.7 mmol/L was associated with significant clinical benefit in a wide range of high-risk individuals irrespective of baseline cholesterol levels (180). However, one could discuss whether a LDL-C level of 1.7 mmol/L is achievable. Surely, statin treatment is not enough. In the case-control study 17 out of 34 subjects received rosuvastatin and 15 subjects received atorvastatin, respectively. In both groups, all subjects except from four received the highest dose of rosuvastatin or atorvastatin. But even in our case-control study, where the LDL-C was lowered by an estimated median of 67.2 %, the median LDL-C level was 3.2 mmol/L. This information could be used as an incentive upon clinicians to increase, if tolerated, supplemental medication and combination treatment in FH subjects.

As earlier shown in **table 3**, there are differences between statins regarding their lipid-lowering effect. For instance, the lowest dose of rosuvastatin, 5 mg, lowers LDL-C levels by approximately 39 % in comparison with the highest dose of lovastatin, 80 mg, which lowers LDL-C levels by approximately 42 % (150, 151). Rosuvastatin and atorvastatin are examples of high-potency statins, and lovastatin is

not (189). In the retrospective data collection study, 13 out of 30 subjects received lovastatin in the group of deceased FH subjects. In the group of non-deceased FH subjects, no one received lovastatin; most of them were either using atorvastatin or rosuvastatin, with 90 and 36 out of 147 subjects, respectively. Hence, in addition to the statistically significant difference in number of years of statin treatment between non-deceased susceptible FH subjects and deceased FH subjects with early CHD event and between resistant and deceased FH subjects with late or no CHD event, the lipid-lowering effect of statin treatment could be lower in the deceased FH subjects in comparison with non-deceased FH subjects.

In general, both in FH and the general population, men have a higher risk of CHD at all ages (11). In view of the fact that male gender in itself is considered CHD risk factor due to among other factors the protective property of oestrogen in women, this could probably contribute to explain the more aggressive treatment in men than in women. We have shown that combination of drugs in our data material is more common in FH men than in FH women. Yet, literature shows that women do respond to lipid-lowering drugs to the same extent as men do (11). Since statins contribute to the reduction of some potential risk factors, but does not affect others to the extent of which combination treatment with for instance niacin and fibrates do (110), FH women could be at higher risk for developing CHD.

In addition to the effect of lowering LDL-C levels, the CHD risk in individuals is influenced by TG and HDL-C levels (170, 171). Statins first and foremost reduce total cholesterol and LDL-C levels. With combination treatment HDL-C levels usually are increased and TG levels are lowered more than with monotherapy of statins (170, 171). Therapeutic intervention should aim at focusing on correcting other signs of dyslipidemia in FH subjects as well as normalisation of LDL-C. For instance, niacin plays an important role in lowering Lp(a) levels (97, 110) and is currently the most effective HDL-C increasing therapy (152).

Omega-3 supplements

Three out of four non-deceased FH subjects in our studies, regardless of subgroup and in both our studies, reported a regularly intake of omega-3 supplements. Out of the deceased FH subjects, especially those with early CHD event, only a small number (15.4 %) reported use of omega-3 supplements. Hence, there was a significant difference between deceased FH subjects with early CHD event and non-deceased susceptible FH subjects, but also towards deceased FH subjects with late or no CHD event in the use of omega-3 supplements. Interestingly, this was one of the few differences between the two groups of deceased FH subjects. Our FH population was not characterized by increased TG levels so even though we do not know the dosage of omega-3 supplements used by the FH subjects it is likely to assume that they followed the Lipid Clinic recommendation of daily intake of cod liver oil or capsules, supplying a total of 0.6-1 g EPA/DHA per day, respectively.

The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico–Heart Failure (GISSI-HF) study, a large-scale clinical trial, recently showed that omega-3 FAs (850-882 mg/d) reduced mortality in patients with chronic heart failure who were already receiving recommended therapies (190, 191). The favourable effects of omega-3 FAs in GISSI-HF suggest that marine fish oils and omega-3 supplements could confer protection in heart failure mainly through their antiarrhythmic action and in part by influencing the mechanisms related to heart failure progression (191). In the GISSI-Prevenzione trial in 1999 (192) dietary supplementation with omega-3 FAs (1 g/d) was shown to lead to a statistically significant beneficial effect on death, MI and stroke. Interestingly, the dose of omega-3 FAs given in both GISSI trials is somewhat similar to the omega-3 FA intake recommended by the Lipid Clinic. Furthermore, Kris-Etherton et al argue in their 2003 review that evidence from prospective secondary prevention studies suggests that EPA/DHA intake ranging from 0.5 to 1.8 g/d (either as fatty fish or supplements) significantly reduces subsequent cardiac and all-cause mortality (193).

Seen together with our results, it is tempting to suggest that the intake of omega-3 FAs can have influenced early CHD event and perhaps also early death in the deceased FH subjects with early CHD event. Many investigator groups and literature recommend omega-3 intake through the diet and/or as a supplement in subjects with FH; however, some of the recommendations are more well-founded than others (25, 65). However, the NICE guidelines on FH does not support recommendation of omega-3 supplements in FH due to lack of adequate data (79).

Regarding diet registered with SmartDiet, no differences were found between the different groups in our two studies.

5.2.3 Factors involved in inflammation, clotting and thrombosis

Coagulation markers

In the case-control study no differences between the susceptible group and the resistant group were found regarding APC resistance, Protein S, Protein C and vWF. Nor were differences found between female FH subjects and male FH subjects. However, FH subjects had significantly higher vWF antigen and activity in comparison with controls. The same was the case with Protein S, both free and total, and Protein C. However, all values were within the range of reference, besides, low values of Protein S and C, respectively, are associated with increased thrombosis. Yet, it is somewhat interesting that the FH subjects had higher vWF antigen and activity, given the key role of vWF in arterial thrombus formation. However, according to Vischer et al in a recent review study, in the general population evidence indicates that vWF levels are a poor predictor of CHD (144). On the other hand, Wannamethee et al suggested in a recent study based on data from the British Regional Heart Study that vWF, among other haemostatic markers, were associated with a significant increased risk of death from MI/CHD (194).

C-reactive protein

No significant differences were found concerning median level of CRP, except between the susceptible and resistant female groups where the median CRP levels were 0.60 and 0.99 mg/L, respectively. Still, there was a tendency towards significance when analysis was run without the individual with CRP level of 13.00 mg/L, $P = 0.080$. However, use of oestrogen and/or progesterone is associated with increased levels of CRP (129). We do not know to what extent female FH subjects used hormone contraceptives or menopause oestrogen treatment, or if there was a difference between the groups of female FH subjects.

Statins and ezetimibe are shown to reduce CRP (6, 126-128, 195). Li and Fang found in their review a reducing effect of different statins on CRP of 15-40 % (6). In a recent study of 44 subjects with mild hypercholesterolemia Kostakou et al found that both simvastatin and ezetimibe after three months had reduced CRP significantly (195). However, Joynt et al found no relation between CRP level and statin use (196).

In a 2010 meta-analysis, the Emerging Risk Factors Collaboration suggests that CRP levels are as consistent within individuals from year to year as blood pressure and other parameters (120). In addition, they emphasise the association of CRP with CHD, possibly suggesting that lowering CRP levels are important in preventing CHD (120).

Fibrinogen

FH subjects had significantly higher median fibrinogen level in comparison with controls. However, both values were within reference range.

5.2.4 Other parameters

BMI

We found a statistically significant difference in BMI between FH subjects and controls with a median of 27.1 kg/m² and 23.3 kg/m², respectively. In FH subjects in the retrospective data collection study a similar tendency towards elevated BMI was observed, with a median BMI of 27.0 kg/m² in the susceptible group and 27.6 kg/m² in the resistant group. This proposes the question of whether it has been a bigger increase in overweight people in the FH population than people in the general population. A cross-sectional study by Hopkins et al (90) showed even higher average BMI in their FH subjects of investigation, suggesting that this is not a Norwegian phenomenon. On the other hand, an observation study by Meyer and Tverdal from 2005 (197) shows that there has been a decrease in BMI in the Norwegian population from the 1960s to the 1990s and that adult Norwegian men in the 1990s had an BMI of 26.5 kg/m² in average. Taking into consideration that Meyer and Tverdal's data are more than ten years old, the FH subject and the general population BMI average potentially are quite similar. Our study had few participants; hence one could consider it to be more probable that the ten controls had too low BMI in preference to the theory of the FH subject weighting too much.

Blood pressure

According to NCEP ATP III subjects with high-normal blood pressure defined as blood pressure levels at 130-139 mmHg systolic and/or 85-89 mmHg diastolic, respectively, are at increased risk for CHD in comparison with those with optimal blood pressure levels (11). The median blood pressure levels in the susceptible and resistant groups in the case-control study were below categorical hypertension. However, the median blood pressure levels in both groups fit into the NCEP ATP III definition of high-normal blood pressure. A significantly greater proportion of the susceptible subjects were medicated with β -blockers in comparison with the resistant

subjects; however, there was no significant difference between the groups regarding the number of confirmed hypertensive subjects.

There are several potential interpretations of these findings. First, a greater number of persons in the susceptible group were medicated in comparison with the resistant group. Hence, the levels in cases would be systematically affected by the medication they received. Second, when the median blood pressure levels in the susceptible group and resistant group were not different, one could suggest that neither of the groups reached desirable levels of blood pressure.

Our levels of systolic and diastolic blood pressure in FH subjects are in line with the findings in the cohort study on 2,400 FH subjects of Jansen et al (86). However, they registered a significantly higher blood pressure in subjects with CAD in comparison to those without CAD.

6. Conclusion and clinical implications

In the present studies we have shown that

- a. in the retrospective data assessment study, susceptible FH subjects had more severe CHD risk factor profile in comparison with resistant FH subjects in terms of significantly higher median Lp(a) level, pre-treatment total cholesterol level and TG level even though they were more intensively medically treated
- b. deceased FH subjects had a more severe CHD risk factor profile compared with non-deceased FH subjects due to significantly higher median total cholesterol level, LDL-C level at the same time as they experienced a shorter duration of medical treatment, a smaller reduction in total cholesterol – and fewer had a regularly intake of omega-3 FAs
- c. in the case-control study FH subjects and controls did not differ in coagulation factor profile, CRP levels or fibrinogen levels more than within the range of reference, nor were there differences between susceptible and resistant patients
- d. female FH subjects had a more severe CHD risk factor profile in comparison with male FH subjects due to significantly higher median total cholesterol level and LDL-C level at the same time as they were less intensively medically treated

In conclusion, our results may suggest that compared with the lipid-lowering treatment that was offered ten years ago today's lipid-lowering treatment reduces the risk of fatal CHD in FH subjects. Furthermore, the results indicate that regularly intake of omega-3 FAs may contribute to a reduced risk of CHD and death. Even though the treatment seems to have improved substantially, there is still a potential for improvement concerning reaching the treatment goals. Furthermore, our results may also indicate that female FH subjects – and in particular susceptible female FH subjects (FH subjects already having suffered from a cardiovascular event) – may

need to be followed up more closely, and more extensively treated with lipid-lowering medication and combination medication. Of particular interest are the elevated Lp(a) values registered in susceptible women. The results may suggest that FH women with elevated Lp(a) levels could be at a higher risk of developing CHD than other FH patients. Routinely screening of Lp(a) in FH patients together with other blood routine parameters, especially in women, followed by early initiation of niacin treatment in addition to statins may be of importance to reduce the increased CHD risk possible mediated by Lp(a).

7. Future perspectives

The present studies have generated new knowledge and hence new questions and hypotheses.

Only a small number of differences in coagulation markers between the susceptible and resistant groups and in FH subjects versus controls were found, most likely due to the small number of participants included in this study. However, it has previously been shown that peripheral blood mononuclear cells isolated from FH patients with early CAD (n = 6), or with present xanthomas and xanthelasms (n = 10) release more proinflammatory cytokines than FH subjects without CAD (n = 16) and without xanthomas and xanthelasms (n = 12), respectively, indicating that by the use of more sensitive inflammatory markers one might be able to identify new biomarkers (163).

We therefore wish to proceed this project by analysing the circulating level and the gene expression of different – more sensitive – inflammatory markers which are thought to contribute in the atherosclerotic process.

It is known that the phenotypic variation between FH subjects with the same genotype cannot be explained solely by their cholesterol levels. Future research should therefore be directed at identifying and determining which FH subjects that are at particularly high risk of premature CHD through the investigation of which biochemical, genetic and environmental factors that 1) may predict risk and 2) by modulation of which the risk is lowered. Subsequently, individual treatment regimens can be developed on basis of these findings in order to implement early and more intensive treatment for the prevention of CHD and potential fatal events in FH subjects who are at the highest risk of developing premature CHD.

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Appendices

- Appendix 1** Approval from the Regional Committee of Medical Ethics
- Appendix 2** Approval from the Norwegian Directorate of Health
- Appendix 3** Approval from the Privacy Ombudsman at Rikshospitalet, Oslo University Hospital
- Appendix 4** Inclusion and exclusion criteria for susceptible FH subjects
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Appendix 1. Approval from the Regional Committee of Medical Ethics



UNIVERSITETET I OSLO DET MEDISINSKE FAKULTET

Overlege Kjetil Retterstøl
Lipidklinikken
Rikshospitalet HF
Sognvannsveien 20
0027 Oslo

Regional komité for medisinsk og helsefaglig
forskningsetikk Sør-Øst D (REK Sør-Øst D)
Postboks 1130 Blindern
NO-0318 Oslo

Telefon: 22 85 05 93

Telefaks: 22 85 05 90

E-post: i.m.middelthon@medisin.uio.no

Nettadresse: www.etikkom.no

Dato: 11.03.09

Deres ref.:

Vår ref.: S-09150d, 2009/1857

"Betydningen av inflammasjonsmarkører hos personer med familiær hyperkolesterolemi (FH); sammenligning mellom personer med FH med tidlig hjertesykdom og FH personer med sen eller ingen hjertesykdom."
Søknad om opprettelse av forskningsbiobank

Komiteen behandlet søknaden 24.02.09. Prosjektet er vurdert etter lov om behandling av etikk og redelighet i forskning av 30. juni 2006, jfr. Kunnskapsdepartementets forskrift av 8. juni 2007, Lov om humanmedisinsk bruk av bioteknologi m.m. av 15. juni 2007 og retningslinjer av 27. juni 2007 for de regionale komiteer for medisinsk og helsefaglig forskningsetikk.

Prosjekttema:

I studien skal man sammenligne forekomst av ulike betennelsesstoffer i blodet og i genuttrykket i blodceller hos personer med familiær hyperkolesterolemi (FH), for å kunne finne markører i plasma som kan si noe om risikoen for tidlig debut av hjerte-kar sykdom. Prosjektet er delt i en retrospektiv og en prospektiv del. Data fra 100 pasienter skal inkluderes i studien.

Forskningsetisk vurdering:

Studiens design fremstår som noe uklart for komiteen, men det antas at det ikke er de samme pasientene som inngår i den retrospektive og prospektive delen av studien. Dette betyr, slik komiteen oppfatter det, at man bare har tenkt å innhente samtykke fra pasientene som skal avgi blodprøve.

Komiteen anbefaler derfor at de retrospektive dataene hentes ut og anonymiseres av helsepersonell med lovlig tilgang til journalene, før de leveres til masterstudenten for bearbeiding. Dersom man ikke gjør det slik, er det komiteens oppfatning at man bør søke Helsedirektoratet om fritak fra taushetsplikt for denne delen av studien. Komiteen har ingen innvendinger mot at dette innvilges.

Komiteen har følgende merknader til søknad om opprettelse av forskningsbiobank:

Komiteen har ingen innvendinger mot opprettelse av forskningsbiobank og videresender søknaden om opprettelse av denne sammen med kopi av dette vedtaket til Helsedirektoratet for endelig godkjenning, når en eventuell godkjenning av prosjektsøknaden foreligger.

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Det medisinske fakultet

Side 2 av 2

Vedtak:

Vedtak utsettes. Det bes om tilbakemelding på merknaden som er anført, før endelig vedtak kan fattes. Komiteens leder tar stilling til godkjenning av prosjektet etter mottatt svar.

Vedtaket var enstemmig

Med vennlig hilsen

Stein A. Evensen (sign.)
Professor dr.med.
leder


Ingrid Middelthon
Komitésekretær



UNIVERSITETET I OSLO
DET MEDISINSKE FAKULTET

Overlege Kjetil Retterstøl
Lipidklinikken
Rikshospitalet HF
Sognvannsveien 20
0027 Oslo

**Regional komité for medisinsk og helsefaglig
forskningsetikk Sør-Øst D (REK Sør-Øst D)**
Postboks 1130 Blindern
NO-0318 Oslo

Telefon: 22 85 05 93
Telefaks: 22 85 05 90

Dato: 12.01.10
Deres ref.:
Vår ref.: S-09150d, 2009/1857, 2009/1071

E-post: j.m.middelthon@medisin.uio.no
Nettadresse: www.etikkom.no

Vedr. klage på avslag på søknad om fritak fra taushetsplikt for masterstudent i studien "Betydningen av inflammasjonsmarkører hos personer med familiær hyperkolesterolemi (FH); sammenligning mellom personer med FH med tidlig hjertesykdom og FH personer med sen eller ingen hjertesykdom."

Vi viser til klage på avlag om fritak fra taushetsplikt av 14.09.09 og e-post fra REK Sør-Øst D av 28.09.09 hvor det gis melding om at fritak fra taushetsplikt innvilges. Ved en feil er det dessverre ikke sendt ut formelt vedtaksbrev hvor fritak fra taushetsplikt innvilges. Det beklager vi.

Prosjektleder er overlege dr. med. Kjetil Retterstøl.

Forskningsansvarlig er Oslo universitetssykehus, avd. Rikshospitalet.

Vedtak:

Komiteen innvilger fritak fra taushetsplikt i prosjektet jf. helseforskningsloven § 35. Komiteen mener at prosjektet er av vesentlig interesse for samfunnet. Hensynet til deltakernes velferd og integritet vurderes å ivaretas godt slik prosjektet er lagt opp. Forskningsansvarlig institusjon tar ansvar for hvem som gis tilgang til disse dataene og at og at utlevering skjer iht. krav til informasjonssikkerhet.

Tillatelsen er gitt under forutsetning av at prosjektet gjennomføres slik det er beskrevet i søknaden, oppdatert protokoll og de bestemmelser som følger av helseforskningsloven med forskrifter.

Komiteen forutsetter at data oppbevares i avidentifisert form. Det vil si at opplysningene oppbevares uten direkte personidentifiserbare parametre, men hvor man kan finne tilbake til den personen opplysningen stammer fra ved hjelp av en nøkkel eller kode.

Forskningsprosjektets data skal oppbevares forsvarlig, se personopplysningsforskriften kapittel 2, og Helsedirektoratets veileder for «Personvern og informasjonssikkerhet i forskningsprosjekter innenfor helse- og omsorgssektoren», <http://www.norsk-helsenett.no/informasjonssikkerhet/bransjenormen/Personvern%20og%20informasjonssikkerhet%20i%20forskningprosjekter%20v1.pdf>

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Det medisinske fakultet

Side 2 av 2


Dersom det skal gjøres endringer i prosjektet i forhold til de opplysninger som er gitt i søknaden må prosjektleder sende endringsmelding til REK. Vi gjør oppmerksom på at hvis endringene er vesentlige må prosjektleder sende ny søknad, eller REK kan pålegge at dette gjøres.

Prosjektet skal sende sluttmelding til REK Sør-Øst D, jf. helseforskningsloven § 12.

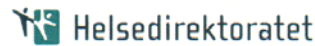
For øvrig gjelder de vilkår som er satt i forbindelse med tidligere godkjenning av prosjektet.

Med vennlig hilsen

Stein A. Evensen (sign.)
prof. dr.med.
leder


Ingrid Middelthon
seniorrådgiver

Appendix 2. Approval from the Norwegian Directorate of Health



Overlege Kjetil Retterstøl
Rikshospitalet HF
Lipidklinikken
Sognsvannveien 20
0027 OSLO

Deres ref.:
Saksbehandler: JKK
Vår ref.: 09/2545
Dato: 08.06.2009

Betydningen av inflammasjonsmarkører hos personer med familiær hyperkolesterolemi - Sammenligning personer med og uten hjertesykdom

Vi viser til brev fra REK Sør Øst D 6.april 2009 med melding 2717 av 28. januar 2009 om forskningsbiobank om ovenstående og til kopi av brev fra REK Sør Øst D 3. april 2009 der det framgår at prosjektet under visse forutsetninger godkjennes og opprettelse av forskningsbiobanken tilrås.

HelseDirektoratet er delegert å vurdere meldinger om opprettelse av forskningsbiobanker i hht. biobankloven § 4.

Under forutsetning av at REK Sør Østs merknader tas til følge, har direktoratet ingen innsigelser til at forskningsbiobanken opprettes i henhold til biobankloven.

Direktoratet forutsetter at opprettelsen av den planlagte forskningsbiobanken oppfyller nødvendige krav til godkjenning, konsesjon m.v. i henhold til annet relevant regelverk, herunder bioteknologiloven, helseregisterloven og legemiddeloven.

Direktoratet har registrert at meldingen om forskningsbiobanken er sendt til Nasjonalt folkehelseinstitutt som har fått ansvaret for å føre et offentlig tilgjengelig register over landets biobanker, jf. biobankloven § 6.

Vennlig hilsen

Ragnhild Castberg e.f.
avdelingsdirektør


Janne Kristin Kjøllesdal
seniorrådgiver

Dokumentet er godkjent elektronisk

Kopi: REK Sør Øst D 2009/1857
Biobankregisteret Melding 2717

HelseDirektoratet • Divisjon spesialisthelsetjenester

Avd. bioteknologi og generelle helselover
Janne Kristin Kjøllesdal, tlf.: 24 16 30 64

Postboks 7000 St. Olavs plass, 0130 Oslo • Besøksadresse: Universitetsgata 2, Oslo • Tlf.: 810 20 050
Faks: 24 16 30 01 • Org. nr.: 983 544 622 • postmottak@helsedir.no • www.helsedirektoratet.no

Appendix 3. Approval from the Privacy Ombudsman at Rikshospitalet, OUS



RIKSHOSPITALET

INTERNE TJENESTER

IT-avdelingen

NOTAT

Til: Kjetil Retterstøl, overlege dr. med, Lipidklinikken

Kopi:

Fra: Anette Engum, personvernombud for forskning

Saksbehandler:

Dato: 08.05.09

Offentlighet: Ikke unntatt offentlighet

Sak: Tiltråding av forskningsstudie unntatt konsesjon

Saksnummer/
Personvernnummer: 09/2733

Besøksadr: Forskningsveien 2 B, Oslo
Postadr: Rikshospitalet HF, 0027 Oslo
Sentralbord: 23 07 00 00
Direktelinje: 23 07 50 34
Epost: firmapost@rikshospitalet.no
personvern@rikshospitalet.no

Tiltråding til innsamling og databehandling av personopplysninger i forskningsstudien "Betydningen av inflammasjonsmarkører hos personer med familiær hyperkolesterolemi (FH); sammenligning mellom personer med FH med tidlig hjertesykdom og FH personer med sen eller ingen sykdom"

Personvernombudet har vurdert det til at den planlagte databehandlingen av personopplysninger tilfredsstillende forutsetningene for melding gitt i personopplysningsforskriften § 7-27 og derfor er unntatt konsesjon. Personvernombudet har myndighet til å foreta denne avgjørelsen på vegne av Datatilsynet.

Det tilrås at prosjektet igangsettes med følgende betingelser:

- Data lagres aidentifisert på en av sykehusets forskningsservere (O:Forskning, Forskernett eller MEDinsight). Annen lagringsform forutsetter gjennomføring av en risikovurdering som må godkjennes av personvernombud. Se referanser.
- Kryssliste som kobler aidentifiserte data med personopplysninger lagres separat på prosjektleders avlåste kontor.
- Data slettes eller anonymiseres (ved at krysslisten slettes) senest 31.12.27.
- Studien må vurderes og godkjennes av Regional komité for medisinsk og helsefaglig forskningsetikk (REK), og eventuelle merknader må følges. Kopi av tilråding fra personvernombudet vedlegges søknaden til REK.
- Søknad om opprettelse av forskningsbiobank sendes Helsedirektoratet via Regional komité for medisinsk og helsefaglig forskningsetikk (REK-Sør). Kjetil Retterstøl ved Lipidklinikken settes som ansvarshavende for biobanken. Pasientinformasjonsskrivet oppdateres i henhold til dette.
- Pasientinformasjonsskrivet oppdateres slik at det fremgår at prosjektet er tilrådd av personvernombudet ved Oslo universitetssykehus HF – Rikshospitalet (ikke NSD).

Kontaktperson for prosjektet skal hvert tredje år sende personvernombudet ny melding som bekrefter at databehandlingen skjer i overensstemmelse med opprinnelig formål og

helseregisterlovens regler. Hvis formålet eller databehandlingen endres må personvernombudet informeres om dette. Studien er registrert i Rikshospitalets offentlig tilgjengelig database over forsknings- og kvalitetsstudier <http://forpro>.

Med vennlig hilsen

(sign.)
Anette Engum
personvernombud for forskning

Oslo universitetssykehus HF – enheten Rikshospitalet

Referanser

[1-ADM.2.6.1 Risikovurdering av informasjonssikkerhet](#)

[1-FOR.4.05 Lagring, arkivering og sletting av helse- og personopplysninger i forskningsstudier og kvalitetssikring](#)

[1-FOR.4.09 Utforming av samtykke og informasjonsskriv ved ekstern og intern databehandlingsansvarlig](#)

[1-FOR.11.0.2 Mal for forespørsel om deltakelse i forskningsprosjekt](#)

Appendix 4. Inclusion and exclusion criteria for susceptible FH subjects

Inclusion criteria susceptible group

1. Proven LDL receptor or ApoB or PCSK9 mutation
2. Aged 18 years or older
3. Clinical diagnosis of CHD prior to:
 - 44 years for male non-smokers
 - 41 years for male smokers / ex-smokers
 - 55 years for female non-smokers
 - 51 years for female smokers / ex-smokers

For susceptible, smokers / ex-smokers are those patients who were a smoker or ex-smoker at the time of their CHD.

- Smoker is defined as any cigarette smoking over the previous month
- Ex-smoker is defined as no cigarette smoking in the previous month but has previously smoked

Clinical diagnosis of CHD is defined by the presence of at least one of the following symptoms:

- a) Myocardial infarction proven by at least 2 of the following:
 - I. Classical symptoms (>15 minutes)
 - II. Specific ECG abnormalities
 - III. Elevated cardiac enzymes (>2x upper limit of normal)
- b) Percutaneous coronary intervention or other invasive procedures
- c) Coronary artery bypass grafting
- d) Angina pectoris diagnosed as classical symptoms in combination with at least one unequivocal result of one of the following:
 - I. Exercise test
 - II. Nuclear scintigram
 - III. Dobutamine stress ultrasound
 - IV. More than 70 % stenosis on a coronary angiogram
4. The volunteer, their parents and all four grandparents must be white Caucasian. All four grandparents must have been born in the country of study
5. Willing to provide a blood sample
6. Completed written informed consent, including consent for their DNA to be used for anonymous genotyping with a potential commercial application

Exclusion criteria susceptible group

1. Diabetes mellitus prior to the diagnosis of CHD
2. Homozygous FH
3. Pre-treatment triglyceride levels >5mmol/L
4. Body mass index (BMI) > 40
5. Quarter or half blood relative (sibling (including half sibling), parent, offspring, grandparent, aunt or uncle) already participating in the trial in the same group (i. e. a resistant volunteer can not be enrolled on the study if they have a qualifying blood relative already enrolled in the trial in the resistant group however they may be enrolled if their relative is in the susceptible group)
6. Current pregnancy (that the volunteer is aware of) or lactation
7. A known genetic disorder other than FH or ApoB or PCSK9 mutation
8. A medical condition that, in the opinion of the investigator, would make it inadvisable for the volunteer to participate in the trial
9. Volunteers who, in the opinion of the investigator, should not participate in the study

Appendix 5. Inclusion and exclusion criteria for resistant FH subjects

Inclusion criteria resistant group

1. Proven LDL receptor or ApoB or PCSK9 mutation
2. No clinical diagnosis of CHD or CVD prior to
 - 49 years for male non-smokers
 - 46 years for male smokers / ex-smokers
 - 60 years for female non-smokers
 - 56 years for female smokers / ex-smokers

For resistant, smokers / ex-smokers are those patients who are a smoker or ex-smoker at the time of the study

 - Smoker is defined as any cigarette smoking over the previous month
 - Ex-smoker is defined as no cigarette smoking in the previous month but has previously smoked

Clinical diagnosis of CHD is defined by the presence of at least one of the following symptoms:

 - a) Myocardial infarction proven by at least 2 of the following:
 - I. Classical symptoms (>15 minutes)
 - II. Specific ECG abnormalities
 - III. Elevated cardiac enzymes (>2x upper limit of normal)
 - b) Percutaneous coronary intervention or other invasive procedures
 - c) Coronary artery bypass grafting
 - d) Angina pectoris diagnosed as classical symptoms in combination with at least one unequivocal result of one of the following:
 - I. Exercise test
 - II. Nuclear scintigram
 - III. Dobutamine stress ultrasound
 - IV. More than 70 % stenosis on a coronary angiogram
 - e) Ischaemic stroke demonstrated by CT or MRI scan
 - f) Documented transient ischaemic attack
 - g) Peripheral arterial bypass graft
 - h) Peripheral percutaneous transluminal angioplasty or other percutaneous invasive intervention
 - i) Intermittent claudication defined as classical symptoms on combination with at least one unequivocal result of one of the following:
 - I. Ankle/arm index <0.9
 - II. Stenosis (>50 %) on an angiogram or duplex scan
3. Pre-treatment LDL cholesterol >6.5 mmol/L
 If no pre-treatment LDL cholesterol levels are available then one of the following criteria (in order of preference) may be used for selection:
 - I. Off-treatment LDL cholesterol >6.5 mmol/L. Off-treatment is defined as no lipid-lowering medication for at least 6 weeks
 - II. Pre-treatment total cholesterol >9.0 mmol/L
 - III. Off-treatment total cholesterol >9.0 mmol/L. Off-treatment is defined as no lipid-lowering medication for at least 6 weeks
4. The volunteer, their parents and all four grandparents must be white Caucasian. All four grandparents must have been born in the country of study
5. Willing to provide a blood sample
6. Completed written informed consent, including consent for their DNA to be used for anonymous genotyping with a potential commercial application

Exclusion criteria resistant group

1. Quarter or half blood relative (sibling (including half sibling), parent, offspring, grandparent, aunt or uncle) already participating in the trial in the same group (i. e. a resistant volunteer can not be enrolled on the study if they have a qualifying blood relative already enrolled in the trial in the resistant group however they may be enrolled if their relative is in the susceptible group)
2. Current pregnancy (that the volunteer is aware of) or lactation
3. A known genetic disorder other than FH or ApoB or PCSK9 mutation
4. A medical condition that, in the opinion of the investigator, would make it inadvisable for the volunteer to participate in the trial
5. Volunteers who, in the opinion of the investigator, should not participate in the study

Appendix 6. Information letter and written informed consent given the FH subjects in the case-control study



Pasientinformasjon og samtykkeerklæring

Dette er en forespørsel om å delta i et forskningsprosjekt hvor vi ser på betydningen av betennelselignende stoffer (inflammasjonsmarkører) hos personer med familiær hyperkolesterolemi (FH); sammenligning mellom personer med FH med tidlig hjertesykdom og FH personer med sen eller ingen hjertesykdom”

Før du bestemmer deg for om du vil delta, er det viktig at du forstår hvorfor studien gjennomføres, hva den innebærer og hvilke fordeler, risikoer og ubehag som kan være forbundet med den. Du bør lese denne informasjonen nøye og spør gjerne en av de prosjektansvarlige om ting du er usikker på.

Bakgrunn

Vitenskapelige rapporter har vist at hjerte-karsykdom også er en betennelsesprosess, som involverer blodets celler. Betennelse kan for eksempel utløses av høyt kolesterol i blodet, men også andre faktorer kan starte denne betennelsesprosessen. Vi vil undersøke om blodcellene produserer forskjellige betennelses stoffer hos personer med tidlig og sen eller ingen hjertesykdom og om dette er relatert til kolesterol nivået. Hjerte- karsykdom er en av de hyppigste årsakene til sykdom hos voksne i dag. Ved å kunne påvirke denne betennelsesprosessen vil man kunne redusere kolesterolavleiring i blodåreveggen og dermed kanskje påvirke risikoen for hjerte- karsykdom.

Hovedhensikten med denne studien er å sammenligne forekomsten av ulike betennelsesstoffer både i blodet og i genuttrykket i blodceller hos personer med FH for å kunne finne markører i plasma som kan si noe om risikoen for tidlig debut av hjerte- karsykdom.

Hvem vi søker

- Kvinner og menn med FH i alderen 20-80 år både de som har eller har hatt hjertesykdom og de som aldri har hatt hjertesykdom.

Hva vil dette bety for deg?

- Et oppmøte på Lipidklinikken på Rikshospitalet for å ta blodprøve en gang (ett stikk)
- Du små møte fastende, dette betyr at du ikke skal spise eller drikke noe annet enn vann de siste 12 timer før besøket.
- Du må ikke røyke den morgenen du skal møte til besøk, og ikke drikke alkohol de siste 24 timene før visitten.
- Dersom du bruker legemidler, skal du fortsette med dem som vanlig.

Deltagelse er frivillig

Det er frivillig å være med i studien. Du kan når som helst trekke deg uten å oppgi grunn og kreve din prøve destruert, samt personopplysningene slettet, uten at det får noen følger for den ordinære behandlingen. Prøven kan dog ikke kreves destruert hvis opplysningene inngår i et større arbeide eller inngår i vitenskapelige arbeider. Deltakelse medfører ingen alvorlige smerter eller psykiske påkjenninger. Noen synes det er ubehagelig å kjenne stikket ved å ta blodprøve.

Hva skjer med blod og informasjon som samles inn om deg?

Blodprøven fra deg vil vi bruke til å undersøke betydningen av betennelsesstoffer (inflammasjonsmarkører) i blodet for utvikling av hjerte/karsykdom. Blodprøven fra deg vil inngå i en biobank, og vil bli forskriftsmessig lagret i aidentifisert tilstand, og vil kun bli brukt for å belyse sammenhengen mellom inflammasjon (betennelse) og hjertesykdom og vil bli destruert senest i 2027.

Forskerne er underlagt taushetsplikt, og alle opplysninger som nedtegnes blir behandlet strengt konfidensielt. Alle forskningsdata vil være aidentifiserte. Opplysninger som fremkommer i sluttrapporten og i artikler vil ikke kunne tilbakeføres til enkeltpersoner. Dersom du trekker deg fra studien vil evt. blodprøver bli destruert og alle data slettet. Prøven kan dog ikke kreves destruert hvis opplysningene inngår i et større arbeide eller inngår i vitenskapelige arbeider. Vi gjør oppmerksom på at kontrollmyndigheter vil kunne ha behov for å sjekke at opplysninger gitt i studien stemmer med opplysninger i din journal for å kontrollere studiens kvalitet, og ønsker å ha denne muligheten i 15 år etter at siste pasient har gått ut av studien, dvs. til 31.12.2027. Etter denne dato vil alle opplysninger bli anonymisert eller makulert.

Andre viktige opplysninger

- Gravide og ammende kan ikke delta i studien.
- Prosjektet finansieres av egne driftsmidler fra Universitetet i Oslo. Det vil bli søkt støtte til prosjektet fra ulike forskningsfond. De prosjektansvarlige har ingen økonomiske interesser i prosjektet.
- Etter gjeldende regler er studien blitt vurdert av Regional komité for medisinsk forskningsetikk, og Statens legemiddelverk, og meldt til Personvernombudet for forskning, Norsk samfunnsviteskapelig datajeneste AS, og til Sosial- og Helsedirektoratet.
- Etter planen skal siste pasient være ute av studien senest 31.12.12.
- Ved evt. spørsmål før, under eller etter studien kan du kontakte en av de prosjektansvarlige (se nedenfor).
- Ansvarshavende for prosjektet er førsteamanuensis Kirsten B Holven, Avdeling for Ernæringsvitenskap, Institutt for Medisinske basalfag, UiO (e-post: kirsten.holven@medisin.uio.no, tlf 22851361) og overlege Kjetil Retterstøl, Lipidklinikken, Rikshospitalet (e-post: kjetil.retterstol@rikshospitalet.no).

Ytterligere informasjon om studien finnes i kapittel A – utdypende forklaring av hva studien innebærer.

Ytterligere informasjon om biobank, personvern og forsikring finnes i kapittel B – Personvern, biobank, økonomi og forsikring.

Samtykkcerklæring følger etter kapittel B.

Kapittel A- utdypende forklaring av hva studien innebærer

Inklusjon/eksklusjonskriterier:

Følgende forhold må være oppfylt for å delta i studien:

Inklusjonskriterier:

- Menn og kvinner med familiær hyperkolesterolemi
- Alder 20-80 år

Eksklusjonskriterier:

- Gravide og ammende

Kapittel B - Personvern, biobank, økonomi og forsikring

Personvern

Opplysninger som vi får i forbindelse med målingene og prøvene som blir tatt av deg (se over) blir registrert hos oss. Blodprøven fra deg vil vi bruke til å undersøke hvordan blodcellene produserer forskjellige betennelses stoffer hos personer med tidlig og sen eller ingen hjertesykdom og om dette er relatert til kolesterol nivået. Hjerte- karsykdom er en av de hyppigste årsakene til sykdom hos voksne i dag. Ved å kunne påvirke denne betennelsesprosessen vil man kunne redusere kolesterolavleiring i blodåreveggen og dermed kanskje påvirke risikoen for hjerte- karsykdom. Forskerne er underlagt taushetsplikt, og alle opplysninger som nedtegnes blir behandlet strengt konfidensielt. Det blir ikke kopling mot andre registre som har opplysninger om deg. Einar Hysing ved Oslo Universitets sykehus er databehandlingsansvarlig etter personopplysningsloven /helseregisterloven.. Etter gjeldende regler er studien vurdert av Regional komité for medisinsk forskning, og meldt til Norsk samfunnsvitenskapelig datatjeneste AS og til Sosial og helsedirektoratet.

Biobank

Blodprøvene som blir tatt og informasjonen utledet av dette materialet vil bli lagret i en forskningsbiobank ved Lipidklinikken/Universitetet i Oslo. Hvis du sier ja til å delta i studien, gir du også samtykke til at det biologiske materialet og analyseresultater inngår i biobanken. Blodprøven vil bli forskriftsmessig lagret i aidentifisert tilstand, og vil kun bli brukt for å studere betydningen av betennelseslignende stoffer (inflammasjonsmarkører) hos personer med familiær hyperkolesterolemi (FH) med tidlig hjertesykdom og FH personer med sen eller ingen hjerte-sykdom". Overlege Kjetil Retterstøl Lipidklinikken, Oslo Universitetssykehus/ Førsteamanuensis Kirsten Holven, Avdeling for Ernæringsvitenskap, Universitetet i Oslo er ansvarshavende for forskningsbiobanken. Biobanken varer til 2027. Etter dette vil materiale og opplysninger bli destruert og slettet etter interne retningslinjer.

Utlevering av materiale og opplysninger til andre

Hvis du sier ja til å delta i studien, gir du også ditt samtykke til at prøver og aidentifiserte opplysninger utleveres til de forskningsinstitusjonene som er samarbeidspartnerne i prosjektet.

Rett til innsyn og sletting av opplysninger om deg og sletting av prøver

Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger. Prøven kan dog ikke kreves destruert hvis opplysningene inngår i et større arbeide eller inngår i vitenskapelige arbeider.

Økonomi

- Prosjektet er et samarbeidsprosjekt mellom Lipidklinikken, Oslo Universitetssykehus og Avdeling for Ernæringsvitenskap, Oslo Universitet. Prosjektet finansieres av egne driftsmidler Avdeling for Ernæringsvitenskap, Oslo Universitet. Det vil bli søkt støtte til prosjektet fra ulike forskningsfond. De prosjektansvarlige har ingen økonomiske interesser i prosjektet.

Forsikring

- Dersom et uhell eller en komplikasjon skulle inntreffe, er deltagerne forsikret gjennom pasientskaderstatningsordningen

Informasjon om utfallet av studien

Resultatene fra studien vil bli publisert, og deltagerne vil få informasjon om hvor publisering skjer.

- Ansvarshavende for prosjektet er førsteamanuensis Kirsten B Holven, Avdeling for Ernæringsvitenskap, Institutt for Medisinske basalfag, UiO (e-post: kirsten.holven@medisin.uio.no, tlf 22851361) og overlege Kjetil Retterstøl, Lipidklinikken, Rikshospitalet (e-post: kjetil.retterstol@rikshospitalet.no).

Samtykke til deltakelse i studien

Jeg er villig til å delta i studien

(Signert av prosjektdeltaker, dato)

Jeg bekrefter å ha gitt informasjon om studien

(Signert av prosjektansvarlig, dato)

Appendix 7. Information letter and written informed consent given the control subjects in the case-control study



Studieinformasjon og samtykkeerklæring

Dette er en forespørsel om å delta i en referansegruppe til et forskningsprosjekt hvor vi ser på betydningen av betennelseslignende stoffer (inflammasjonsmarkører) hos personer med familiær hyperkolesterolemi (FH); sammenligning mellom personer med FH med tidlig hjertesykdom og FH-personer med sen eller ingen hjertesykdom.

Dersom man ikke skulle finne noen forskjell mellom FH-personer med tidlig hjertesykdom og personer med FH med sen eller ingen hjertesykdom, kan det likevel være et annerledes nivå av inflammasjonsmarkørene vi ønsker å måle hos den samlede gruppen av FH-personer sammenliknet med personer uten FH. Det er derfor ønskelig å inkludere en referansegruppe, matchet opp mot utvalget av FH-personer, på 10 friske personer.

Før du bestemmer deg for om du vil delta, er det viktig at du forstår hvorfor studien gjennomføres, hva den innebærer og hvilke fordeler, risikoer og ubehag som kan være forbundet med den. Du bør lese denne informasjonen nøye og spør gjerne en av de prosjektansvarlige om ting du er usikker på.

Bakgrunn

Vitenskapelige rapporter har vist at hjerte-karsykdom også er en betennelsesprosess, som involverer blodets celler. Betennelse kan for eksempel utløses av høyt kolesterol i blodet, men også andre faktorer kan starte denne betennelsesprosessen. Vi vil undersøke om blodcellene produserer forskjellige betennelsesstoffer hos personer med tidlig og sen eller ingen hjertesykdom og om dette er relatert til kolesterolnivået. Hjerte- karsykdom er en av de hyppigste årsakene til sykdom hos voksne i dag. Ved å kunne påvirke denne betennelsesprosessen vil man kunne redusere kolesterolavleiring i blodåreveggen og dermed kanskje påvirke risikoen for hjerte- karsykdom.

Hovedhensikten med denne studien er å sammenligne forekomsten av ulike betennelsesstoffer både i blodet og i genuttrykket i blodceller hos personer med FH for å kunne finne markører i plasma som kan si noe om risikoen for tidlig debut av hjerte- karsykdom. For å sammenlikne om gruppene av personer med FH har forhøyede verdier av inflammasjonsmarkører trenger vi også en gruppe med friske matchede kontroller.

Hvem vi søker

- Friske personer i alderen 20-80 år som aldri har hatt hjerte- karsykdom

Hva vil dette bety for deg?

- Et oppmøte på Lipidklinikken på Rikshospitalet for å ta blodprøve en gang (ett stikk)
- Du må møte fastende, dette betyr at du ikke skal spise eller drikke noe annet enn vann de siste 12 timer før besøket.
- Du må ikke røyke den morgenen du skal møte til besøk, og ikke drikke alkohol de siste 24 timene før visitten.
- Dersom du bruker legemidler, skal du fortsette med dem som vanlig.

Deltagelse er frivillig

Det er frivillig å være med i studien. Du kan når som helst trekke deg uten å oppgi grunn og kreve din prøve destruert, samt personopplysningene slettet, uten at det får noen følger for eventuelle senere studier eller senere kontakt. Prøven kan dog ikke kreves destruert hvis opplysningene inngår i et større

arbeide eller inngår i vitenskapelige arbeider. Deltakelse medfører ingen alvorlige smerter eller psykiske påkjenninger. Noen synes det er ubehagelig å kjenne stikket ved å ta blodprøve.

Hva skjer med blod og informasjon som samles inn om deg?

Blodprøven fra deg vil vi bruke til å undersøke betydningen av betennelsesstoffer (inflammasjonsmarkører) i blodet for utvikling av hjerte/karsykdom. Blodprøven fra deg vil inngå i en biobank, og vil bli forskriftsmessig lagret i aidentifisert tilstand, og vil kun bli brukt for å belyse sammenhengen mellom inflammasjon (betennelse) og hjertesykdom og vil bli destruert senest i 2027. Forskerne er underlagt taushetsplikt, og alle opplysninger som nedtegnes blir behandlet strengt konfidensielt. Alle forskningsdata vil være aidentifiserte. Opplysninger som fremkommer i sluttrapporten og i artikler vil ikke kunne tilbakeføres til enkeltpersoner. Dersom du trekker deg fra studien vil evt. blodprøver bli destruert og alle data slettet. Prøven kan dog ikke kreves destruert hvis opplysningene inngår i et større arbeide eller inngår i vitenskapelige arbeider. Vi gjør oppmerksom på at kontrollmyndigheter vil kunne ha behov for å sjekke at opplysninger gitt i studien stemmer med opplysninger i din journal for å kontrollere studiens kvalitet, og ønsker å ha denne muligheten i 15 år etter at siste pasient har gått ut av studien, dvs. til 31.12.2027. Etter denne dato vil alle opplysninger bli anonymisert eller makulert.

Andre viktige opplysninger

- Gravide og ammende kan ikke delta i studien.
- Prosjektet finansieres av egne driftsmidler fra Universitetet i Oslo. Det vil bli søkt støtte til prosjektet fra ulike forskningsfond. De prosjektansvarlige har ingen økonomiske interesser i prosjektet.
- Etter gjeldende regler er studien blitt vurdert av Regional komité for medisinsk forskningsetikk, og Statens legemiddelverk, og meldt til Personvernombudet for forskning, Norsk samfunnsvitenskapelig datatjeneste AS, og til Helsedirektoratet.
- Etter planen skal siste pasient være ute av studien senest 31.12.12.
- Ved evt. spørsmål før, under eller etter studien kan du kontakte en av de prosjektansvarlige (se nedenfor).
- Ansvarshavende for prosjektet er professor Kirsten B. Holven, Avdeling for Ernæringsvitenskap, Institutt for Medisinske basalfag, UiO (e-post: kirsten.holven@medisin.uio.no, tlf 22851361) og overlege Kjetil Retterstøl, Lipidklinikken, Rikshospitalet (e-post: kjetil.retterstol@rikshospitalet.no).

Ytterligere informasjon om studien finnes i kapittel A – utdypende forklaring av hva studien innebærer.

Ytterligere informasjon om biobank, personvern og forsikring finnes i kapittel B – Personvern, biobank, økonomi og forsikring.

Samtykkeerklæring følger etter kapittel B.

Kapittel A- utdypende forklaring av hva studien innebærer

Inklusjon/eksklusjonskriterier:

Følgende forhold må være oppfylt for å delta i referansegruppe i studien:

Inklusjonskriterier:

- Friske menn og kvinner som aldri har hatt hjerte- karsykdom
- Alder 20-80 år

Eksklusjonskriterier:

- Gravide og ammende

Kapittel B - Personvern, biobank, økonomi og forsikring

Personvern

Opplysninger som vi får i forbindelse med målingene og prøvene som blir tatt av deg (se over) blir registrert hos oss. Blodprøven fra deg vil vi bruke til å undersøke hvordan blodcellene produserer forskjellige betennelsesstoffer hos personer med tidlig og sen eller ingen hjertesykdom og om dette er relatert til kolesterolnivået. Hjerte- karsykdom er en av de hyppigste årsakene til sykdom hos voksne i dag. Ved å kunne påvirke denne betennelsesprosessen vil man kunne redusere kolesterolavleiring i blodåreveggen og dermed kanskje påvirke risikoen for hjerte- karsykdom.

Forskerne er underlagt taushetsplikt, og alle opplysninger som nedtegnes blir behandlet strengt konfidensielt. Det blir ikke kopling mot andre registre som har opplysninger om deg. Einar Hysing ved Oslo Universitetssykehus er databehandlingsansvarlig etter personopplysningsloven/helseregisterloven.. Etter gjeldende regler er studien vurdert av Regional komité for medisinsk forskning, og meldt til Norsk samfunnsvitenskapelig datatjeneste AS og til Helsedirektoratet.

Biobank

Blodprøvene som blir tatt og informasjonen utledet av dette materialet vil bli lagret i en forskningsbiobank ved Lipidklinikken/Universitetet i Oslo. Hvis du sier ja til å delta i studien, gir du også samtykke til at det biologiske materialet og analyseresultater inngår i biobanken. Blodprøven vil bli forskriftsmessig lagret i aidentifisert tilstand, og vil kun bli brukt for å studere betydningen av betennelselignende stoffer (inflammasjonsmarkører) hos personer med familiær hyperkolesterolemi (FH) med tidlig hjertesykdom og FH-personer med sen eller ingen hjerte-sykdom". Overlege Kjetil Retterstøl Lipidklinikken, Oslo Universitetssykehus/ Førsteamanuensis Kirsten Holven, Avdeling for Ernæringsvitenskap, Universitetet i Oslo er ansvarshavende for forskningsbiobanken. Biobanken varer til 2027. Etter dette vil materiale og opplysninger bli destruert og slettet etter interne retningslinjer.

Utlevering av materiale og opplysninger til andre

Hvis du sier ja til å delta i studien, gir du også ditt samtykke til at prøver og aidentifiserte opplysninger utleveres til de forskningsinstitusjonene som er samarbeidspartnerne i prosjektet.

Retten til innsyn og sletting av opplysninger om deg og sletting av prøver

Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger. Prøven kan dog ikke kreves destruert hvis opplysningene inngår i et større arbeide eller inngår i vitenskapelige arbeider.

Økonomi

- Prosjektet er et samarbeidsprosjekt mellom Lipidklinikken, Oslo Universitetssykehus og Avdeling for Ernæringsvitenskap, Universitetet i Oslo. Prosjektet finansieres av egne driftsmidler Avdeling for Ernæringsvitenskap, Universitetet i Oslo. Det vil bli søkt støtte til prosjektet fra ulike forskningsfond. De prosjektansvarlige har ingen økonomiske interesser i prosjektet.

Forsikring

- Dersom et uhell eller en komplikasjon skulle inntreffe, er deltagerne forsikret gjennom pasientskadeerstatningsordningen.

Informasjon om utfallet av studien

Resultatene fra studien vil bli publisert, og deltagerne vil få informasjon om hvor publisering skjer.

- Ansvarshavende for prosjektet er professor Kirsten B Holven, Avdeling for Ernæringsvitenskap, Institutt for Medisinske basalfag, UiO (e-post: kirsten.holven@medisin.uio.no, tlf 22851361) og overlege Kjetil Retterstøl, Lipidklinikken, Rikshospitalet (e-post: kjetil.retterstol@rikshospitalet.no).

Samtykke til deltakelse i studien

Jeg er villig til å delta i studien

(Signert av prosjektdeltaker, dato)

Jeg bekrefter å ha gitt informasjon om studien

(Signert av prosjektansvarlig, dato)

Appendix 8. SmartDiet food questionnaire

De gode rådene finner du her

Mettet fett er kolesteroløkende. Reduser derfor inntaket av matvarer med mye mettet fett. Velg i stedet matvarer med umettet fett som kan senke kolesterolet.

Drink mager melk, ½ liter skummet, søt eller sur, daglig. Dersom du ikke drikker melk daglig, kan det føre til et for lavt inntak av kalsium.

Alle fløte- og rømmetyper inneholder mye mettet fett og anbefales ikke i hverdagskostholdet. Cultura, skummet kultur, lettmeik, ekstra lett melk, skummet melk, yoghurt, mager Crème Fraiche (10 % fett) og Kesam (1 % fett) kan brukes i matlaging, til sauser og dressing.

Ost er en kilde til store mengder mettet fett. Velg lettere eller mager ost (ost med mindre enn 10 % fett) til hverdags. Ikke bruk lettere ost som pålegg på mer enn en tredel av dagens brødkiver. Vær også oppmerksom på mengde og type ost du bruker i matlagingen. Velg gjerne planteoljebaserte oster som pålegg og i matlagingen.

Fett kjøtt er også en kilde til store mengder mettet fett. Velg kjøtt med mindre enn 10 % fett både som middagsmat og som pålegg. Skjær bort alt synlig fett, og spis minst mulig oppblandede kjøttprodukter. Velg for eksempel karbonadedeig eller kylling-/svinekjøttdeig fremfor kjøttdeig. Fjern skinnet på kylling, kalkun og annet fjærkre. Velg skinkeprodukter som pålegg fremfor salami, fårepølse og lignende.

Spis alle typer fisk til middag flere ganger i uken. Fet fisk som makrell, sild, laks og ørret inneholder umettet fett (omega-3) og er derfor spesielt gunstig. Spis fisk som pålegg daglig. Ta i tillegg 1 skje tran, eventuelt 2 fiskeoljekapsler, daglig året rundt.

Bruk gjerne majonespålegg daglig, men i moderate mengder. De fleste majonesprodukter inneholder mye olje og derfor mye fett (og kalorier), men fettet er umettet og derfor gunstig.

Myk plantemargarin er en god kilde til umettet fett. Velg typer med mer enn 70 % umettet fett. Velg gjerne margarin med plantesteroler. Plantesteroler er gunstig for kolesterolet. Ved bruk av medikamentet Ezetrol® (ezetimib) forventes imidlertid ikke plantesteroler å gi noen ytterligere kolesterolreduksjon.

Bruk gjerne olje, flytende eller myk plantemargarin i matlagingen (velg typer med mer enn 70 % umettet fett). Spis mindre stekt mat. Velg heller kokt eller

ovnsstekt mat, da vil behovet for fett i matlagingen reduseres.

Grove kornprodukter er viktig i hverdagskostholdet. Spis mye av alle sorter fiberrike kornprodukter. Havre er spesielt gunstig og bør brukes regelmessig. Brødet bør inneholde mer enn 6 gram fiber pr 100 gram brød. Se også etter Brødskala'n på emballasjen.

Husk "5-om-dagen". Spis minst tre porsjoner grønnsaker og to porsjoner frukt hver dag. Fyll halve middagstallerkenen med grønnsaker, både rå og lettkokte. Spis frukt og grønnsaker som mellommåltid, som pålegg og som pynt på pålegget. Vær raus med porsjonene. Erter, bønner og linser kan med fordel spises ofte.

En porsjon poteter, ris eller pasta daglig er et fint tilbehør til middagen.

Bruk minst mulig sukker, sukkerholdig mat og drikk, som kjeks, kaker, is, søtt pålegg, sukker-godt, sjokolade, juice, nektar, saft og brus. Med unntak av fruktjuice gir disse produktene ingen eller få næringsstoffer, men kan bidra til økt vekt. Sukker (inkludert fruktsukker) kan også øke triglyseridverdiene.

Nøtter og mandler inneholder gunstig umettet fett, men er veldig kaloririke. Bruk det derfor gjerne, men i begrenset mengde. Kokosnøtten og chillinøttene inneholder mye mettet fett og bør derfor unngås.

Kaffebønnen inneholder fettstoffer som øker kolesterolet. Velg derfor pulverkaffe (inneholder ikke fett) eller kaffe som blir filtrert, da filteret fjerner det meste av fettstoffene. Husk at kaffe tilsatt melk (for eksempel café latte, cappuccino) kan være en kilde til mettet fett avhengig av melketyper som brukes og mengde kaffe som drikkes.

Alkohol inneholder mye kalorier og kan derfor føre til vektøkning. Alkohol kan også øke triglyseridverdiene.

Eggeplommen inneholder mye kolesterol. Begrens inntaket til to eggeplommer per uke. Den største årsaken til økning av kolesterolet i blodet er likevel matvarer rike på mettet fett.

SmartDiet™

26 spørsmål om ditt kosthold og din livsstil

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Les spørsmålene og de angitte svarmulighetene nøye!

Sett kryss ved det svaret som passer best med det du vanligvis spiser.

Kommentarer:

Antall poeng: _____

Kostholdsvurdering

27 poeng eller mindre:	Du bør forbedre kostholdet ditt på mange punkter, for å gjøre det mer helse- og hjertevennlig.
28-35 poeng:	Du kan forbedre kostholdet ditt på en del punkter, slik at det blir mer helse- og hjertevennlig.
36 poeng eller mer:	Du har sunne kostholdsvaner.

Spørreskjemaet vil ikke nødvendigvis gi et komplett bilde av ditt kosthold. Du kan få mer informasjon om kostholdet i heftet "Kostbehandling ved høye blodlipider hos voksne" (Lipidklinikken 2006).

Spørsmål 1-15 med unntak av spørsmål 10 er evaluert i forhold til veid kostholdsregistrering.

Kilde: Svilaas A, Strøm EC, Svilaas T, Borgejordet Å, Thoresen M, Ose L. SmartDiet™, a health educational tool. Reproducibility and validity of a short food questionnaire for assessment of dietary habits. Nutr Metab Cardiovasc Dis 2002; 12: 60-70. Tredje revidering av skjemaet utgitt i mai 2009.

Navn:

Fødselsdato: Dato for besvarelsen:

Navn på fastlege:

Adresse til fastlege:

Sett ett kryss til hvert spørsmål ved å krysse av i sirkelen ved det alternativet som passer best med det du vanligvis spiser.
 Vær oppmerksom på at spørsmålene veksler mellom å spørre etter daglig og ukentlig forbruk.

1. Melk (sur/søt) og yoghurt

Hvor mange glass melk drikker/bruker du daglig som drikke, i matlagingen, på gryn, i grøt, i dessert, i kaffe/te o.l.? Antall:.....

Hvor mange små beger med yoghurt (ca 1 dl) spiser du i løpet av en uke? Antall:.....

Hvilken type melk bruker du oftest?

- Helmelk • Kulturmilk • Kefir • Kaffemelk 5 % fett
- Lettmelk • Cultura • Biola naturell (syrt lettmelk) • Ekstra Lett melk • Melk med smak
- Skummet melk • Skummet kultur melk • Biola bærdrick 0,1 % fett
- Drikker/bruker mindre enn 1 liter melk i uken eller bruker aldri

2. Fløte, rømme o.l.

Hvilken type bruker du oftest i matlagingen, i dressing, i dip, i kaker, i kaffe/te o.l.

- Kremfløte • Crème Fraiche • Seterromme • Pisket krem
- Matfløte • Lettromme • Crème Fraiche lett
- Kaffefløte • Ekstra lett rømme • Vikingmelk • Kesam • Matyoghurt • Crème Fraiche 10 % fett ..
- Bruker ikke dette ukentlig eller bruker aldri

3. Ost på brødmaten, i matlaging, på pizza o.l.

Hvor mye ost som pålegg, regnet i osteskiver eller i spiseskjeer (for smørbar ost), spiser du daglig? Antall:.....

Til hvor mange middager per uke bruker du ost? (eks pizza, lasagne, i saus, i salat o.l.) Antall:.....

Hvilken type ost bruker du oftest?

- Hvitost • Nøkkelost • Gudbrandsdalsost (G35) • Ekte geitost • Flotemysost • Edamer • Gräddost • "Dessert oster" • Smørbare fete oster • Mozzarella • Revet pizza-/pastaost • Taffelost • Burgerost • Snøfrisk • Parmesan
- Lettere hvitost • Lettere nøkkelost • Lettere fløtemysost • Lettere Gudbrandsdalsost • Lettere smørbare oster • Mozzarella • Fetaost • Prim med vaniljesmak
- Ost med raps- og solsikkeolje (Vita Gul o.l.) • Cottage cheese • Gammalost • Pultost • Mager mysost • Prim • Mager prim • "Så lett" ost 10 % fett
- Bruker ost kun en gang i uken eller bruker aldri

4. Kjøttpålegg

Hvilken type kjøttpålegg bruker du oftest?

- Salami • Lett salami • Servelat • Fårepølse • Stabburpølse • Morrpølse • Haugpølse • Reinsdyrpølse • Falukorv • Fleskepølse • Sylte • Lammerull • Paté • Fenalår • Leverpostei (vanlig)
- Kokt/røkt skinke • Hamburgerrygg • Krydderskinke • Pastramiskinke • Roastbiff • Bankekjøtt • Kylling- og kalkunpålegg • Lett servelat • Kalverull • Spekeskinke uten fettrand • Oljebaserte posteier (Vita, Mills, Delikat, Gilde) • Mager leverpostei
- Bruker kjøttpålegg kun en gang i uken eller bruker aldri

5. Kjøtt til middag

Hvilken type kjøtt bruker du oftest?

- Familiedeig • Medisterdeig • Grillpølse • Wienerpølse • Kjøttpølse • Medisterpølse • Knakkpølse • Nakkekoteletter med fettrand • Lammekoteletter • Medisterkake • Wienerschnitzel • Bacon • Flesk • Grillben • Fårekjøtt
- Kjøttdeig (okse, lam) • Kyllingpølse • Lettpølse • Kjøpte karbonader • Hamburger • Kebabkjøtt • Kjøttkaker • Kjøttpudding • Kamkoteletter med fettrand • Nakkekoteletter uten fettrand • Kylling, kalkun og høne uten skinn • Bayonneskinke med fettrand • Hamburgerrygg med fettrand
- Karbonadedeig • Kjøttdeig (svin, kylling) • Biff • Filet (kylling, svin, okse, lam) • Viltkjøtt • Stek uten fettrand • Bogskinke • Kamkoteletter uten fettrand • Kjøtt uten synlig fett • Kylling, kalkun og høne uten skinn • "Go og mager" pølser • Vita pølser
- Spiser kjøtt kun en gang i uken eller spiser aldri

6. Fiskepålegg

Hvor ofte har du fisk som pålegg eller i salater til lunsj?

- Eksempel: Laks • Makrell • Sild • Sardiner • Brisling • Tunfisk • Reker • Krabbe • Crab-sticks • Fiskepudding • Fiskekaker o.l.*
- På inntil 1 brødkive i uken eller aldri
- På 2-4 brødkiver i uken
- På 5 eller flere brødkiver per uke

7. Fisk til middag

Hvor mange ganger i uken spiser du fisk, fiskemat og/eller fiskeretter?

- Inntil en gang i uken eller aldri
- 2 ganger i uken
- 3 eller flere ganger i uken

Til hvor mange av disse middagene spiser du fet fisk ukentlig? Antall:.....

Eksempel: Ørret • Laks • Makrell • Kveite • Sild

8. Majones, remulade og kaviar

Hvor ofte bruker du majonesprodukter, remulade og/eller kaviar på brødmaten?

- Eksempel: Majones • Rekesalat • Italiensk salat • Crab-stick salat • Skagensalat • Frokostsalat • Remulade • Kaviar/kaviamix o.l.*
- På inntil 1 brødkive i uken eller aldri
- På 2-7 brødkiver i uken
- På 8 eller flere brødkiver per uke

9. Smør eller margarin på brødmaten

Hvilken type bruker du oftest?

- Meierismør og alle andre typer smør • Smøregod • Bremyk • Brelett • Brelett Oliven • Melange margarin • Per margarin • Soft margarin uten salt og melk • Letta
- Soft Flora (beger) • Soft Light • Soya margarin • Soya lett margarin • Oliven margarin • Olivero • Solsikke margarin • Soft Ekstra • Brelett omega-3 margarin
- Vita • Vita lett • Vita Pro-aktiv • Becel Pro-activ • Münsterland Organic Margarin
- Bruker vanligvis ikke smør eller margarin på brødmaten

10. Plantesteroler

Bruker du et produkt som inneholder plantesteroler? Ja Nei

Eksempel: Vita Pro-aktiv • Becel Pro-aktiv

11. Fett i matlagingen

Hvilken type fett bruker du oftest til steking, baking, i saus, som dressing o.l.

- Meierismør og alle andre typer smør • Bremyk • Smøregod • Melange margarin (kube) • Per margarin • Soft Flora stekemargarin (kube) • Soya stekemargarin • Palmeolje
- Soft Flora (beger) • Soya margarin • Solsikke margarin • Oliven margarin • Olivero • Soft Ekstra
- Olje • Flytende margarin • Vita
- Bruker vanligvis ikke fett i matlagingen

12. Brød, knekkebrød og andre kornprodukter

Hvor mange skiver brød, rundstykker eller knekkebrød spiser du daglig? Antall:.....

Hvor mange porsjoner havregrøt, kornblanding eller andre typer frokostblandinger spiser du ukentlig? Antall:.....

Hvilken type brød og kornprodukter spiser du oftest?

- Kneippbrød • Firkornbrød • Landbrød • Jegerbrød • Loff • Fine rundstykker • Baguetter • Ciabatta • Lyst knekkebrød • Riskaker • Puffet ris • Cornflakes • Havrenøtter • Frokostkorn (med sjokolade, honning, sukker) o.l.
- Rugbrød • Pumpenikkel • Bakers havre-, spelt- og byggbrød • Vita brød • Goman havrebrød • Mesterbakeren grovbrød • Birkebeinerbrød • Mørke knekkebrød • Rugsprø • Fiberrik • Havregryn • Weetabix • Havrefras • Shredded wheat o.l.
- Spiser ikke brød, knekkebrød eller andre kornprodukter

13. Grønnsaker, frukt og bær

Hvor mange porsjoner grønnsaker, frukt og bær spiser du daglig?

- 1 porsjon = 150 g som tilsvarer ca 2 gulrøtter eller ca et stort eple*
- Mindre enn 2 porsjoner (< 300 g)
- 2-4 porsjoner (300-600 g)
- Mer enn 4 porsjoner (> 600 g)

Hvor mange av disse porsjonene er grønnsaker? Antall:.....

Hvor mange ganger i uken spiser du salat til lunsj? Antall:.....

14. Søtt pålegg og søt drikke

Hvor ofte bruker du søtt pålegg eller søt drikke med sukker eller frukt sukker?

- Eksempel: Syltetøy • Marmelade • Prim • Geitost • Sjokoladepålegg • Honning • Brus • Saft • Fruktjuice/juice • Nektar o.l.*
- 0-1 ganger daglig
- 2 ganger daglig
- 3 eller flere ganger daglig

15. Sjokolade, snacks, kaker, kjeks o.l.

Hvor ofte spiser du snacks?

- Eksempel: Sjokolade • Fløteis • Potetgull • Ostepop • Baconcrisp • Tortilla chips • Kaker • Kjeks • Smågodt o.l.*
- 0-1 ganger ukentlig
- 2 ganger ukentlig
- 3 eller flere ganger ukentlig

Totalt antall poeng: =====

16. Belgvekster

Spiser du belgvekster ukentlig? Ja Nei

Eksempel: Hvite tomatbønner • Brune bønner • Kikerter • Linser • Erter • Sukkererter o.l.

17. Potet, ris og pasta

Hvor mange porsjoner poteter, ris og/eller pasta spiser du daglig?

- En porsjon tilsvarer 2 poteter eller 2 dl kokt ris eller 2 dl kokt pasta/spaghetti*
- Spiser ikke 0-1 porsjon 2 porsjoner 3 porsjoner eller fler

Hva spiser du oftest? Potet Ris Pasta

18. Nøtter, mandler o.l.

Spiser du nøtter/mandler ukentlig? Ja Nei

Spiser du avokado eller oliven ukentlig? Ja Nei

19. Kaffe

Drikker du kaffe? Ja Nei

Hvis ja, hvilken type?

Eksempel: Cappuccino • Café latte • Presskannekaffe • Kokekaffe • Traktekaffe • Pulverkaffe o.l.

20. Alkohol

Drikker du alkohol? Ja Nei

- Hvis ja, hvor mange enheter drikker du til sammen per uke? Mindre enn 1 1-7 8-14 15 enheter eller flere

*1 enhet =
1 glass vin (125 ml)
1 glass øl (0.33 l)
4 cl brennevin*

21. Egg

Hvor mange egg, inkludert i matlaging, spiser du per uke? Antall:.....

1. Måltidsmønster

Hvor mange måltider, inkludert mellommåltider, spiser du daglig?

- 1-2 måltider 3 måltider
- 4 måltider 5 eller flere måltider

2. Høyde, vekt og midjemål

Høyde: cm Vekt: kg

Ønsker du å gå ned i vekt? Nei Ja

Hvis ja, hvor mange kilo ønsker du å gå ned i vekt?

Midjemål: cm (Fylles ut av helsearbeider)

3. Røyk/snus

Røyker du? Nei Ja Ja, selskapsrøyker

Hvis ja, hvor mange sigaretter/piper røyker du i gjennomsnitt per dag? Antall

Snuser du? Nei Ja

Hvis ja, hvor mange porsjoner snuser du i gjennomsnitt per dag? Antall

4. Mosjon

Hvor ofte mosjonerer du i minst 30 minutter slik at du blir lett andpusten eller svett?

- Eksempel: Rask gange • Løping • Skigåing • Svømming • Sykling o.l.*
- Sjeldnere enn 1 gang per uke eller aldri
- 1 til 2 ganger per uke
- 3 eller flere ganger per uke

Hvilken type mosjon bedriver du?

5. Kosttilskudd

Bruker du kosttilskudd?

- Nei Tran Fiskeoljekapsler/omega3-kapsler
- Multivitaminpreparat Annet