Cardiovascular abnormalities in familial combined hyperlipidemia

Citation for published version (APA):

Keulen, E. T. P. (2001). *Cardiovascular abnormalities in familial combined hyperlipidemia*. [Doctoral Thesis, Maastricht University]. Datawyse / Universitaire Pers Maastricht.
<https://doi.org/10.26481/dis.20011220ek>

Document status and date:

Published: 01/01/2001

DOI:

[10.26481/dis.20011220ek](https://doi.org/10.26481/dis.20011220ek)

Document Version:

Publisher's PDF, also known as Version of record

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

www.umlib.nl/taverne-license

Take down policy

If you believe that this document breaches copyright please contact us at:

repository@maastrichtuniversity.nl

providing details and we will investigate your claim.

Cardiovascular abnormalities in Familial Combined Hyperlipidemia

© Eric Keulen, Maastricht 2001

ISBN 90 5278 330 6

Cover design: Dimitri Delnoye

Production: Datawyse | Universitaire Pers Maastricht

Financial support by the Netherlands Heart Foundation for the publication of this thesis is gratefully acknowledged.

Printing of this thesis was financially supported by Amgen bv. (Breda), Astra Zeneca, Aventis, Bayer bv., Bristol-Myers Squibb, Byk Nederland bv., Merck Sharp & Dohme bv., Novartis Pharma bv., Novo Nordisk Pharma bv., Pfizer bv. and Roche.

Cardiovascular abnormalities in Familial Combined Hyperlipidemia

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit Maastricht,
op gezag van Prof. dr. A.C. Nieuwenhuijzen Kruseman,
rector magnificus,
volgens het besluit van het College van Decanen,
in het openbaar te verdedigen
op donderdag 20 december 2001 om 14.00 uur

door

Eric Theodorus Petronella Keulen

geboren op 31 maart 1972 te Geleen

Promotor

Prof.dr. T.W.A. de Bruin

Beoordelingscommissie

Prof.dr. P.W. de Leeuw (voorzitter)

Prof.dr. H.J.G.M. Crijns

Prof.dr. M.J.A.P. Daemen

Prof.dr. D.E. Grobbee (Universiteit Utrecht, Julius Instituut voor
Patiëntgebonden onderzoek)

Prof.dr. C.D.A. Stehouwer (Vrije Universiteit Amsterdam)

Life is what happens to you
while you're busy
making other plans
John Lennon

Aan: Bianca en Eva

Contents

	Abbreviations	9
Chapter 1	General introduction and aims of the thesis	11
Chapter 2	Subjects and general methods and epidemiological aspects of Familial Combined Hyperlipidemia	21
Chapter 3	Pathophysiological and genetic mechanisms in Familial Combined Hyperlipidemia, our current understanding	37
Chapter 4	Familial Dyslipidemic Hypertension Syndrome: further delineation in Familial Combined Hyperlipidemia, and the role of abdominal fat mass	53
Chapter 5	QT-interval (QTc) is prolonged in Familial Combined Hyperlipidemia: association with insulin resistance	69
Chapter 6	Pro-coagulant endothelium in Familial Combined Hyperlipidemia	83
Chapter 7	Reduced structural and functional skin capillaries in Familial Combined Hyperlipidemia: association with increased remnant-like lipoprotein cholesterol levels	91
Chapter 8	Increased intima media thickness in Familial Combined Hyperlipidemia: association with apolipoprotein B	105
Chapter 9.	General discussion and conclusions	119
Chapter 10.	Summary	129
Chapter 11.	Samenvatting	137
	List of publications	143
	Dankwoord	147
	Curriculum Vitae	153

Abbreviations

ADD1	alpha adducin A gene
ALP	atherogenic lipoprotein phenotype
Apo	apolipoprotein
ASP	acylation stimulatory protein
BC	basal number of capillaries
BMI	body mass index
CABG	coronary artery bypass graft
CAD	coronary artery disease
CI	confidence interval
CR	capillary recruitment
CV	coefficient of variation
CVA	cerebrovascular accident
CVD	cardiovascular disease
DBP	diastolic blood pressure
DH	dyslipidemic hypertension
FBG	fasting blood glucose concentration
FCHL	familial combined hyperlipidemia
FDH	familial dyslipidemic hypertension
FFA	free fatty acids
FI	fasting insulin
HC	hypercholesterolemia
HDL	high-density lipoprotein
HL	hepatic lipase
HL-relative	hyperlipidemic relative
HOMA	homeostasis model assessment (calculated marker for insulin resistance)
HSL	hormone sensitive lipase
HT	hypertriglyceridemia
IDL	intermediate density lipoprotein
IMT	intima media thickness
IRS	insulin resistance syndrome
LCAT	lecithin: cholesterol acyltransferase
LDBF	laser Doppler basal flow
LDF	laser Doppler fluxmetry
LDL	low-density lipoprotein
LDPF	laser Doppler peak flow
LDPRH	laser Doppler post-occlusive reactive hyperemia
Lp(a)	lipoprotein(a)
LPL	lipoprotein lipase
MAP	mean arterial pressure
MI	myocardial infarction
NEFA	non-esterified fatty acids
NHLBI	National Heart, Lung and Blood Institute

NL-relative	normolipidemic relative
OR	odds ratio
PAI-1	plasminogen activator inhibitor 1
POC	post-occlusive number of capillaries
PP	pulse pressure
PPAR	peroxisome proliferator-activated receptor
PTCA	percutane transluminal coronary angioplasty
PU	perfusion units
QTc	heart-rate corrected QT-interval
RF	radiofrequency
RLP	remnant-like lipoprotein particles
SBP	systolic blood pressure
sdLDL	small dense low-density lipoprotein
TC	total cholesterol
TFPI	tissue factor pathway inhibitor
TG	plasma triglyceride
TNF	tumor necrosis factor
t-PA	tissue type plasminogen activator
VIF	variance inflation factor
VLDL	very-low-density lipoprotein
vWF	von Willebrand factor
WHR	waist to hip ratio

Chapter 1

General introduction and aims of the thesis

ETP Keulen

1.1 Cardiovascular disease and cardiovascular risk factors

Cardiovascular disease (CVD) is a major cause of death in our western society. In 1995, cardiovascular diseases combined were responsible for 39% of all deaths and 16% of all hospital admissions in the Netherlands¹. Over the last decades, epidemiological studies have together written a cohesive story that convincingly links a number of important risk factors to the development of CVD²⁻⁵. The Framingham study, initiated in 1948 in Framingham, Massachusetts, USA, has developed into one of the most important longitudinal cohort studies of risk of cardiovascular disease, in both men and women. This study and other longitudinal studies have identified many of the major cardiovascular risk factors and have refined assessment of absolute risk that is slowly adapted into clinical practice.

Major cardiovascular risk factors, including increasing age, male gender³, previous history of cardiovascular disease⁶, positive family history of cardiovascular disease⁷, smoking⁸, hypertension, elevated total (TC)⁹ and low-density lipoprotein (LDL) cholesterol¹⁰, reduced high-density lipoprotein (HDL) cholesterol¹¹ and diabetes mellitus¹², are currently known. Newer risk factors, also known as 'non-classical' risk factors, like hypertriglyceridemia^{4,13}, hyperinsulinemia^{14,15}, elevated apolipoprotein B concentrations^{16,17}, elevated lipoprotein (a)¹⁸, homocysteine¹⁹, C-reactive protein²⁰, fibrinogen²¹, von Willebrand factor²² and plasminogen activator inhibitor 1²¹ concentrations have emerged over the past years. These newer risk factors often provide smaller increases in absolute cardiovascular risk than major risk factors, but are potentially important in clinical management of premature atherosclerosis. The increased number of classical and non-classical risk factors will, over time, provide us with the possibility to establish the cardiovascular risk profile and absolute cardiovascular risk of an individual more clearly. Not every classical or newly discovered risk factor can be adequately reduced or treated with current dietary or medical therapy. Therefore, knowledge of classical and non-classical risk factors may provide us with increased insight in cardiovascular risk profile assessment and possible atherogenic pathways, but leave us with limited therapeutic options to decrease the risk of subsequent cardiovascular events.

In clinical practice a distinction can be made between cardiovascular risk factors with potential therapeutic options and risk factors without therapeutic options. Despite the many cardiovascular risk factors currently known, few major risk factors have the biggest impact on subsequent cardiovascular events. For instance, without accounting for the impact of other risk factors, 42.7% of all subjects who died of CVD in the United States had a total cholesterol ≥ 5.2 mmol/L²³. Furthermore, of the subjects who died of CVD, 32.1% were obese; 28.9% had a systolic blood pressure above 140 mmHg, 25.1% were current or former smokers and 13.1% were diagnosed as having diabetes mellitus²³.

Since a decade, more attention has and still is been paid to genetically determined risk factors of cardiovascular disease, using linkage and association studies and identification of contributing genetic polymorphisms. Several genetic polymorphisms, specifically in genes participating in classical risk factor pathways, have been associated with increased risk of cardiovascular disease. Identification of gene-environment interactions will be important to identify subgroups at higher cardiovascular risk²⁴. Furthermore, genetic risk factor screening in cardiovascular disease is absolutely necessary to improve our understanding of biochemical atherogenic pathways eventually leading to subsequent clinical events.

1.2 Familial Combined Hyperlipidemia and cardiovascular risk

Multiple combinations of the above mentioned major cardiovascular risk factors will lead to an exponentially increased risk of early cardiovascular disease^{2-5,25,26}. Moreover, classical, non-classical and genetic risk factors together often cluster in familial disorders. Increased clustering of cardiovascular risk factors and cardiovascular events in families can provide us with a powerful strategy to identify genetic contributors to specific cardiovascular diseases.

One of the familial diseases with increased risk of cardiovascular disease is Familial Combined Hyperlipidemia (FCHL). FCHL, originally delineated in survivors of myocardial infarction, is one of the most common genetic lipid disorders²⁷⁻³⁰ affecting approximately 10% of the survivors of myocardial infarction^{27,29,31}, whereas the population frequency is estimated from 1:200 to 1:100 or 0.5-1.0%^{27,29,32}. FCHL is therefore associated with a substantially increased risk of CVD in index cases. Recently, two reports also addressed the seriously increased risk of fatal and non-fatal coronary artery disease in relatives of FCHL probands and found overall odds ratios of 1.7 and 5.1, respectively^{33,34}. The differences in odds ratios may relate to the different risk of CVD in the control population in each study^{33,34}.

FCHL presents, by definition, with a multiple lipoprotein phenotype among relatives in a family, i.e. elevated very-low-density lipoproteins (VLDL, type IV hyperlipidemia), elevated low-density lipoproteins (LDL, type IIa hyperlipidemia), or both (type IIb hyperlipidemia)²⁷. Moreover, the pattern of hyperlipidemia may change within one individual over time. Lipid levels are usually only moderately elevated in FCHL, with typical total cholesterol concentrations between 6 and 9 mmol/L and fasting triglyceride concentrations ranging from 2 to 6 mmol/L. Patients with FCHL are characterised by an overproduction of apolipoprotein B containing lipoproteins, including VLDL^{35,36}, LDL³⁷, and small dense LDL particles^{38,41}, in combination with a relative impairment of lipoprotein catabolism⁴². Furthermore, FCHL affected patients are frequently insulin resistant^{43,44}. Clearly, the aggregate of these abnormalities results in an unfavourable, atherogenic risk profile. However, these lipid-related cardiovascular risk factors alone may not fully explain the high CVD risk in FCHL.

1.3 Atherosclerosis

Established cardiovascular risk factors will, over time, result in advanced atherosclerosis and subsequent cardiovascular events. For a long time, atherosclerosis was viewed as a steadily progressive process in which fairly inert components relentlessly collected in the vessel wall until an artery developed stenosis, eventually leading to occlusion and subsequent cardiovascular events. Nowadays, it has been established that stable atherosclerotic lesions with a thick fibrous cap and protruding into the vascular lumen, are not the major cause of acute clinical events. Instead, most clinical events arise from lesions causing only mild to moderate angiographic stenosis or even from lesions that are invisible on angiography^{45,46}. Furthermore, it is currently known that lesion progression can be slowed, stopped and even regressed. Atherosclerosis is presently understood as a very dynamic process, with many different cellular elements that play a role in different stages of the process⁴⁷. Atherosclerosis has been divided in several stages, especially depending on the cellular elements involved and progression and regression characteristics of the lesions^{48,49}. However, one must bear in mind the continuous process of atherosclerosis⁵⁰. Moreover, several different stages of the atherosclerotic process will occur next to each other in the human vasculature. In the prevalent view, atherosclerosis is considered as an immune, inflammatory or healing response of the intima to injury, also known as the 'response to injury' theory, first presented by Russell Ross^{20,45,50,51}. The endothelium, long considered merely as a semi-permeable membrane, is now viewed a critical layer to maintain blood flow and vascular integrity. Healthy endothelium has anti-atherosclerotic properties and tends to favour vasodilatation, antithrombosis, fibrinolysis, and monocyte disadhesion⁵². Endothelial dysfunction is now regarded as the first step in the development of atherosclerosis. In principle, endothelial dysfunction is a reversible state. Dysfunctional endothelium is characterised by increased uptake of LDL and monocyte recruitment into the vessel wall, which are both pivotal initiating events in atherosclerosis⁵³. Atherosclerosis is viewed as a disorder restricted to the intima layer of the arterial vessel wall. Accumulation of lipid-filled macrophages, or foam cells, in the intima represents the bulk of the initial lesion named fatty streak. Chemo-attraction of smooth muscle cells of the media layer and subsequent deposition of connective tissue matrix will lead to a fibro-fatty lesion. As the lesions progress, the cells in the lesions undergo rearrangements that may lead to the advanced atherosclerotic lesions or fibrous plaque⁵⁰. The fibrous plaque is characteristically covered by a dense cap of fibrous tissue overlaying a cholesterol-rich inner layer. Unstable lesions are characterised by a lipid-rich core, a thin fibrous cap, few smooth muscle cells and fibroblasts, and a large number of foam cells, particularly at the shoulders of the caps. Unstable lesions are prone to rupture and lead to hemorrhage in the vessel wall or thrombotic occlusions, with subsequent ischemia and clinical cardiovascular events⁴⁵. Thus, before development of clinically manifest atherosclerosis and subsequent events, subclinical abnormalities in the different vascular beds will occur. Early detection of initial atherosclerotic lesions should give the physician more time to prevent

further deterioration of the process and the occurrence of devastating cardiovascular events.

1.4 Aims of the study

Although FCHL is now known for more than 25 years, most of the research has been focussed upon the pathophysiology of lipid abnormalities and insulin resistance found in FCHL. Moreover, a lot of effort has been directed to identify the genes contributing to FCHL. First described as single gene disease, FCHL is nowadays regarded as a clinical entity caused by one or two major genes and a range of modifier genes. Most of the genetic researches have been focussed at genes involved in the lipid or insulin pathways. However, despite the high cardiovascular risk in FCHL families, little is known about early manifestations of vascular abnormalities, and the atherosclerotic process in FCHL. Furthermore, the presently known high risk of developing a cardiovascular event in FCHL can not be solely explained by the lipid-related cardiovascular risk factors studied so far.

Therefore, the overall objective of this thesis is:

- A to describe cardiovascular risk factors that potentially contribute to the high risk of cardiovascular disease in Familial Combined Hyperlipidemia.
- B and to characterise early cardiovascular abnormalities in a range of vascular beds to provide us with a better view of early manifestations of the atherosclerotic process in subjects affected with Familial Combined Hyperlipidemia.

In this light, we studied:

- 1) The association of dyslipidemia and hypertension, in which abdominal fat mass is important (**chapter 4**).
- 2) The heart rate corrected QT-interval (QTc), and its association with insulin resistance (**chapter 5**).
- 3) Abnormalities in endothelium-derived proteins of the coagulation and fibrinolytic systems (**chapter 6**).
- 4) Capillary structure and function, and the potential association with hyperlipidemia, blood pressure values and insulin resistance (**chapter 7**).
- 5) Intima media thickness, and the potential association with hyperlipidemia, blood pressure values and insulin resistance (**chapter 8**).

Because a single biochemical or genetic marker for FCHL is unavailable at present, family studies are still required to establish the diagnosis. In **chapter 2**, the family survey methodology is described, including descriptive epidemiological aspects of environmental factors in ascertained FCHL families. Moreover, the range of different measurement methodologies used in each subsequent chapter is described. **Chapter 3** summarises our current pathophysiological view of lipid abnormalities and insulin resistance found in FCHL. Furthermore a brief overview on FCHL modifier genes identified up till now is presented.

References

1. Reitsma JB, Dalstra JA, Bonsel GJ, van der Meulen JHP, Koster RW, Gunning-Schepers LJ, Tijssen JGP. Cardiovascular disease in the Netherlands, 1975 to 1995: decline in mortality, but increasing numbers of patients with chronic conditions. *Heart* 1999;82:52-56.
2. Yusuf HR, Giles WH, Croft JB, Anda RF, Casper ML. Impact of multiple risk factor profiles on determining cardiovascular disease risk. *Preventive Medicine* 1998;27:1-9.
3. Castelli WP. Epidemiology of coronary heart disease: the Framingham study. *American Journal of Medicine* 1984;76:4-12.
4. Assmann G, Schulte H. Relation of high-density lipoprotein cholesterol and triglycerides to incidence of atherosclerotic coronary artery disease (the PROCAM experience). Prospective Cardiovascular Munster study. *American Journal of Cardiology* 1992;70:733-737.
5. Stamler J, Dyer AR, Shekelle RB, Neaton J, Stamler R. Relationship of baseline major risk factors to coronary and all-cause mortality, and to longevity: findings from long-term follow-up of Chicago cohorts. *Cardiology* 1993;82:191-222.
6. Rosengren A, Hagman M, Wedel H, Wilhelmsen L. Serum cholesterol and long-term prognosis in middle-aged men with myocardial infarction and angina pectoris. A 16-year follow-up of the Primary Prevention Study in Goteborg, Sweden. *European Heart Journal* 1997;18:754-761.
7. Eaton CB, Bostom AG, Yanek L, Laurino JP, McQuade W, Huma A, Selhub J. Family history and premature coronary heart disease. *Journal of the American Board of Family Practice* 1996;9:312-318.
8. Lakier JB. Smoking and cardiovascular disease. *American Journal of Medicine* 1992;93:8S-12S.
9. Phillips NR, Waters D, Havel RJ. Plasma Lipoproteins and Progression of Coronary Artery Disease Evaluated by Angiography and Clinical Events. *Circulation* 1993;88:2762-2770.
10. Kraus RM. Low-density lipoprotein subclasses and risk of coronary artery disease. *Current Opinion in Lipidology* 1991;2:248-252.
11. Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD, Jacobs-Jr DR, Bangdiwala S, Tyroler HA. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation* 1989;79:8-15.
12. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *New England Journal of Medicine* 1998;339:229-234.
13. Gotto-Jr AM. Triglyceride: the forgotten risk factor. *Circulation* 1998;97:1027-1028.
14. Fontbonne A, Charless MA, Thibault N. Hyperinsulinemia as a predictor of coronary heart disease mortality in a healthy population: the Paris prospective study, 15 years follow-up. *Diabetologia* 1998;34:356-361.

15. Feskens EJM, Kromhout D. Hyperinsulinemia, risk factors, and coronary artery disease. *Arteriosclerosis Thrombosis and Vascular Biology* 1994;14:1641-1647.
16. Lamarche B, Tchernof A, Mauriege P, Cantin B, Dagenais, Lupien PJ, Depress JP. Fasting insulin and apolipoprotein B levels and low-density lipoprotein particle size as risk factors for ischemic heart disease. *Journal of American Medical Association* 1998;279:1955-1961.
17. Lamarche B, Lemieux I, Despres JP. The small, dense LDL phenotype and the risk of coronary heart disease: epidemiology, patho-physiology and therapeutic aspects. *Diabetes Metabolism* 1999;25:199-211.
18. Scanu AM. Update on lipoprotein (a). *Current Opinion in Lipidology* 1991;2:253-258.
19. Duell PB, Malinow MR. Homocyst(e)ine: an important risk factor for atherosclerotic vascular disease. *Current Opinion of Lipidology* 1997;8:28-34.
20. Ross R. Atherosclerosis--an inflammatory disease. *New England Journal of Medicine* 1999;340:115-126.
21. Thogersen AM, Jansson JH, Boman K, Nilsson TK, Weinehall L, Huhtasaari F, Hallmans G. High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. *Circulation* 1998;98:2241-2247.
22. Jager A, van Hinsbergh VW, Kostense PJ, Emeis JJ, Yudkin JS, Nijpels G, Dekker JM, Heine RJ, Bouter LM, Stehouwer CD. von Willebrand factor, C-reactive protein, and 5-year mortality in diabetic and nondiabetic subjects: the Hoorn Study. *Arteriosclerosis Thrombosis and Vascular Biology* 1999;19:3071-3078.
23. Gotto-Jr AM, Pownall HJ. Observational epidemiology and risk factors. In: *Manual of lipid disorders*, edited by Redford DC. Williams & Wilkins Inc., 1999;160-191.
24. Talmud PJ, Bujac SR, Hall S, Miller GJ, Humphries SE. Substitution of asparagine for aspartic acid at residue 9 (D9N) of lipoprotein lipase markedly augments risk of ischaemic heart disease in male smokers. *Atherosclerosis* 2000;149:75-81.
25. Turner RC, Millns H, Neil HA, Strathon IM, Manley SE, Matthews DR, Holman RR. Risk factors for coronary artery disease in non-insulin dependent diabetes mellitus: United Kingdom Prospective Diabetes Study (UKPDS: 23). *British Medical Journal* 1998;316:823-828.
26. Anderson KM, Odell PM, Wilson PWF, Kannel WB. Cardiovascular disease risk profiles. *American Heart Journal* 1991;121:293-298.
27. Goldstein JL, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG. Hyperlipidaemia in Coronary Heart Disease. II Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidaemia. *Journal of Clinical Investigation* 1973;52:1544-1568.
28. Rose HG, Kranz P, Weinstock M, Juliano J, Haft JL. Inheritance of combined hyperlipoproteinaemia: evidence for a new lipoprotein phenotype. *American Journal of Medicine* 1973;54:148-160.
29. Nikkilä EA, Aro A. Family study of serum lipids and apolipoproteins in coronary artery disease. *Lancet* 1973;78:954-959.
30. Brunzell JD, Schrott HG, Motulsky AG, Bierman EL. Myocardial infarction in the familial forms of hypertriglyceridemia. *Metabolism* 1976;25:313-320.

31. Genest-Jr JJ, Martin MS, McNamara JR, Ordovas JM, Jenner J, Meyers R, Silberman SR, Wilson PWF, Salem DN, Schaefer EJ. Familial lipoprotein disorders in patients with premature coronary artery disease. *Circulation* 1992;85:2025-2033.
32. Motulsky AG. Current concepts in genetics. The genetic hyperlipidemias. *New England Journal of Medicine* 1976;294:823-827.
33. Austin MA, McKnight B, Edwards KL, Bradley CM, McNeely MJ, Psaty BM, Brunzell JD, Motulsky AG. Cardiovascular disease mortality in familial forms of hypertriglyceridemia: A 20-year prospective study. *Circulation* 2000;101:2777-2782.
34. Voors-Pette C, de Bruin TWA. Excess coronary heart disease in Familial Combined Hyperlipidemia, in relation to genetic factors and central obesity. *Atherosclerosis* 2001;157:481-489.
35. Venkatesan S, Cullen P, Pacy P, Halliday D, Scott J. Stable isotopes show a direct relation between VLDL apoB overproduction and serum triglyceride levels and indicate a metabolically and biochemically coherent basis for familial combined hyperlipidemia. *Arteriosclerosis Thrombosis and Vascular Biology* 1993;13:1110-1118.
36. Castro Cabezas M, de Bruin TWA, de Valk HW, Shoulders CC, Jansen H, Erkelens DW. Impaired fatty acid metabolism in familial combined hyperlipidemia. A mechanism associating hepatic apolipoprotein B overproduction and insulin resistance. *Journal of Clinical Investigation* 1993;92:160-168.
37. Kissebah AH, Alfarsi S, Evans DJ. Low density lipoprotein metabolism in familial combined hyperlipidemia. Mechanism of the multiple lipoprotein phenotypic expression. *Arteriosclerosis* 1984;4:614-624.
38. Hennekens CH. Increasing burden of cardiovascular disease; current knowledge and future directions for research on risk factors. *Circulation* 1998;97:1095-1102.
39. Bredie SJ, Kiemeny LA, de Haan AF, Demacker PN, Stalenhoef AF. Inherited susceptibility determines the distribution of dense low-density lipoprotein subfraction profiles in familial combined hyperlipidemia. *American Journal of Human Genetics* 1996;58:812-822.
40. Bredie SJ, Demacker PN, Stalenhoef AF. Metabolic and genetic aspects of familial combined hyperlipidaemia with emphasis on low-density lipoprotein heterogeneity. *European Journal of Clinical Investigation* 1997;27:802-811.
41. Hokanson JE, Krauss RM, Albers JJ, Austin MA, Brunzell JD. LDL physical and chemical properties in familial combined hyperlipidemia. *Arteriosclerosis Thrombosis and Vascular Biology* 1995;15:452-459.
42. Cabezas MC, de Bruin TWA, Jansen H, Kock LA, Kortlandt W, Erkelens DW. Impaired chylomicron remnant clearance in familial combined hyperlipidemia. *Arteriosclerosis and Thrombosis* 1993;13:804-814.
43. Bredie SJH, Tack CJJ, Smits P, Stalenhoef AFH. Non-obese patients with familial combined hyperlipidemia are insulin resistant as compared with their non-affected relatives. *Arteriosclerosis Thrombosis and Vascular Biology* 1997;17:1465-1471.
44. Hunt SC, Wu LL, Hopkins PN, Stults BM, Kuida H, Ramirez ME, Lalouel JM, Williams RR. Apolipoprotein, low density lipoprotein subfraction, and insulin associations with familial combined hyperlipidemia. Study of Utah patients with familial dyslipidemic hypertension. *Arteriosclerosis* 1989;9:335-344.

45. Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes. *New England Journal of Medicine* 1992;326:242-250.
46. Brown BG, Zhao X-O, Sacco DE, Albers JJ. Lipid lowering and plaque regression: new insights into prevention of plaque disruption and clinical events in coronary disease. *Circulation* 1993;87:1781-1791.
47. Lusis AJ. Atherosclerosis. *Nature* 2000;407:233-241.
48. Stary HC, Chandler AB, Glagov S, Guyton JR, Insull W, Rosenfeld ME, Schaeffer SA, Schwartz CJ, Wagner WD, Wissler RW. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation* 1994;89:2462-2478.
49. Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W, Rosenfeld ME, Schwartz CJ, Wagner WD, Wissler RW. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Arteriosclerosis Thrombosis and Vascular Biology* 1995;15:1512-1531.
50. Ross R. Cell biology of atherosclerosis. *Annual Review of Physiology* 1995;57:791-804.
51. Ross R. Atherosclerosis is an inflammatory disease. *American Heart Journal* 1999;138:S419-S420.
52. Vogel RA. Coronary risk factors, endothelial function, and atherosclerosis: a review. *Clinical Cardiology* 1997;20:426-432.
53. Fuster V, Gotto AM, Libby P, Loscalzo J, McGill HC. 27th Bethesda Conference: matching the intensity of risk factor management with the hazard for coronary disease events. Task Force 1. Pathogenesis of coronary disease: the biologic role of risk factors. *Journal of American College of Cardiology* 1996;27:964-976.

Chapter 2

Subjects and general
methods
Epidemiological aspects
of Familial Combined
Hyperlipidemia
Specific methodology

ETP Keulen

2.1 Subjects and general methods

2.1.1 Introduction

Up till now, no unique marker is available to identify a single subject as affected with Familial Combined Hyperlipidemia (FCHL). Therefore, a family study has to be conducted to establish the diagnosis of FCHL. Furthermore, in the framework of genetic and metabolic studies in FCHL, a family survey is needed. In this chapter we will address the collection of FCHL families used in the different studies described in the subsequent chapters of this thesis. Furthermore, the nature and frequency of hyperlipidemia in 'Maastricht' FCHL families and the occurrence and clustering of main cardiovascular risk factors (hypertension, obesity and smoking) is described. Finally, the main survey method used in each following chapter is briefly mentioned.

2.1.2 Family survey

In 1991, at the department of Internal Medicine and Endocrinology of the University Hospital Utrecht, Tjerk de Bruin and colleagues started with a family survey of Caucasian FCHL index patients (proband). Twenty-seven unrelated Caucasian probands were identified at the Lipid Clinic. In total, during the period 1991 to 1997, 95% of living relatives over 18 years of age have been collected ($n=470$), to confirm the diagnosis of FCHL and in the framework of metabolic and genetic studies. Part of these FCHL relatives were used for the blood pressure analyses as described in **chapter 4**.

Since 1998, family surveys for FCHL were conducted at the Department of Internal Medicine and Endocrinology of the University Hospital Maastricht. Thirty-nine unrelated Caucasian probands were identified at the out-patient clinic of the Department of Internal Medicine and their families were subsequently invited to participate in our FCHL research program. In total, during the period 1998 to 2001, 95 % of living relatives of 18 years of age and older have been collected ($n=396$) to confirm the diagnosis of FCHL.

In both places, married-in spouses and partners were asked to participate in our research project to establish a control group (Utrecht, $n=215$; Maastricht, $n=144$). All spouses had a negative history for familial premature cardiovascular disease (CVD) or familial hyperlipidemia. Still a single spouse individual could suffer from CVD or have been diagnosed with hyperlipidemia in the past. Part of FCHL affected subjects and spouses from 'Maastricht' families were used for the analysis in **chapters 5 to 8**.

2.1.3 Diagnosing FCHL

To establish the diagnosis of FCHL in a family, each of the following criteria were met: first, a proband with a primary hyperlipidemia with varying phenotypic expression including fasting total plasma cholesterol (TC) >6.5 mmol/L (250 mg/dL) and/or a fasting plasma triglyceride (TG) concentration >2.0 mmol/L (180 mg/dL); second, at least one first-degree relative with a different hyperlipidemic phenotype (Fredrickson Classification IIa, IIb or IV) from the proband; third, a positive family history of premature cardiovascular disease (CVD). Premature CVD was defined as the occurrence of a myocardial infarction (MI) or cerebrovascular incidence before the age of 60 years in at least one first degree relative of the proband, or the proband him-/herself. Secondary causes of hyperlipidemia (renal or hepatic insufficiency and hypothyroidism), presence of the apo E2/E2 genotype (thus, familial dysbetalipoproteinemia), and subjects with tendon xanthomas or a diagnosis matching familial hypercholesterolemia were excluded. A family tree of a FCHL family is depicted in Figure 2.1. Actual measured lipid values at the screening visit, all untreated, were used to diagnose FCHL relatives as hyperlipidemic or normolipidemic. As stated above, hyperlipidemia was defined as a total cholesterol >6.5 mmol/L or fasting plasma triglyceride concentration >2.0 mmol/L, or a combination of both. Otherwise a relative was diagnosed as normolipidemic.

Figure 2.1. FCHL pedigree

Legend: HC = total cholesterol >6.5 mmol/L; HT = fasting plasma triglyceride >2.0 mmol/L; HC + HT = combination of both; MI = myocardial infarction.

2.1.4 Materials and methods

Clinical measurements were performed at the clinical research unit in the morning (08⁰⁰ - 11⁰⁰) after an overnight fast (12-14 h). Subjects had refrained from smoking and had not drunk coffee or tea in the morning. Participants also had abstained from alcohol for at least 72 hours. Any lipid lowering medication had been withdrawn for two weeks before blood samples were collected. At the screening visit, a standardised questionnaire for smoking habits, use of alcohol, habitual physical activity, history of cardiovascular disease and use of medication was obtained from all participating subjects. A detailed family history was obtained from all probands. Subsequently, blood pressure values (OMRON Health Care GmbH, Hamburg, Germany) and antropometric parameters, i.e. weight, height, body mass index (BMI), waist and hip circumference, waist to hip ratio (WHR), were obtained. After all physical tests were performed fasting venous blood was collected in pre-cooled EDTA (1-mg/ml) Vacutainer tubes for measurements of lipid, lipoprotein, glucose and insulin concentrations, using commercially available methods (for details, see method sections in following chapters)

2.2 Epidemiological aspects of Familial Combined Hyperlipidemia

2.2.1 The epidemiology of cardiovascular risk factors in FCHL

In the present analyses, a standardised questionnaire, antropometric parameters, blood pressure values, lipid and glucose concentrations were obtained from 35 Maastricht FCHL families, consisting of 362 relatives and 126 married-in spouses, as mentioned above.

Any lipid lowering medication had been withdrawn for two weeks prior to the study. Hyperlipidemia was defined as stated before. Hypertension was defined as either systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg, or the current use of antihypertensive medication^{1,2}. Overweight was defined as body mass index >27.5 kg/m² and obesity as BMI >30 kg/m².

The characteristics of the subjects studied are presented in Table 2.1. In these Dutch FCHL families, 143 out of 362 (39.5%) relatives were currently diagnosed with hyperlipidemia compared to 23 out of 126 (18.3%) spouses ($p<0.001$). Hyperlipidemic FCHL (HL) relatives were significantly older than normolipidemic (NL) relatives, with significantly higher BMI, WHR, waist-circumference, systolic (SBP) and diastolic (DBP) blood pressure and total cholesterol, fasting plasma triglyceride, low-density lipoprotein cholesterol, apolipoprotein B, glucose and insulin concentrations, and significantly lower high-density lipoprotein cholesterol levels. Comparing HL relatives versus spouses revealed the same significant differences as in comparison to NL relatives. Spouses are of the same age as the HL relatives. NL relatives were significantly younger than spouses, and showed lower WHR and lower plasma total cholesterol concentration.

The different hyperlipidemic phenotypes (i.e. HC, HT and HC + HT) were almost equally distributed among affected FCHL relatives (Figure 2.2). As already mentioned in the preceding chapter, many risk factors contributing to cardiovascular events are currently known. Major cardiovascular risk factors, like increasing age, hyperlipidemia, hypertension, obesity and current smoking, alone or in combination, have an important contribution to a high cardiovascular risk. Increasing age not only increases the absolute risk of cardiovascular events, but has also been associated with an increased prevalence of hyperlipidemia and hypertension in a general population. In our FCHL population, an increasing prevalence of hyperlipidemia (Figure 2.3) and hypertension (chapter 4) with increasing age was observed, far in excess of our spouse population. The prevalence of hyperlipidemia in FCHL families steeply increased from 15% (13 out of 86 subjects) under age 30 years up till 71.4% (25 out of 35 subjects) in the age category 60 – 69 years. A striking observation was that in FCHL subjects, hyperlipidemia occurs almost 20 years earlier than in the population; $p<0.01$ for trend (Figure 2.3).

Table 2.1. Characteristics of 362 FCHL relatives from 35 FCHL families and 126 married-in spouses.

	Hyperlipidemic relatives (n=143)	Normolipidemic relatives (n=219)	Spouses (n=126)
Age (years)	48.9 ± 13.1 [†]	37.6 ± 14.3 [†]	49.5 ± 14.2
BMI (kg/m ²)	27.6 ± 3.9 ^{*†}	24.6 ± 4.4	25.5 ± 3.4
WHR	0.94 ± 0.08 ^{*†}	0.87 ± 0.08 [‡]	0.89 ± 0.09
Waist (cm)	97.0 ± 11.1 ^{*†}	87.4 ± 13.0	90.3 ± 11.0
SBP (mmHg)	141.7 ± 21.8 ^{*†}	127.3 ± 17.7	133.4 ± 21.3
DBP (mmHg)	88.2 ± 12.3 ^{*†}	81.0 ± 10.1	83.8 ± 11.0
TC (mmol/L)	6.9 ± 1.5 ^{*†}	4.9 ± 0.9 [‡]	5.4 ± 1.0
HDL-C (mmol/L)	0.87 ± 0.24 ^{*†}	0.99 ± 0.23	1.04 ± 0.25
LDL-C (mmol/L)	4.6 ± 1.7 ^{*†}	3.5 ± 0.8	3.8 ± 1.2
TG (mmol/L)	2.8 ± 2.4 ^{*†}	1.1 ± 0.4	1.3 ± 1.2
Apo B (g/L)	1.4 ± 0.3 ^{*†}	1.0 ± 0.2	1.1 ± 0.2
Apo A1 (g/L)	1.4 ± 0.3 [*]	1.4 ± 0.3	1.5 ± 0.3
Glycerol (mmol/L)	77.9 ± 30.1	76.0 ± 33.2	71.7 ± 31.5
FFA (mmol/L)	0.5 ± 0.2	0.5 ± 0.2	0.4 ± 0.2
Glucose (mmol/L)	5.4 ± 1.2 ^{*†}	4.9 ± 0.7	5.0 ± 1.2
Insulin (μU/mL)	10.5 ± 7.6 ^{*†}	5.9 ± 4.4	6.1 ± 5.4

* = HL vs. Spouses, $p < 0.01$; [†] = HL vs. NL, $p < 0.001$; [‡] = NL vs. Spouses, $p < 0.01$
Differences in blood pressure values are age-corrected.

Figure 2.2. Distribution of hyperlipidemic phenotypes in FCHL-affected relatives.
Legend: HC = total cholesterol >6.5 mmol/L; HT = fasting plasma triglyceride >2.0 mmol/L; HC + HT = combination of both.

Figure 2.3. Age-dependent prevalence of hyperlipidemia (HL) in 35 FCHL-families ($n = 362$) and 126 married-in spouses.

Subsequently we evaluated the prevalence of several cardiovascular risk factors in HL- and NL-relatives and the spouse group (Table 2.2). Hypertension, overweight and obesity occurred more frequently in HL-relatives compared to spouses ($p < 0.01$ for all three), whereas no differences were observed in prevalence of smoking or alcohol use, nor in the amount of cigarettes smoked (pack years) or consumed alcohol units per week. Hypertension, overweight and obesity also occurred more often in HL-relatives than in NL-relatives, even after age-correction ($p < 0.001$). The percentage of NL-relatives using alcohol was significantly higher in comparison to HL-relatives. However, no difference in the amount of alcohol consumed per week was found.

It is plausible to assume that each single risk factor increases the risk of developing cardiovascular disease. Moreover, multiple combinations of these risk cardiovascular risk factors shall lead to an exponentially increased risk of cardiovascular events. For that reason we analysed the clustering of several risk factors in our study populations. A significant clustering of main cardiovascular risk factors (hyperlipidemia, hypertension, obesity and current smoking) in all FCHL family members was observed compared to spouses; $p < 0.05$ for trend (Figure 2.4). Clearly, the aggregate of these abnormalities results in an unfavourable atherogenic risk profile, and hence increased risk of developing subsequent cardiovascular events. This is underlined by the high prevalence of cardiovascular disease (myocardial infarction, CABG, PTCA and cerebrovascular accident) found in HL-relatives compared to spouses: 21.0% vs. 6.3%, $p < 0.005$ (Figure 2.5).

Table 2.2. Distribution of cardiovascular risk factors in FCHL relatives and spouses.

	Spouses (n=126)	Hyperlipidemic relatives (n=143)	Normolipidemic relatives (n=219)
Overweight (BMI > 27.5)	36 (28.6%)	66 (46.2%)*†	45 (20.6%)
Obesity (BMI > 30.0)	12 (9.5%)	37 (25.9%)*†	24 (11.0%)
Hypertension	45 (35.7%)	96 (67.1%)*†	55 (25.2%)
Smoking	38 (30.2%)	45 (31.5%)	74 (33.9%)
Smoking (packyear)	9.6 ± 11.5	11.3 ± 14.3	6.2 ± 10.2
Alcohol	98 (77.8%)	100 (69.9%)*†	178 (81.7%)
Alcohol (U/week)	8.5 ± 9.5	8.9 ± 9.3	8.2 ± 8.6

* = HL vs. Spouses, $p < 0.01$; † = HL vs. NL, $p < 0.001$; Differences in hypertension prevalence and packyears smoking values have been age-corrected.

$p < 0.05$ for trend

Figure 2.4. Clustering of cardiovascular risk factors in 35 FCHL-families ($n = 362$) and 126 married-in spouses.

Figure 2.5. Prevalence of cardiovascular disease in hyperlipidemic FCHL relatives (n = 143) and 126 married-in spouses.
Legend: CVD = cardiovascular disease.

In conclusion, in a cross-sectional survey almost 40% of relatives from Dutch FCHL families were hyperlipidemic, with an equal distribution over the three hyperlipidemic phenotypes (HC, HT or the combination). The hyperlipidemia in FCHL is expressed 20 years earlier than in spouses. Furthermore, a clustering of cardiovascular risk factors is observed in FCHL relatives, further aggravating the risk of developing subsequent cardiovascular events. Environmental factors alone might play a minor role in explaining the high cardiovascular risk observed in FCHL, as no differences in smoking or alcohol habits were found. It is more plausible to assume that FCHL subjects are more susceptible to the unfavourable life style in the Western world. This can be caused by more pronounced gene-environment interactions.

2.3 Specific methodology

2.3.1 Most important methodology in each subsequent chapter

The main methodologies used in the investigations and analyses in each subsequent chapter are briefly described below. In **chapter 4**, FCHL relatives and spouses ascertained in Utrecht were used for the analyses. Part of FCHL affected subjects, and spouses from 'Maastricht' families and healthy controls were used for the analysis in **chapters 5 to 8**.

Chapter 4. Blood pressure

FCHL has been proposed as the leading cause of dyslipidemia in Familial Dyslipidemic Hypertension (FDH)³. Furthermore, it is very likely that the presence of both hyperlipidemia and hypertension contribute to the high risk of early cardiovascular disease in FCHL. In this chapter we quantified the simultaneous occurrence of hypertension and combined hyperlipidemia in FCHL families and evaluated a potential common pathogenic ancestry for FDH and FCHL. Therefore, blood pressure was measured twice with a mercury sphygmomanometer in a standardised fashion, with the subject in sitting position after 10 minutes of rest. Cuff size was adjusted to the circumference of the arm, and the arm was placed with the cuff at heart-level. Systolic (SBP) and diastolic blood (DBP) pressures were defined according to Korotkoff sounds I and V. Hypertension has been defined as either SBP >140 mmHg and/or DBP >90 mmHg, or the current use of antihypertensive medication^{1,2}.

Chapter 5. Electrocardiogram and heart-rate corrected QT-interval.

Heart rate corrected QT-interval (QTc) prolongation has been associated with increased risk of coronary heart disease death⁴⁻⁶ and has been proposed as additional feature of the insulin resistance syndrome⁷. In this chapter we determined whether FCHL subjects had prolonged QTc times relative to spouse controls and investigated the potential relationship of QTc with hyperlipidemia and surrogate markers of the insulin resistance syndrome. Therefore, a standard resting 12-lead electrocardiography was obtained, using a Marquette 12 SL ECG (GE Marquette Medical Systems, Milwaukee, WI, USA). QT intervals were automatically measured in all 12 leads. The beginning of the QT-interval was defined as the first deflection of the QRS complex, the end as the point of maximal change in the slope as the T-wave merges with the baseline. Heart rate adjusted QT (QTc) interval was automatically calculated with a modified Bazett formula, using heart rate instead of RR interval for adjustment.

Chapter 6. Endothelium-derived pro-coagulant and anti-fibrinolytic factors

The increased risk of early cardiovascular events in FCHL is not fully explained by classical risk factors such as hyperlipidemia, insulin resistance and hypertension. Abnormal hemostatic properties of endothelium are potentially relevant to the

development of clinical cardiovascular events in FCHL⁸⁻¹⁰. Endothelium produces pro-coagulant and anti-coagulant substances (pro-coagulant: von Willebrand factor (vWf); anti-coagulant: tissue factor pathway inhibitor (TFPI) and thrombomodulin) and components of the fibrinolytic system that promote or inhibit fibrinolysis (tissue type plasminogen activator (t-PA) and plasminogen activator inhibitor 1 (PAI-1), respectively). In this study venous blood was collected in pre-cooled tubes containing 3.25% sodium citrate (dilution 1:10) to measure vWf, TFPI, soluble thrombomodulin and PAI-1, using commercially available assays. Blood collected in the Biopool®stabilyte™ devices was used to measure t-pa levels. Venous congestion was minimised as much as possible and blood between 2 and 10 ml after start of collection was drawn into the requested tubes. Plasma levels of vWf were determined by enzyme-linked fluorescent assay (normal range 60-200%; Coefficient of Variation (CV) 4.2%, Reader bioMérieux, France). Plasma levels of truncated TFPI were measured using an Elisa (range 40-85 ng/mL, CV 5-7%, American Diagnostica Inc., Greenwich, USA). Thrombomodulin determination was performed with an enzyme immuno assay (normal range 50-120 ng/mL, mean 76 ± 22 ng/mL, CV 8-10%, Diagnostica Stago, France). Levels of PAI-1 were determined by a specific chromogenic assay (range 1-30 U/mL, median 8 U/mL, CV 3%, Spectrolyse®, Biopool, Sweden). Plasma levels of t-PA were measured with an enzyme immuno assay (range 0-30 ng/mL, CV 5%, Biopool, Sweden).

Chapter 7. Capillary density and function

In subjects with hypertension or type 2 diabetes, several abnormalities in the microvasculature have been reported^{11,12}. Furthermore, impaired capillary recruitment has been proposed as a link between elevated blood pressure and insulin resistance, in both hypertensive¹³ and normotensive subjects¹⁴, although this was not confirmed in subjects with type 2 diabetes or impaired glucose tolerance¹⁵. As a result, the possible relationship between insulin resistance and microvascular abnormalities is not clear. Moreover, the possible role of plasma lipids, lipoproteins and remnant lipoprotein particles in microvascular abnormalities have not yet been taken into account in the above mentioned studies. Furthermore, little is known about microvasculature in hyperlipidemic diseases.

Therefore, the aim of the study described in chapter 7 was to determine whether abnormalities in capillary structure and function were present in FCHL, and furthermore to evaluate the potential association of observed microvascular abnormalities with plasma lipids and remnant-like lipoprotein particles, blood pressure values, and surrogate markers of insulin resistance. After an acclimatisation period of at least 20 minutes in a quiet, temperature-controlled room, the capillary density and laser Doppler fluxmetry were measured before and after 4 minutes of arterial occlusion. Capillary density, using capillary microscopy, was measured approximately 6 mm proximal to the finger nail fold of the right 4th finger. Finger nail fold capillaries were visualised using a Wild-Leitz microscope. Light from the microscope that penetrates the skin is scattered, reflected multiple times, and depolarised. If no strong absorbing parts are

present, the reflected light image will be homogeneously bright. However, the 100-Watt mercury lamp has a peak emission about 420 nm, matching the hemoglobin absorption spectrum. Consequently, the red blood cells appear dark in a bright background. Images (1.6 mm^2) were videotaped (Panasonic) for off-line analysis. The day-to-day coefficient of variation was 8%, as determined in ten subjects on two occasions.

Thermoregulatory skin perfusion was simultaneously determined, using laser-Doppler fluxmetry (LDF, Periflux PF3, Perimed, Sweden), at the dorsum of the middle phalanx of the right 3rd finger. The light (632.8 nm) of the He-Ne laser in the laser-Doppler device is reflected and scattered by the skin tissue and moving red blood cells. The light beams scattered by moving red blood cells undergo a frequency shift according to the Doppler effect. This frequency shift is a measure of red blood cell flux (red cell velocity times concentration) and equals red blood cell flow¹⁶. All measurements were performed using the standard probe PF308, filter setting: 12 kHz ('wide band') and time constant 0.2 s. He-Ne laser light penetrates the skin to a depth of about 1-mm, and hence the total flux values include signals from capillaries, arterioles, venous plexus and arterio-venous shunts¹⁷. The day-to-day coefficient of variation of post-occlusive reactive hyperaemia was 16%, as determined in twenty subjects on two occasions.

Chapter 8. Intima media thickness of common carotid artery

FCHL is the most common genetic dyslipidemia in humans, affecting 0.5-2.0% of the population¹⁸. FCHL-affected subjects have a markedly increased risk of premature cardiovascular disease¹⁸⁻²⁰. However, the clinical cardiovascular events are preceded by silent atherosclerotic changes in the vessel wall. Ultrasound measurement of intima media thickness (IMT) in the carotid wall is currently used as marker of silent atherosclerosis. The aim of the study described in chapter 8 was to assess the amount of silent atherosclerosis, by ultrasound carotid IMT measurement, in FCHL-affected subjects and age- and gender-matched healthy controls. In addition, we investigated the potential relationship of IMT with lipid- and lipoprotein concentrations, blood pressure values, and surrogate markers of insulin resistance.

The IMT measurements were performed using an Ultramark 4+ (Ultramark 4+, ATL, Bothel, Washington, USA), with a linear array transducer of 7.5 MHz. All subjects were measured in a supine position in a quiet room. During the measurements the head was tilted to the contra-lateral side at an angle of 45°. The automated radiofrequency (RF) method used to assess IMT has been described in detail before²¹. Briefly, a B-mode image was obtained from the common carotid artery (CCA) in a longitudinal section just before the widening of the bulb in the bifurcation. An M-line was positioned perpendicular to the posterior wall approximately 10-20 mm proximal to the carotid bulb, showing a clear intima-media complex during the whole measurement. Subsequently, the ultrasound system was switched to M-mode and ECG-triggered RF data collection was started at a sample frequency of 18.4 MHz, an M-line update frequency of 800 Hz, for a time period of 5 seconds, corresponding to 4-6 heartbeats. For each heartbeat, the derivative with respect to depth of the

averaged amplitude distribution was considered to distinguish wall signals from other signals. Subsequently, the locations of the intima and the media-adventitia edge were determined and IMT was defined as the distance between both edges. The ratio of intima to adventitia was set at 1.0 to suppress detection of false edges. The resolution for wall thickness detection was approximately $40\text{ }\mu\text{m}^{21}$. Only the posterior wall was investigated because here the reflections from the blood-intima and media-adventitia transition are distinctly visible, whereas at the anterior wall the trailing edge of the adventitia may obscure the media and intima signals. Each heartbeat within a recording resulted in an estimate of IMT. Measurements were repeated seven times at both left and right CCA. The average of the median values for each measurement obtained on both sides was taken as a representative IMT value. Ultrasound M-mode and RF signals method to measure IMT instead of off-line B-mode measurement has been validated previously²². The intra-observer variability was below 6.5% as determined in 10 subjects on two different occasions. The inter-observer variability was 4.6 % as determined in 10 subjects.

References

1. The sixth report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure. *Archives of Internal Medicine* 1997;157:2413-2446.
2. 1999 World Health Organization-International Society of Hypertension Guidelines for the Management of Hypertension. Guidelines Subcommittee. *Journal of Hypertension* 1999;17:151-183.
3. Williams RR, Hunt SC, Hopkins PN, Stults BM, Wu LL, Hasstedt SJ, Barlow GK, Stephenson SH, Lalouel JM, Kuida H. Familial Dyslipidemic Hypertension. Evidence from 58 Utah families for a syndrome present in approximately 12% of patients with essential hypertension. *Journal of the American Medical Association* 1988;259:3579-3586.
4. Schwartz PJ, Wolf S. QT interval prolongation as predictor of sudden death in patients with myocardial infarction. *Circulation* 1978;57:1074-1077.
5. Schouten EG, Dekker JM, Meppelink P, Kok FJ, Vandenbroucke JP, Pool J. QT interval prolongation predicts cardiovascular mortality in an apparently healthy population. *Circulation* 1991;84:1516-1523.
6. Dekker JM, Schouten EG, Klootwijk P, Pool J, Kromhout D. Association between QT interval and coronary heart disease in middle-aged and elderly men. The Zutphen Study. *Circulation* 1994;90:779-785.
7. Dekker JM, Feskens EJ, Schouten EG, Klootwijk P, Pool J, Kromhout D. QTc duration is associated with levels of insulin and glucose intolerance. The Zutphen Elderly Study. *Diabetes* 1996;45:376-380.
8. Hamsten A, de Faire U, Walldius G, Dahlen G, Szamosi A, Landou C, Blomback M, Wiman B. Plasminogen activator inhibitor in plasma: risk factor for recurrent myocardial infarction. *Lancet* 1987;2:3-9.
9. Jager A, van Hinsbergh VW, Kostense PJ, Emeis JJ, Yudkin JS, Nijpels G, Dekker JM, Heine RJ, Bouter LM, Stehouwer CD. von Willebrand factor, C-reactive protein, and 5-year mortality in diabetic and nondiabetic subjects: the Hoorn Study. *Arteriosclerosis Thrombosis and Vascular Biology* 1999;19:3071-3078.
10. Thogersen AM, Jansson JH, Boman K, Nilsson TK, Weinehall L, Huhtasaari F, Hallmans G. High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. *Circulation* 1998;98:2241-2247.
11. Shore AC, Tooke JE. Microvascular function in human essential hypertension. *Journal of Hypertension* 1994;12:717-728.
12. Jaap AJ, Hammersley MS, Shore AC, Tooke JE. Reduced microvascular hyperaemia in subjects at risk of developing type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 1994;37:214-216.
13. Serne EH, Gans ROB, ter Maaten J, ter Wee PM, Donker AM, Stehouwer CDA. Capillary recruitment is impaired in essential hypertension and relates to insulin's metabolic and vascular actions. *Cardiovascular Research* 2001;49:161-168.

14. Serne EH, Stehouwer CD, ter Maaten J, ter Wee PM, Rauwerda JA, Donker AJ, Gans RO. Microvascular function relates to insulin sensitivity and blood pressure in normal subjects. *Circulation* 1999;99:896-902.
15. Jaap AJ, Shore AC, Stockman AJ, Tooke JE. Skin capillary density in subjects with impaired glucose tolerance and patients with type 2 diabetes. *Diabetic Medicine* 1996;13:160-164.
16. Driessen G, Rutten W, Inhoffen W, Scheidt H, Heidtmann H. Is the laser Doppler flow signal a measure of microcirculatory cell flux? *International Journal of Microcirculation in Clinical Experiments* 1990;9:141-161.
17. Nilsson GE, Tenland T, Oberg PA. Evaluation of a laser Doppler flowmeter for measurement of tissue blood flow. *IEEE Transactions on Biomedical Engineering* 1980;27:597-604.
18. Goldstein JL, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG. Hyperlipidaemia in Coronary Heart Disease. II Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidaemia. *Journal of Clinical Investigation* 1973;52:1544-1568.
19. Austin MA, McKnight B, Edwards KL, Bradley CM, McNeely MJ, Psaty BM, Brunzell JD, Motulsky AG. Cardiovascular disease mortality in familial forms of hypertriglyceridemia: A 20-year prospective study. *Circulation* 2000;101:2777-2782.
20. Voors-Pette C, de Bruin TWA. Excess coronary heart disease in Familial Combined Hyperlipidemia, in relation to genetic factors and central obesity. *Atherosclerosis* 2001;157:481-489.
21. Hoeks AG, Willekes C, Boutouyrie P, Brands PJ, Willigers JM, Reneman RS. Automated detection of local artery wall thickness based on M-line signal processing. *Ultrasound in Medicine and Biology* 1997;23:1017-1023.
22. Willekes C, Hoeks A, Bots M, Brands P, Willigers J, Reneman R. Evaluation of off-line automated intima-media thickness detection of the common carotid artery based on M-line signal processing. *Ultrasound in Medicine and Biology* 1999;25:57-64.

Chapter 3

Pathophysiological and genetic mechanisms in Familial Combined Hyperlipidemia: our current understanding

ETP Keulen, TWA de Bruin

3.1 Introduction

Familial Combined Hyperlipidemia (FCHL) is one of the most common genetic lipid disorders in humans¹⁻⁴, affecting approximately 10% of survivors of early myocardial infarction (i.e. before 60 years of age)^{1,3,5}, whereas the population frequency is estimated from 1:200 to 1:100 or 0.5-1.0%^{1,3,6}. FCHL, within one family, may present with elevated very low-density lipoprotein (VLDL, type IV hyperlipidemia), elevated low-density lipoprotein (LDL, partly resembling type IIa hyperlipidemia), or both (type IIb hyperlipidemia)¹. Moreover, the pattern of hyperlipidemia (i.e. hypercholesterolemia and hypertriglyceridemia) may change within one individual, and is not often seen before adulthood¹. Despite the high risk of cardiovascular disease (CVD), lipid levels are only moderately elevated in FCHL, with typical total cholesterol (TC) concentrations between 6.0 and 9.0 mmol/L and fasting triglyceride (TG) concentrations of 2.0 to 6.0 mmol/L.

Although FCHL was first described in 1973 by Goldstein and colleagues¹, the fundamental basis of FCHL remains to be elucidated. As no unique biochemical or genetic marker for FCHL is available yet, family studies are required to establish the diagnosis of FCHL in a single patient⁷. Generally, we accept a diagnosis of FCHL if the index patient and a first-degree relative have different hyperlipidemic phenotypes, in which at least one of the subjects shows a combined hyperlipidemia, and if there is an unequivocal family history of hyperlipidemia and premature CVD.

Patients with FCHL are characterised by an overproduction of apolipoprotein B (apo B) containing lipoproteins, including VLDL^{8,9}, LDL¹⁰, and small dense LDL particles¹¹⁻¹⁴, in combination with a relative impairment of lipoprotein catabolism¹⁵. These mechanisms contribute to elevated plasma concentrations of apo B, the structural apoprotein in these lipoproteins. Furthermore, FCHL subjects are often insulin resistant¹⁶⁻²⁰. Clearly, the aggregate of these abnormalities results in an unfavourable, atherogenic risk profile and increases the risk of developing cardiovascular events. A recent report by Austin and colleagues showed that the 20-year cardiovascular mortality in FCHL kindred was a factor 1.7 higher than in spouse controls²¹. However, this may represent an underestimation of the true risk, because spouse controls showed a remarkably high cardiovascular mortality of 9.8% and total mortality of 29.4%. Furthermore, Voors-Pette and colleague showed that FCHL men had a 5.3 times higher risk of developing non-fatal coronary artery disease²².

Despite the high risk on CVD mortality and morbidity, and the corresponding serious impact on our health care system, FCHL is often not recognised by the physician in daily clinical practice. This chapter will summarise our current understanding of the pathophysiology of FCHL, especially focusing on lipid metabolism and insulin resistance. Furthermore, the role of genetic susceptibility and interaction with environment will be addressed briefly.

3.2 Lipid metabolism

Several studies have shown abnormalities in lipoprotein metabolism, including increased hepatic secretion of apolipoprotein B (apo B) containing lipoproteins (very low-density lipoproteins, the large VLDL1 and smaller VLDL2)^{8,10,23}, leading to a combined increase of cholesterol and triglyceride concentrations in plasma. It has also been suggested that increased VLDL1 residence time in the circulation contributes to hypertriglyceridemia in FCHL. Two subgroups of apo B are known: apo B100 and apo B48. Apo B100 is present in VLDL particles produced by the liver, as well as in intermediate density lipoprotein (IDL) and LDL particles, which are both derived from VLDL catabolism. Apo B48 is present in chylomicrons produced in the gut, and the chylomicron remnants. As mentioned above, in FCHL affected subjects increased apo B concentrations are often found. As each lipoprotein contains only one apo B molecule, an increased apo B concentration is an indicator of increased numbers of circulating lipoproteins. Furthermore, all apo B-containing lipoproteins are potentially atherogenic, where apo B also has been shown to be a better risk indicator of CVD compared to other lipid levels or smoking, diabetes mellitus and hypertension²⁴.

Although apo B overproduction in FCHL is known for several years, the underlying mechanism, for instance, increased lipid supply, especially free fatty acids or an intrinsic defect in hepatocytes, whether or not related to insulin resistance, is not known yet. Because of the elevated plasma apo B levels, the apo B gene has been suggested as primary candidate gene for FCHL. However, no genetic abnormalities involved in this apo B overproduction have been detected in FCHL so far. Furthermore, apo B gene defects have been ruled out by linkage analysis in FCHL kindred as primary major gene, despite the effect on lipid levels²⁵⁻²⁸.

After secretion, endogenous (from liver) and exogenous (from gut) derived lipoprotein particles are catabolised by a common lipolytic pathway represented by lipoprotein lipase (LPL). Because of the action of the LPL-enzyme, fatty acids are liberated from the lipoproteins and passed to the adipose tissue or skeletal muscle cells to be stored or oxidised. By doing this, LPL converts VLDL into smaller VLDL-remnants or IDL, and finally LDL, and chylomicrons are converted into chylomicron remnants. ApoB100 and apoB48 containing lipoproteins compete for LPL action, where LPL at low concentrations seems to have a preference for apoB48 containing particles and LPL at high concentrations seems to have an equal preference for either apoB48 or apoB100 containing particles²⁹.

Diminished post-heparin LPL activity and mass values have been reported in FCHL subjects³⁰⁻³⁴. Moreover, normal insulin-mediated activation of LPL is diminished in FCHL subjects, which contribute to reduced clearance of triglyceride-rich lipoproteins¹⁵. However, single LPL-gene mutations have been infrequently found in FCHL subjects^{35,36}. Interestingly, such single LPL-gene mutation has a significant additional effect on lipid and lipoprotein phenotype

expression^{35,36}. Linkage analysis revealed no significant relationship between the LPL-gene and FCHL^{34,35,37}. These data indicate that the LPL-gene does not represent the major single gene causing FCHL, but is a variability gene or a minor gene in FCHL, with potentially important gene-environment interactions, which might contribute to increased risk of CVD³⁸.

Hepatic lipase (HL) is a candidate gene for FCHL as well. HL resides in the sinusoidal space in the liver and is involved in the conversion of HDL particles, predominantly acting as a phospholipase, and in processing and removal of remnant lipoproteins. Low activity of HL has been associated with high levels of triglycerides and HDL-cholesterol and reduced LDL particle size³⁹. Furthermore, the HL gene was also linked to LDL particle size, HDL-cholesterol and TG levels in FCHL families and an association of a HL promoter polymorphism with HDL-cholesterol levels was observed^{20,40,41}.

Impaired insulin-mediated LPL activation and reduced LPL mass values both contribute to less optimal lipolysis and increased circulation times of TG containing lipoproteins. Moreover, in FCHL a reduced clearance of chylomicron remnants has been observed¹⁵. Both phenomena can eventually lead to the formation of smaller, denser LDL particles. Small, dense LDL and its immediate precursor, IDL, have been associated with increased risk of CVD⁴²⁻⁴⁴. A preponderance of small LDL particles, called LDL phenotype B, is associated with a more atherogenic lipoprotein profile than a preponderance of larger, buoyant, LDL (type A phenotype). The presence of small dense LDL particles, which occurs in combination with increased plasma TG and apo B concentrations and with reduced HDL-cholesterol levels, has been termed the 'atherogenic lipoprotein phenotype' (ALP) and is independently associated with an increased risk for CVD⁴². Reduced levels of HDL-cholesterol^{7,9} and a preponderance of small, dense LDL particles^{13,44} also frequently, but not always, occur in patients with FCHL, although they are not considered as criteria for the diagnosis of disease. Allayee and colleagues provided evidence that minor FCHL loci also contribute to LDL particle size, and suggested that ALP and FCHL share a subset of their genetic determinants⁴⁵.

Clearly, the frequent occurrence of small dense LDL in FCHL subjects raises the risk of developing early and severe atherosclerosis and subsequent events. Lipoprotein(a) or Lp(a) is a lipoprotein with a LDL-like moiety and the addition of apo (a). Increased Lp(a) concentrations have been associated with increased CVD risk. Therefore, Lp(a) might play an important role in FCHL as well. In both FCHL and normolipidemic subjects a connection between the metabolism of Lp(a) and triglyceride-rich lipoproteins has been suggested⁴⁶. However, no significant differences in Lp(a) concentrations between FCHL and healthy normolipidemic subjects have been observed^{46,47}. Furthermore, several genome scans in FCHL populations failed to show a significant LOD peak in the Lp(a) gene region^{48,49}. So, it is unlikely that Lp(a) contributes to the expression of FCHL or to the high CVD risk observed in FCHL.

In summary, FCHL harbours a complex phenotype with increased concentrations of atherogenic lipoproteins. Furthermore, an increased flux of atherogenic lipoproteins in the vessels, with in addition, increased residence time, suggests an increased exposure of endothelium to atherogenic lipoproteins in FCHL affected subjects.

3.3 Adipose tissue

Over the past few years the possible role of adipose tissue in the expression of FCHL has emerged. FCHL affected subjects often show increases in body weight, waist to hip ratio (WHR) and waist-circumference. Although manifest obesity (body mass index (BMI) $>30 \text{ kg/m}^2$) is, as secondary cause of hyperlipidemia, an exclusion criteria for FCHL in our laboratory, the increased WHR and waist-circumference indicate increased amounts of adipose tissue, and, perhaps, loss of muscle mass as well⁵⁰. Several hormonal and enzymatic systems in adipose tissue have been studied in relationship to FCHL and will be discussed below.

Leptin is a protein hormone produced predominantly by adipocytes. Serum leptin concentrations in healthy individuals are positively correlated with body fat content and BMI. In FCHL women, serum leptin concentrations out of proportion to BMI have been reported⁵¹. A study by Haluzik and colleagues however, failed to demonstrate a significant difference in leptin levels between FCHL subjects and healthy controls⁵². Also no significant relationship between serum leptin concentrations and lipid levels were observed⁵². Van der Kallen and colleagues have reported evidence that a human chromosome 1p locus, harbouring the leptin receptor gene, contributes to plasma leptin concentrations, adiposity and body weight in FCHL subjects⁵³. This supports the hypothesis that the leptin receptor gene plays a role in the observed increase in weight and waist-circumference observed in FCHL subjects. As will be pointed out below, an increased visceral fat mass appears to play a pivotal role in the development of the insulin resistant, hyperlipidemic and hypertensive phenotype found in FCHL.

In the postprandial state, storage of TG will take place in adipose tissue. Before entering the adipocyte, TG from VLDL and chylomicrons will have to be hydrolysed by LPL into free fatty acids (FFA) and glycerol. In the adipocyte, intracellular re-esterification to TG takes place, which is mediated by the action of acylation stimulatory protein (ASP)⁵⁴. The possible role of ASP in the development of hyperlipidemia in FCHL is still under study. Impaired FFA 'trapping' has been observed in adipose tissue of subjects with hyper-apo B as well⁵⁵.

In the fasting state, adipocyte TG is lipolysed by hormone sensitive lipase (HSL) for energy delivery to the bloodstream. The lipolytic effect of HSL, which is normally inhibited by postprandial hyperinsulinemia and enhanced by catecholamines, is partly impaired in FCHL⁵⁵⁻⁵⁷. A reduction in maximum enzymatic activity of HSL has also been correlated with serum lipid abnormalities in Swedish FCHL families⁵⁶, although not confirmed in Finnish FCHL families⁵⁸. Both defect in HSL reaction to insulin and an impaired FFA re-esterification in FCHL may result in an increased flux of FFA to the liver and consequently in an

increased hepatic VLDL synthesis. Although increased FFA flux to the liver is an established metabolic entity in FCHL, genetic analysis have failed to demonstrate an important contribution of the HSL gene to the expression of FCHL³⁷. An alternative hypothesis is that insulin resistance might play an important role in the observed increased FFA flux from adipose tissue to the liver.

3.4 Insulin resistance

Because insulin has several important actions on lipid metabolism, the existence of insulin resistance may be an important factor modulating FCHL phenotypes. Several studies have shown that FCHL subjects are equally insulin resistant for glucose disposal to subjects with diabetes mellitus¹⁶⁻²⁰. Moreover, even in normolipidemic non-affected subjects of FCHL families insulin resistance has been found⁵⁹, indicating a possible role of insulin resistance in the development of some features of FCHL.

As pointed out before, an impaired insulin-mediated activation of LPL results in a reduced clearance of triglyceride-rich proteins. Additionally, insulin resistance has been associated with increased levels of small dense LDL particles, which has also often been observed in FCHL. Furthermore, an impaired FFA suppression during hyperinsulinemic euglycemic clamp^{20,59,60} and after oral fat load⁶¹ has been observed in FCHL. Also, *in vitro* stimulation of adipose tissue derived from FCHL affected subjects, showed an impaired FFA suppression after stimulation with insulin⁵⁷.

However, increased fasting plasma insulin concentrations may also be secondary to impaired postprandial FFA metabolism. Increased FFA-flux from visceral fat to the liver not only promotes VLDL production, but also gluconeogenesis. Increased production of glucose is a trigger to the pancreas to secrete insulin in order to maintain normoglycemia, and can thus result in increased fasting plasma insulin concentrations. Although the occurrence of diabetes mellitus is not enhanced in FCHL kindred, increased prevalence of impaired glucose tolerance and higher insulin and glucose area under the oral glucose tolerance curve have been observed.

Because of the remarkable resemblance of the metabolic profile observed in FCHL and the Insulin Resistance Syndrome (IRS), FCHL has been postulated as being part of IRS instead of being a solely clinical entity. At present, it is clear that IRS plays an important role in the clinical and biochemical expression of FCHL. However, whether IRS also plays a role in the development of FCHL or is merely a consequence of FCHL remains to be elucidated. Furthermore, the observed clinical and biochemical abnormalities in FCHL cannot be explained by IRS alone, because normolipidemic relatives have been reported to be insulin resistant as well⁵⁹.

3.5 Genetic susceptibility

Originally, it was thought that FCHL was a dominantly inherited hyperlipidemia closely associated with cardiovascular disease. However, subsequent research has not supported this concept. One hypothesis is that affected families have an oligogenic hyperlipidemia, causing variable phenotypes among family members, in relation to environmental effects. Because of its complex phenotype, FCHL is likely to be a heterogeneous disorder, influenced by several susceptibility genes and environmental factors. Till now, no major genes have been located, but some modifier genes are known.

As pointed out above, mutations in apo B gene, LPL gene, HL gene and HSL gene have an effect on lipid and lipoprotein phenotype expression, but do not represent the major gene causing FCHL. One of the first proposed major candidate-regions causing FCHL was the apo AI-CIII-AIV gene cluster on chromosome 11⁶², that was further confirmed in other populations^{63,64}. However, not all FCHL populations confirmed this finding⁶⁵. A more detailed analysis revealed a specific high risk combination of haplotypes based on three polymorphic markers within this cluster: XmnI, MspI and SstI, indicating that two different susceptibility loci for FCHL exist within this gene complex⁶⁶. Furthermore, the SstI locus was recently shown to be related to non-fatal CVD risk in FCHL subjects²².

Recently, linkage studies in a large number of Finnish FCHL families revealed evidence of a major gene for FCHL on chromosome 1q21-q23⁶⁷. A study in another FCHL population confirmed⁶⁸ this finding using a novel analytical strategy. However, different strategies in genetic analyses (affected-only vs. sibpair analyses), renders comparisons between the several studies difficult^{67,69}. For instance, analyses of Dutch pedigrees using the Finnish affected-only strategy also revealed linkage to this chromosomal region (TWA de Bruin, P Pajukanta, FCHL workshop, ESCI meeting, 2-5 May 2001, Barcelona, Spain). A novel gene (*Hyplip 1*) in HcB19 mice has been mapped to a portion of the mouse genome that is syntenic to the human chromosome 1q21-q23. Recently, the region on chromosome 1q has been narrowed down to 1q21 by fine-mapping the *Hyplip1* locus in the mouse and defining the borders of the region of conserved synteny between human and mouse. This region lies approximately within 5-10 Mb of the peak marker (D1S104) for linkage to FCHL⁷⁰, which, however, also harbours several other interesting candidate genes (P Pajukanta, FCHL workshop, ESCI meeting, 2-5 May 2001, Barcelona, Spain).

A genome scan in Dutch FCHL families revealed another candidate modifier gene: lecithin:cholesterol acyltransferase (LCAT)⁷¹, an enzyme involved in cholesterol transfer between lipoprotein particles⁴⁸. Furthermore, candidate gene studies also have shown that PPAR γ ⁷², and tumor necrosis factor receptor superfamily member 1B (TNFRSF1B)⁷³ have some effect on the FCHL phenotype, but seem not to be the primary cause of FCHL.

Despite all these major and important efforts, much work has to be done to truly clarify the genetic background of FCHL. Additional candidate gene studies

should be performed since many candidates for both 'major' and 'modifier' effects remain to be examined. Furthermore, 'major' genes are likely to be heterogeneous. The potential overlap of genetic factors between FCHL, type 2 diabetes mellitus and ALP suggests that collaboration between the groups studying these traits will be beneficial.

3.6 Conclusion

Since its first description in 1973, major efforts have been taken to unravel the genetic and metabolic pathophysiology underlying FCHL. Currently, the increased free fatty acid flux from visceral or subcutaneous adipose tissue to the liver is an important metabolic theory in FCHL, that explains - in part - the increased production of VLDL particles, and hence hyperlipidemia. Several modifier genes have been detected, which have especially an effect on the hyperlipidemic phenotype observed in FCHL. The recently published human genome map^{74,75} may make it easier to fine-map and detect possible important genes underneath an interesting LOD-peak for FCHL. However, despite the knowledge of the chromosomal map of a gene, the exact role of many genes has to be established. Moreover, gene-gene and gene-environment interaction probably also will play a major role in many multi-genic diseases like FCHL. It is likely that gene expression profile from affected organs of FCHL subjects will further increase our insight and understanding of FCHL at the cellular level^{76,77}.

References

1. Goldstein JL, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG. Hyperlipidaemia in Coronary Heart Disease. II Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidaemia. *Journal of Clinical Investigation* 1973;52:1544-1568.
2. Rose HG, Kranz P, Weinstock M, Juliano J, Haft JJ. Inheritance of combined hyperlipoproteinaemia: evidence for a new lipoprotein phenotype. *American Journal of Medicine* 1973;54:148-160.
3. Nikkilä EA, Aro A. Family study of serum lipids and apolipoproteins in coronary artery disease. *Lancet* 1973;78:954-959.
4. Brunzell JD, Schrott HG, Motulsky AG, Bierman EL. Myocardial infarction in the familial forms of hypertriglyceridemia. *Metabolism* 1976;25:313-320.
5. Genest-Jr JJ, Martin MS, McNamara JR, Ordovas JM, Jenner J, Myers RH, Silberman SR, Wilson PW, Salem DN, Schaefer EJ. Familial lipoprotein disorders in patients with premature coronary artery disease. *Circulation* 1992;85:2025-2033.
6. Motulsky AG. Current concepts in genetics. The genetic hyperlipidemias. *New England Journal of Medicine* 1976;294:823-827.
7. Grundy SM, Chait A, Brunzell JD. Familial combined hyperlipidaemia workshop. *Arteriosclerosis* 1987;7:203-207.
8. Venkatesan S, Cullen P, Pacy P, Halliday D, Scott J. Stable isotopes show a direct relation between VLDL apoB overproduction and serum triglyceride levels and indicate a metabolically and biochemically coherent basis for familial combined hyperlipidemia. *Arteriosclerosis Thrombosis and Vascular Biology* 1993;13:1110-1118.
9. Castro Cabezas M, de Bruin TWA, de Valk HW, Shoulders CC, Jansen H, Erkelens DW. Impaired fatty acid metabolism in familial combined hyperlipidemia. A mechanism associating hepatic apolipoprotein B overproduction and insulin resistance. *Journal of Clinical Investigation* 1993;92:160-168.
10. Kissebah AH, Alfarsi S, Evans DJ. Low density lipoprotein metabolism in familial combined hyperlipidemia. Mechanism of the multiple lipoprotein phenotypic expression. *Arteriosclerosis* 1984;4:614-624.
11. Hennekens CH. Increasing burden of cardiovascular disease; current knowledge and future directions for research on risk factors. *Circulation* 1998;97:1095-1102.
12. Bredie SJ, Kiemeny LA, de Haan AF, Demacker PN, Stalenhoef AF. Inherited susceptibility determines the distribution of dense low-density lipoprotein subfraction profiles in familial combined hyperlipidemia. *American Journal of Human Genetics* 1996;58:812-822.
13. Bredie SJ, Demacker PN, Stalenhoef AF. Metabolic and genetic aspects of familial combined hyperlipidaemia with emphasis on low-density lipoprotein heterogeneity. *European Journal of Clinical Investigation* 1997;27:802-811.
14. Hokanson JE, Krauss RM, Albers JJ, Austin MA, Brunzell JD. LDL physical and chemical properties in familial combined hyperlipidemia. *Arteriosclerosis Thrombosis Vascular Biology* 1995;15:452-459.

15. Cabezas MC, de Bruin TWA, Jansen H, Kock LA, Kortlandt W, Erkelens DW. Impaired chylomicron remnant clearance in familial combined hyperlipidemia. *Arteriosclerosis and Thrombosis* 1993;13:804-814.
16. Bredie SJH, Tack CJJ, Smits P, Stalenhoef AFH. Non-obese patients with familial combined hyperlipidemia are insulin resistant as compared with their non-affected relatives. *Arteriosclerosis Thrombosis and Vascular Biology* 1997;17:1465-1471.
17. Hunt SC, Wu LL, Hopkins PN, Stults BM, Kuida H, Ramirez ME, Lalouel JM, Williams RR. Apolipoprotein, low density lipoprotein subfraction, and insulin associations with familial combined hyperlipidemia. Study of Utah patients with familial dyslipidemic hypertension. *Arteriosclerosis* 1989;9:335-344.
18. Ascaso JF, Merchante A, Lorente RI, Real JT, Martinez VJ, Carmena R. A study of insulin resistance using the minimal model in nondiabetic familial combined hyperlipidemic patients. *Metabolism* 1998;47:508-513.
19. Ascaso JF, Lorente R, Merchante A, Real JT, Priego A, Carmena R. Insulin resistance in patients with familial combined hyperlipidemia and coronary artery disease. *American Journal of Cardiology* 1997;80:1484-1487.
20. Pihlajamäki J, Karjalainen L, Karhapää P, Vauhkonen I, Laakso M. Impaired free fatty acid suppression during hyperinsulinemia is a characteristic finding in familial combined hyperlipidemia, but insulin resistance is observed only in hypertriglyceridemic patients. *Arteriosclerosis Thrombosis and Vascular Biology* 2000;20:164-170.
21. Austin MA, McKnight B, Edwards KL, Bradley CM, McNeely MJ, Psaty BM, Brunzell JD, Motulsky AG. Cardiovascular disease mortality in familial forms of hypertriglyceridemia: A 20-year prospective study. *Circulation* 2000;101:2777-2782.
22. Voors-Pette C, de Bruin TWA. Excess coronary heart disease in Familial Combined Hyperlipidemia, in relation to genetic factors and central obesity. *Atherosclerosis* 2001;157:481-489.
23. Cuchel M, Schaefer EJ, Millar JS, Jones PJ, Dolnikowski GG, Vergani C, Lichtenstein AH. Lovastatin decreases de novo cholesterol synthesis and LDL Apo B-100 production rates in combined-hyperlipidemic males. *Arteriosclerosis Thrombosis and Vascular Biology* 1997;17:1910-1917.
24. Kwiterovich-Jr PO, Coresh J, Smith HH, Bachorik PS, Derby CA, Pearson TA. Comparison of the plasma levels of apolipoproteins B and A-1, and other risk factors in men and women with premature coronary artery disease. *American Journal of Cardiology* 1992;69:1015-1021.
25. Rotter JI, Bu X, Cantor RM, Warden CH, Brown J, Gray RJ, Blanche PJ, Kraus RM, Lusis AJ. Multilocus genetic determinants of LDL particle size in coronary artery disease families. *American Journal of Human Genetics* 1996;58:585-594.
26. Austin MA, Wijsman E, Guo SW, Krauss RM, Brunzell JD, Deeb S. Lack of evidence for linkage between low-density lipoprotein subclass phenotypes and the apolipoprotein B locus in familial combined hyperlipidemia. *Genetic Epidemiology* 1991;8:287-297.
27. Rauh G, Schuster H, Müller B, Schewe S, Keller C, Wolfram G, Zollner N. Genetic evidence from 7 families that the apolipoprotein B gene is not involved in familial combined hyperlipidemia. *Atherosclerosis* 1990;83:81-87.

28. Coresh J, Beatty TH, Kwiterovich-Jr PO, Antonarakis SE. Pedigree and sib-pair linkage analysis suggest the apolipoprotein B gene is not the major gene influencing plasma apolipoprotein B levels. *American Journal of Human Genetics* 1992;50:1038-1045.
29. van Beek AP, van Barlingen HH, de Ruijter Heijstek FC, Jansen H, Erkelens DW, Dallinga-Thie GM, de Bruin TWA. Preferential clearance of apoB-48-containing lipoproteins after heparin-induced lipolysis is modulated by lipoprotein lipase activity. *Journal of Lipid Research* 1998;39:322-332.
30. Babirak S, Brown B, Brunzell J. Familial combined hyperlipidemia and abnormal lipoprotein lipase. *Arteriosclerosis and Thrombosis* 1992;12:1176-1183.
31. Seed M, Mailly F, Vallance D, Doherty E, Winder A, Talmud P, Humphries SE. Lipoprotein lipase activity in patients with combined hyperlipidaemia. *Clinical Investigation* 1994;72:100-106.
32. Nevin DN, Brunzell JD, Deeb SS. The LPL gene in individuals with familial combined hyperlipidemia and decreased LPL activity. *Arteriosclerosis and Thrombosis* 1994;14:869-873.
33. Gagne E, Genest J, Zhang H, Clarke LA, Hayden MR. Analysis of DNA changes in the LPL gene in patients with familial combined hyperlipidemia. *Arteriosclerosis and Thrombosis* 1994;14:1250-1257.
34. Yang WS, Nevin DN, Iwasaki L, Peng R, Brown BG, Brunzell JD, Deeb SS. Regulatory mutations in the human lipoprotein lipase gene in patients with familial combined hyperlipidemia and coronary artery disease. *Journal of Lipid Research* 1996;37:2627-2637.
35. de Bruin TWA, Mailly F, van Barlingen HH, Fisher R, Castro Cabezas M, Talmud P, Dallinga-Thie GM, Humphries SE. Lipoprotein lipase gene mutations D9N and N291S in four pedigrees with familial combined hyperlipidaemia. *European Journal of Clinical Investigation* 1996;26:631-639.
36. Hoffer MJ, Bredie SJ, Boomsma DI, Reymer PW, Kastelein JJ, de Knijff P, Demacker PN, Stalenhoef AF, Havekes LM, Frants RR. The lipoprotein lipase (Asn291-->Ser) mutation is associated with elevated lipid levels in families with familial combined hyperlipidaemia. *Atherosclerosis* 1996;119:159-167.
37. Pajukanta P, Porkka KV, Antikainen M, Taskinen MR, Perola M, Murtomaki Repo S, Ehnholm S, Nuotio I, Suurinkeroinen L, Lahdenkari AT, Syvanen AC, Viikari JS, Ehnholm C, Peltonen L. No evidence of linkage between familial combined hyperlipidemia and genes encoding lipolytic enzymes in Finnish families. *Arteriosclerosis Thrombosis and Vascular Biology* 1997;17:841-850.
38. Talmud PJ, Bujac SR, Hall S, Miller GJ, Humphries SE. Substitution of asparagine for aspartic acid at residue 9 (D9N) of lipoprotein lipase markedly augments risk of ischaemic heart disease in male smokers. *Atherosclerosis* 2000;149:75-81.
39. Zambon A, Austin MA, Brown BG, Hokanson JE, Brunzell JD. Effect of hepatic lipase on LDL in normal men and those with coronary artery disease. *Arteriosclerosis and Thrombosis* 1993;13:147-153.
40. Allayee H, Dominguez KM, Aouizerat BE, Kraus RM, Rotter JJ, Lu J, Cantor RM, de Bruin TWA, Lusis AJ. Contribution of the hepatic lipase gene to the atherogenic lipoprotein phenotype in familial combined hyperlipidemia. *Journal of Lipid Research* 2000;41:245-252.
41. Jansen H, Chu G, Ehnholm C, Dallongeville J, Nicaud V, Talmud PJ. The T allele of the hepatic lipase promoter variant C-480T is associated with increased fasting lipids

- and HDL and increased preprandial and postprandial LpCIII:B : European Atherosclerosis Research Study (EARS) II. *Arteriosclerosis Thrombosis and Vascular Biology* 1999;19:303-308.
42. Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM. Low-density lipoprotein subclass patterns and risk of myocardial infarction. *Journal of American Medical Association* 1988;260:1917-1921.
 43. Austin MA, Brunzell JD, Fitch WL, Krauss RM. Inheritance of low density lipoprotein subclass patterns in familial combined hyperlipidemia. *Arteriosclerosis* 1990;10:520-530.
 44. Jarvik GP, Brunzell JD, Austin MA, Krauss RM, Motulsky AG, Wijsman E. Genetic predictors of FCHL in four large pedigrees. Influence of ApoB level major locus predicted genotype and LDL subclass phenotype. *Arteriosclerosis and Thrombosis* 1994;14:1687-1694.
 45. Allayee H, Aouizerat BE, Cantor RM, Dallinga-Thie GM, Kraus RM, Lanning CD, Rotter JJ, Lusis AJ, de Bruin TWA. Families with familial combined hyperlipidemia and families enriched for coronary artery disease share genetic determinants for the atherogenic lipoprotein phenotype. *American Journal of Human Genetics* 1998;63:577-585.
 46. Castro Cabezas M, de Bruin TWA, Van-Linde-Sibenius TM, Kock LA, Jansen H, Erkelens DW. Lipoprotein(a) plasma concentrations associated with lipolytic activities in eight kindreds with familial combined hyperlipidemia and normolipidemic subjects. *Metabolism* 1993;42:756-761.
 47. Netea RT, Netea MG, Bredie SJ, Demacker PN, Hancu N, Thien T, Stalenhoef AF. Lipoprotein (a) concentrations in patients with familial combined hyperlipidemia and hypertension. *Netherlands Journal of Medicine* 1999;55:39-45.
 48. Aouizerat BE, Allayee H, Bodnar J, Krass KL, Peltonen L, de Bruin TWA, Rotter JJ, Lusis AJ. Novel genes for familial combined hyperlipidemia. *Current Opinion in Lipidology* 1999;10:113-122.
 49. Pajukanta P, Terwilliger JD, Perola M, Hiekkalinna T, Nuotio I, Ellonen P, Parkkonen M, Hartiala J, Ylitalo K, Pihlajamäki J, Porkka K, Laakso M, Viikari J, Ehnholm C, Taskinen MR, Peltonen L. Genomewide scan for familial combined hyperlipidemia genes in Finnish families, suggesting multiple susceptibility loci influencing triglyceride, cholesterol, and apolipoprotein B levels. *American Journal of Human Genetics* 1999;64:1453-1463.
 50. Seidell JC, Cigolini M, Charzewska J, Ellsinger BM, Deslypere JP, Cruz A. Fat distribution in European men: a comparison of anthropometric measurements in relation to cardiovascular risk factors. *International Journal of Obesity Related Metabolic Disorders* 1992;16:17-22.
 51. Jacobson MS, Yoon DJ, Frank GR. Serum leptin is elevated out of proportion to the body mass index in adolescent females with familial combined hyperlipidemia (FCH). *Clinical Pediatrics Philadelphia* 1999;38:49-53.
 52. Haluzik M, Fiedler J, Nedvidkova J, Ceska R. Serum leptin concentrations in patients with combined hyperlipidemia: relationship to serum lipids and lipoproteins. *Physiological Research* 1999;48:363-368.
 53. van der Kallen CJH, Cantor RM, van Greevenbroek MMJ, Geurts JMW, Bouwman FG, Aouizerat BE, Allayee H, Buurman WA, Lusis AJ, Rotter JJ, de Bruin TWA. Genome scan for adiposity in Dutch dyslipidemic families reveals novel quantitative

- trait loci for leptin, body mass index and soluble tumor necrosis factor receptor superfamily 1A. *International Journal of Obesity* 2000;24:1381-1391.
54. Sniderman AD, Wolfson C, Teng B, Franklin FA, Bachorik PS, Kwiterovich-Jr PO. Association of hyperapobetalipoproteinemia with endogenous hypertriglyceridemia and atherosclerosis. *Annals of Internal Medicine* 1982;97:833-839.
 55. van der Kallen CJH, Voors-Pette C, Bouwman FG, Lu JY, van de Hulst RRWJ, Janssen MJ, Keulen ETP, Boeckx WD, Rotter JI, de Bruin TWA. Evidence of insulin resistant lipid metabolism in adipose tissue in familial combined hyperlipidemia, but not type 2 diabetes mellitus. *Atherosclerosis* 2001;submitted.
 56. Reynisdottir S, Eriksson M, Angelin B, Arner P. Impaired activation of adipocyte lipolysis in familial combined hyperlipidemia. *Journal of Clinical Investigation* 1995;95:2161-2169.
 57. Pette C, Janssen MJ, van der Kallen CJH, de Bruin TWA. Specific insulin resistance of adipose tissue in familial combined hyperlipidemia. *Circulation* 1999;100:1-196 (Abstract).
 58. Ylitalo K, Large V, Pajukanta P, Reynisdottir S, Porkka KVK, Vukkilainen J, Nuotio I, Taskinen MR, Arner P. Reduced hormone-sensitive lipase activity is not a major metabolic defect in Finnish FCHL families. *Atherosclerosis* 2000;153:373-381.
 59. Karjalainen L, Pihlajamäki J, Karhapää P, Laakso M. Impaired insulin-stimulated glucose oxidation and free fatty acid suppression in patients with familial combined hyperlipidemia: a precursor defect for dyslipidemia? *Arteriosclerosis Thrombosis and Vascular Biology* 1998;18:1548-1553.
 60. Aitman TJ, Godsland IF, Farren B, Crook D, Wong HJ, Scott J. Defects of insulin action on fatty acid and carbohydrate metabolism in familial combined hyperlipidemia. *Arteriosclerosis Thrombosis and Vascular Biology* 1997;17:748-754.
 61. Meijssen S, Cabezas MC, Twickler TB, Jansen H, Erkelens DW. In vivo evidence of defective postprandial and postabsorptive free fatty acid metabolism in familial combined hyperlipidemia. *Journal of Lipid Research* 2000;41:1096-1102.
 62. Wojciechowski AP, Farrall M, Cullen P, Wilson TM, Buyliss JD, Farren B, Griffin BA, Caslake MJ, Packard CJ, Shepherd AJ. Familial combined hyperlipidaemia linked to the apolipoprotein AI-CII-AIV gene cluster on chromosome 11q23-q24. *Nature* 1991;349:161-164.
 63. Dallinga-Thie GM, van Linde-Sibenius Trip M, Rotter JI, Lusis AJ, de Bruin TWA. Apolipoprotein AI/CIII/AIV gene cluster in familial combined hyperlipidemia: effects on LDL-cholesterol and apolipoproteins B and C-III. *Journal of Lipid Research* 1996;37:136-147.
 64. Coon H, Myers RH, Borecki IB, Arnett DK, Hunt SC, Province MA, Djousse L, Leppert MF. Replication of linkage of familial combined hyperlipidemia to chromosome 1q with additional heterogeneous effect of apolipoprotein A-I/C-III-IV locus - The NHLBI Family Heart Study. *Arteriosclerosis Thrombosis and Vascular Biology* 2000;20:2275-2280.

65. Wijsman EM, Brunzell JD, Jarvik GP, Austin MA, Motulsky AG, Deeb SS. Evidence against linkage of familial combined hyperlipidemia to the apolipoprotein AI-CIII-AIV gene complex. *Arteriosclerosis Thrombosis and Vascular Biology* 1998;18:215-226.
66. Dallinga-Thie GM, van Linde-Sibenius Trip M, Rotter JI, Cantor RM, Bu X, Lusis AJ, de Bruin TWA. Complex genetic contribution of the apoAI-CIII-AIV gene cluster to familial combined hyperlipidemia: identification of different susceptibility haplotypes. *Journal of Clinical Investigation* 1997;99:953-961.
67. Pajukanta P, Nuotio I, Terwilliger JD, Porkka KV, Ylitalo K, Pihlajamaki J, Suomalainen AJ, Syvanen AC, Lehtimaki T, Viikari JS, Laakso M, Taskinen MR, Ehnholm C, Peltonen L. Linkage of familial combined hyperlipidaemia to chromosome 1q21-q23. *Nature Genetics* 1998;18:369-373.
68. Pei W, Baron H, Muller MB, Myksoh B, Knoblauch H, Al Yahyaee SA, Hui R, Wu X, Liu L, Busjahn A, Luft FC, Schuster H. Support for linkage of familial combined hyperlipidemia to chromosome 1q21-q23 in Chinese and German families. *Clinical Genetics* 2000;57:29-34.
69. Aouizerat BE, Allayee H, Cantor RM, Pavis RC, Lanning CD, Lien PZ, Dallinga-Thie GM, de Bruin TWA, Rotter JI, Lusis AJ. A genome scan for familial combined hyperlipidemia reveals evidence of linkage with a locus on chromosome 11. *American Journal of Human Genetics* 1999;65:397-412.
70. Pajukanta P, Bodnar JS, Sallinen R, Chu M, Airaksinen T, Xiao QN, Castellani LW, Sheth SS, Wesman M, Palotie A, Sinsheimer JS, Demant P, Lusis AJ, Peltonen L. Fine mapping of Hyplip1 and the human homolog, a potential locus for FCHL. *Mammalian Genome* 2001;12:238-245.
71. Aouizerat BE, Allayee H, Cantor RM, Dallinga-Thie GM, Lanning CD, de Bruin TWA, Lusis AJ, Rotter JI. Linkage of a candidate gene locus to familial combined hyperlipidemia: lecithin:cholesterol acyltransferase on 16q. *Arteriosclerosis Thrombosis and Vascular Biology* 1999;19:2730-2736.
72. Pihlajamaki J, Miettinen R, Valve R, Karjalainen L, Mykkanen L, Kuusisto J, Deeb S, Auwerx J, Laakso M. The Pro12Ala substitution in the peroxisome proliferator activated receptor gamma 2 is associated with an insulin-sensitive phenotype in families with familial combined hyperlipidemia and in nondiabetic elderly subjects with dyslipidemia. *Atherosclerosis* 2000;151:567-574.
73. Geurts JMW, Janssen RGJH, van Greevenbroek MMJ, van der Kallen CJH, Cantor RM, Bu XD, Aouizerat BE, Allayee H, Rotter JI, de Bruin TWA. Identification of TNFRSF1B as a novel modifier gene in familial combined hyperlipidemia. *Human Molecular Genetics* 2000;9:2067-2074.
74. Human genome project 2001. *Science* 2001;291:1195-1200.
75. Lander ES, Linton LM, Birren B. Initial sequencing and analysis of the human genome. *Nature* 2001;409:860-921.
76. Eurlings PMH, Geurts JMW, van der Kallen CJH, de Bruin TWA. Genetic dissection of familial combined hyperlipidaemia. *European Journal of Clinical Investigation* 2001;31:20: 88 (Abstract).
77. Eurlings PMH, van der Kallen CJH, Geurts JMW, van Greevenbroek MMJ, de Bruin TWA. Genetic dissection of familial combined hyperlipidemia. *Molecular Genetics and Metabolism* 2001;in press.

Chapter 4

Familial Dyslipidemic Hypertension Syndrome: further delineation in Familial Combined Hyperlipidemia, and the role of abdominal fat mass

ETP Keulen, C Voors-Pette, TWA de Bruin

American Journal of Hypertension
2001;14:357-363

Abstract

Familial Combined Hyperlipidemia (FCHL) is the most frequent genetic lipid abnormality in humans, with a 5- to 10-fold increased risk of early myocardial infarction. FCHL has been proposed as the leading cause of dyslipidemia in Familial Dyslipidemic Hypertension (FDH). It was the objective of this study to quantify and analyse the simultaneous occurrence of hypertension and hyperlipidemia in FCHL families. We assessed blood pressure and hyperlipidemia in 27 families with FCHL (235 relatives and 140 spouses, aged 30 to 60 years). Hypertension was defined as a blood pressure more than 140/90 mmHg, or the use of antihypertensive medication. Multiple backward linear regression analysis was used to derive a biological formula describing blood pressure in FCHL families. One-third of 27 FCHL-families were diagnosed with FDH. Sixty-four of 235 (27.2%) relatives had dyslipidemic hypertension (DH), compared to 20 of 140 (14.3%) spouses ($p=0.005$); odds ratio = 2.25 (95% confidence interval 1.29-3.91). Multiple linear regression analysis showed that age, FCHL-status and waist-circumference significantly contributed to systolic blood pressure in females. In conclusion, in FCHL we defined age, waist-circumference and hyperlipidemia as predictors of systolic blood pressure. This study indicates that visceral adipose tissue strongly contributes to the high prevalence of DH in FCHL families. Reduction of visceral fat should be tested as a potential therapeutic intervention for hyperlipidemia and hypertension in FCHL individuals.

Introduction

Hyperlipidemia, hypertension, diabetes mellitus, smoking, and a positive family history of cardiovascular disease are established major risk factors for the development of premature atherosclerosis and subsequent cardiovascular events. Multiple combinations of these cardiovascular risk factors will lead to an exponentially increased risk of early coronary artery disease (CAD)^{1,2}. Hyperlipidemia, hypertension and a positive family history of CAD combined occur as a separate entity: Familial Dyslipidemic Hypertension (FDH), which has been delineated in a large survey on the genetics of essential hypertension in Utah, USA³. Approximately 12% of all adults in families with essential hypertension also expressed dyslipidemia³. Although no formal study was done at that time and early coronary artery events had not been recorded, Familial Combined Hyperlipidemia (FCHL) was proposed to be the most frequent cause of the observed 12% dyslipidemic cases³.

FCHL is, by far, the most common genetic dyslipidemia in humans, with a prevalence of 0.5 to 1.0% in the general population⁴⁻⁷. Ten percent of survivors of early onset coronary artery disease have been reported to be affected with FCHL, resulting in a high relative risk ratio of 10 with regard to early CAD^{4,5}. It is very likely that the presence of both hyperlipidemia and hypertension in FCHL families contribute to such high risk of early CAD. It is the objective of this study to quantify, for the first time, the simultaneous occurrence of hypertension and combined hyperlipidemia in FCHL families and to evaluate a common pathogenic ancestry for FDH and FCHL.

Subjects and methods

Twenty-seven unrelated Caucasian FCHL index patients (proband) were identified at our Lipid Clinic. FCHL probands met each of the following criteria: first, a primary hyperlipidemia including fasting total plasma cholesterol (TC) >6.5 mmol/L (250 mg/dL) and/or fasting plasma triglyceride (TG) concentration >2.3 mmol/L (200 mg/dL); second, at least one first-degree relative with a different hyperlipidemic phenotype (Fredrickson Classification IIa, IIb or IV); third, a positive family history of premature cardiovascular disease (CVD). Premature CVD was defined as the occurrence of a myocardial infarction (MI) or cerebrovascular disease before the age of 60 y in at least one first degree relative of the proband, or the proband him-/herself. Secondary causes of hyperlipidemia (renal or hepatic insufficiency, hypothyroidism and medication), presence of the apo E2/E2 genotype, and subjects with tendon xanthomas or a diagnosis matching familial hyper-cholesterolemia were excluded.

In total, during the period 1991 to 1997, 95% of living relatives over 18 years of age have been collected, (n=470) to confirm the diagnosis of FCHL and in the

framework of metabolic and genetic studies⁸. The structure of these pedigrees has been published earlier⁸. In the report by Williams et al³, Familial Dyslipidemic Hypertension (FDH) was diagnosed as follows: first, hypertension before the age of 60 y; second, another first degree relative before age 60 y with hypertension; and third, dyslipidemia³. In the present study hypertension has been defined as either systolic blood pressure (SBP) >140 mmHg and/or diastolic blood pressure (DBP) >90 mmHg, or the current use of antihypertensive medication^{9,10}. Dyslipidemic Hypertension (DH) was diagnosed according to classical criteria when a subject was both hyperlipidemic and hypertensive³. Because of the upper age cut-off in Williams' report (age 60 y)³, our present study group contained all FCHL relatives and spouses between 30 and 60 years of age, thus including 235 FCHL-relatives and 140 spouses. In the present analysis, subjects younger than 30 years were not included because of a low penetrance of the DH phenotype in that age group (5/127=3.9%). Hypertensive subjects had to exhibit a TC >6.5 mmol/L (250 mg/dL) and/or a fasting plasma TG concentration >2.3 mmol/L (200 mg/dL) before they were included as DH individual. FCHL-relatives were divided into a hyperlipidemic (n=121) and normolipidemic (n=114) group.

Measurements

Clinical measurements were performed at the clinical research unit in the morning (08⁰⁰ – 10⁰⁰) after an overnight fast (12-14 h). Subjects had refrained from smoking and had not drunk coffee in the morning. Participants also had abstained from alcohol for at least 72 hours. A medical history was obtained from all participating subjects and a standard questionnaire was filled out. All patients were weighed in underwear (Mettler electronic scale E1200, Greifensee, Switzerland), the height was determined by stabilometer and the body mass index (BMI) was subsequently calculated as weight in kilograms / (height in meters)². The waist was measured at the level of the umbilicus, the hip circumference was measured at the level of the trochanter major and the waist to hip ratio (WHR) was calculated. Both waist-circumference and hip-circumference were measured with the subject in standing position.

Blood pressure was measured twice with a mercury sphygmomanometer in a standardised fashion, with the subject in sitting position after 10 minutes of rest. Cuff size was adjusted to the circumference of the arm, and the arm was placed with the cuff at heart-level. Systolic (SBP) and diastolic blood (DBP) pressures were defined according to Korotkoff sounds I and V⁹. With SBP and DBP, the mean of two measurements was used in the analyses. Mean arterial pressure (MAP) was defined as $DBP + 1/3(SBP - DBP)$. Pulse pressure (PP) was calculated as SBP minus DBP. The study protocol was approved by the Human Investigations Review Committee and performed according to the Helsinki Declaration. All subjects gave written informed consent.

Laboratory methods

Any lipid lowering medication had been withdrawn during two weeks before blood samples were collected. After an overnight fast (12-14 h) venous blood

was collected in pre-cooled EDTA (1-mg/ml) Vacutainer tubes for measurements of lipids, lipoproteins, glucose and insulin. Total cholesterol and fasting triglyceride concentrations were measured in duplicate by a commercially available colorimetric assay (Monotest Cholesterol kit, Boehringer Mannheim #1442350 and GPO-PAP, Boehringer Mannheim, #701912, respectively). HDL-cholesterol (HDL-C) was determined after phosphotungstate-MgCl₂ precipitation of whole plasma. Fasting plasma LDL-cholesterol (LDL-C) was calculated according to the Friedewald formula: $LDL-C = TC - HDL-C - (TG \times 0.45)^{11}$, if plasma TG concentration was below 4.5 mmol/L (390 mg/dL). Otherwise LDL-C was measured in the density 1.019 to 1.063 fractions, prepared by equilibrium ultracentrifugation. Nonesterified fatty acids (NEFA) were measured in plasma samples by an enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, Germany).

Insulin was measured by competitive radioimmunoassay with the polyclonal insulin antibody Caris 46, insulin tracer labelled with ¹²⁵I (IM.166, Radiochemical Centre, Amersham, England), and insulin standards (WHO: first international reference preparation 66/304). Plasma apolipoprotein B (apo B) and apolipoprotein A-1 (apo A-1) were determined by commercially available immunonephelometric assays, using calibrated standards according to the International Federation for Clinical Chemistry (Behringwerke, Marburg, Germany). Apolipoprotein C-III (apo C-III) was measured using a commercially available single radial immunodiffusion assay (Daiichi Chemicals, Tokyo, Japan) according to manufacturer instructions, as described^{12,13}.

Statistical analyses

All values are expressed as mean $\pm$ standard deviation. Log transformed values for fasting insulin and TG concentration were used in statistical analyses because of non-normal distribution; values are non-logarithmically listed in the tables for practical reasons. Differences between FCHL relatives and the spouse group were calculated using an independent sample T-test; one way ANOVA with Bonferroni correction was used for comparisons between hyperlipidemic relatives, normolipidemic relatives and the spouse group. Age and BMI were entered as independent variables in a multiple linear regression analysis between two groups.

Prevalence of DH and FDH in the study groups was compared using χ^2 -test. An odds ratio for the occurrence of DH was calculated¹⁴. Differences in the prevalence of DH over age-category were compared using χ^2 -test for a combined age-DH variable. To test which variables have an independent influence on blood pressure values, the effects of a variable, adjusted for the effects of the other independent variables, was analysed by backward stepwise multiple linear regression analyses with either SBP, DBP or MAP as dependent variable¹⁴. FCHL status was entered as a dummy variable. Although the number of individuals per family differed, a backward stepwise multiple linear regression analyses showed no significant contribution of one of the families to our results. In all statistical analyses, the statistical package SPSS 8.0 was used.

Results

The characteristics of the subjects studied are presented in Table 4.1.

Table 4.1 Characteristics of FCHL relatives from 27 FCHL families and 140 married-in spouses, age 30-59 y.

	FCHL	Spouses
Number	235	140
M / F	127 / 108	55 / 85
% on antihypertensive medication	7.7	4.3
Age (y.)	41.0 $\pm$ 7.9*	43.4 $\pm$ 8.2
BMI (kg/m ²)	25.3 $\pm$ 3.7	25.4 $\pm$ 3.6
WHR	0.87 $\pm$ 0.09*	0.84 $\pm$ 0.09
Systolic Blood Pressure (mmHg)	127.0 $\pm$ 14.9†	123.0 $\pm$ 15.2
Diastolic Blood Pressure (mmHg)	85.4 $\pm$ 10.7	83.4 $\pm$ 10.7
Mean Arterial Pressure (mmHg)	99.3 $\pm$ 11.2†	96.6 $\pm$ 11.5
Pulse Pressure (mmHg)	41.7 $\pm$ 10.6	39.5 $\pm$ 10.0
TC (mmol/L)	6.2 $\pm$ 1.8*	5.6 $\pm$ 1.0
TG (mmol/L)	2.4 $\pm$ 5.5*	1.6 $\pm$ 0.8
HDL-C (mmol/L)	1.17 $\pm$ 0.33†	1.25 $\pm$ 0.34
LDL-C (mmol/L)	4.02 $\pm$ 1.18*	3.67 $\pm$ 0.96
Apo B (g/L)	1.14 $\pm$ 0.34*	1.01 $\pm$ 0.28
Glucose (mmol/L)	4.8 $\pm$ 0.7	4.8 $\pm$ 0.7
Insulin (μ U/mL)	7.7 $\pm$ 6.8	7.5 $\pm$ 7.9

BMI = body mass index, WHR = waist to hip ratio, TC = total cholesterol (for mg/dL divide by 0.0259), TG = plasma triglyceride (for mg/dL divide by 0.0113), HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, glucose = for mg/dL divide by 0.0555, insulin = for pmol/L multiply with 6.945.

* = $p < 0.01$, † = $p < 0.05$

Systolic blood pressure (SBP) and mean arterial pressure (MAP) were higher in FCHL relatives compared to spouses (127.0 $\pm$ 14.9 vs. 123.0 $\pm$ 15.2 mmHg, ($p < 0.05$) and 99.3 $\pm$ 11.2 vs. 96.6 $\pm$ 11.5 mmHg, ($p < 0.05$), respectively). The pulse pressure (PP) showed a trend towards a significant difference between FCHL relatives and spouses ($p = 0.052$). FCHL family members were significantly younger than the spouses, with significantly higher TC, TG, LDL-C, and apo B concentrations and WHR, and significantly lower HDL-C levels. No differences between FCHL relatives and spouses were observed in plasma glucose or insulin concentrations or in the use of antihypertensive medication: in 7.7% of FCHL relatives versus 4.3% of spouses.

Table 4.2 lists the prevalence of Familial Dyslipidemic Hypertension (FDH) and dyslipidemic hypertension (DH) in each of the 27 studied FCHL pedigrees. Nine of twenty-seven FCHL families (33.3%) were diagnosed with FDH.

Table 4.2 Prevalence of FDH and DH in 27 FCHL-families and 140 married-in spouses, age cut-off 30-60 y.

FCHL Family No.	Presence of FDH	DH (affected / n relatives)	% with DH	Odds ratio (95% CI)
1	Yes	10 / 54	18.5	
2	Yes	4 / 16	25.0	
3	No ^a	1 / 5	20.0	
4	No ^a	1 / 12	8.3	
5	No ^a	1 / 4	25.0	
6	No ^a	1 / 3	33.3	
7	No ^a	1 / 4	25.0	
8	No ^a	1 / 8	12.5	
9	No	0 / 1	0	
10	No	0 / 6	0	
11	Yes	11 / 19	57.9	
12	Yes	4 / 10	40.0	
13	Yes	3 / 8	37.5	
14	Yes	5 / 13	38.5	
15	No ^a	1 / 7	14.3	
16	Yes	7 / 19	36.8	
17	No	0 / 1	0	
18	Yes	5 / 12	41.7	
19	Yes	2 / 6	33.3	
20	No	0 / 4	0	
21	No ^a	1 / 2	50.0	
22	No ^a	1 / 4	25.0	
23	No ^a	1 / 7	14.3	
24	No ^a	1 / 4	25.0	
25	No ^a	1 / 2	50.0	
26	No	0 / 1	0	
27	No	0 / 3	0	
9/27 (33.3 %) families		64 / 235 FCHL relatives*	27.2 %	
		20 / 140 spouses	14.3 %	
				2.25 (1.29 – 3.91)

FDH = Familial Dyslipidemic Hypertension, DH = Dyslipidemic Hypertension, n = total number, a = at least 2 affected relatives required to diagnose FDH, OR = Odds Ratio, CI = confidence interval.

* = $p < 0.01$ FCHL-relative vs. Spouses

Because of the age-cut off (30-60 y), several families became smaller in size (<7 individuals) or even nuclear. This may have underestimated the diagnosis of FDH. Sixty-four out of 235 (27.2%) relatives, aged 30-60 y, were diagnosed with dyslipidemic hypertension, compared to twenty out of 140 (14.3%) spouses, ($p=0.005$). This statistically highly significant difference resulted in a significant odds ratio of 2.25 (95% confidence interval, 1.29-3.91) of dyslipidemic hypertension in FCHL relatives. The incidence of essential hypertension, without

dyslipidemia, in normolipidemic relatives (33 of 114; 28.9%) was comparable to the incidence in normolipidemic spouses (23 of 99; 23.2%), $p=0.36$; NS.

The percentages of FCHL relatives and spouses with dyslipidemic hypertension in each 10-y age-category are depicted in Figure 4.1. A significantly steeper increase with age is shown for the FCHL relatives compared with that observed in the spouse control group, ($p<0.001$ for trend). At age 40 y and over, dyslipidemic hypertension was at least two times as frequent among FCHL relatives as in spouses (Figure 4.1).

After this qualitative analysis we evaluated quantitative aspects of blood pressure in men and women separately. Twenty-four subjects on antihypertensive medication were now excluded from these analyses ($n=11$ FCHL men, $n=7$ FCHL women, $n=2$ spouse men and $n=4$ spouse women). In women, hyperlipidemic relatives had significantly higher age- and BMI-adjusted SBP, DBP and MAP than spouses (SBP, 129.0 ± 16.9 vs. 120.8 ± 14.9 mmHg ($p<0.01$); DBP, 87.0 ± 10.1 vs. 81.4 ± 9.1 mmHg ($p<0.01$) and MAP, 101.0 ± 11.3 vs. 94.5 ± 10.4 mmHg ($p<0.01$), respectively) (Table 4.3). These differences were not observed in hyperlipidemic men. After adjustment for differences in age and BMI, normolipidemic relatives exhibited a significantly higher SBP than spouses (122.2 ± 12.9 vs. 120.8 ± 14.9 mmHg, $p<0.05$). Hyperlipidemic relatives had again higher DBP and MAP than normolipidemic relatives (87.0 ± 10.1 vs. 81.0 ± 9.2 mmHg ($p<0.05$), and 101.0 ± 11.3 vs. 94.7 ± 10.0 mmHg ($p<0.05$), respectively). (Table 4.3).

Figure 4.1 Percent Dyslipidemic Hypertension per 10 y age-category in FCHL relatives and married-in spouses ($p < 0.001$ for trend FCHL relatives versus spouses). Numbers in study: FCHL age 30-39, $n = 107$; age 40-49, $n = 89$; age 50-59, $n = 39$; spouses age 30-39, $n = 50$; age 40-49, $n = 56$; 50-59, $n = 34$.

Table 4.3 Blood pressure and anthropometric values in women age 30-59, without anti-hypertensive medication

	Normolipidemic relatives (n=62)	Hyperlipidemic relatives (n=38)	Spouses (n=81)
Age (years)	38.1 ± 6.9	42.2 ± 8.3	42.9 ± 7.9
BMI (kg/m ²)	23.2 ± 2.9	25.4 ± 3.8	24.7 ± 3.4
WHR	0.80 ± 0.07	0.81 ± 0.08	0.80 ± 0.08
Waist (cm)	80.1 ± 9.4	85.7 ± 10.7	83.0 ± 10.9
SBP (mmHg)	122.2 ± 12.9‡	129.0 ± 16.9*	120.8 ± 14.9
DBP (mmHg)	81.0 ± 9.2	87.0 ± 10.1* †	81.4 ± 9.1
MAP (mmHg)	94.7 ± 10.0	101.0 ± 11.3* †	94.5 ± 10.4
PP (mmHg)	41.2 ± 7.8‡	42.0 ± 12.5	40.6 ± 9.9

BMI = body mass index, WHR = waist to hip ratio, SBP = systolic blood pressure, DBP = diastolic blood pressure, MAP = mean arterial pressure, PP = pulse pressure.

* = HL vs. Spouses, $p < 0.01$; † = HL vs. NL, $p < 0.05$; ‡ = NL vs. Spouses, $p < 0.05$

In women, a multiple linear regression analysis showed that age, FCHL status and waist-circumference significantly contributed to SBP and MAP (Table 4.4). The analysis with DBP as dependent variable showed that age and FCHL status were significant contributors (Table 4.5), and waist-circumference was excluded at the last step of the regression analysis only. The analyses used age, BMI, WHR, waist-circumference, TC, logTG, HDL-C, NEFA, Apo-A1, Apo-B, Apo-CIII, glucose, (log)insulin and FCHL status as independent variables. Exclusion of the variable FCHL status did not result in an improved model for SBP nor DBP ($r^2=0.208$ for SBP and $r^2=0.086$ for DBP, respectively), although total cholesterol was now identified as a contributing factor.

Because waist-circumference was identified as independent variable contributing to blood pressure in women, we analysed the total group of 178 women, including those on medication. Twenty-one out of eighty (26.3%) women with a waist-circumference greater than 85.0 cm were diagnosed with dyslipidemic hypertension, compared to nine out of 98 (9.2%) women with a waist-circumference less than 85.0 cm ($p=0.004$, 2x2 table).

Table 4.4 Results of backward stepwise regression analysis with either Systolic Blood Pressure or Mean Arterial Pressure as dependent variable.

Systolic Blood Pressure

Variable	β	β SE	Stand. β	P	Model r^2
					0.217*
Intercept	66.711	10.281		< 0.001	
Age	0.674	0.150	0.359	< 0.001	
Affected FCHL	7.279	3.138	0.187	< 0.05	
Waist	0.311	0.109	0.224	< 0.01	

Mean Arterial Pressure

Variable	β	β SE	Stand. β	P	Model r^2
					0.157*
Intercept	66.262	7.379		< 0.001	
Age	0.362	0.110	0.266	< 0.001	
Affected FCHL	5.422	2.247	0.193	< 0.05	
Waist	0.171	0.082	0.170	< 0.05	

β SE: β standard error; stand β : standardised β ; affected FCHL = hyperlipidemic FCHL status; *: $p < 0.001$

Table 4.5 Results of backward stepwise regression analysis with Diastolic Blood Pressure as dependent variable

Diastolic Blood Pressure

Variable	β	β SE	Stand. β	P	Model r^2
					0.091*
Intercept	71.097	4.232		< 0.001	
Age	0.263	0.101	0.214	< 0.01	
Affected FCHL	5.723	2.093	0.224	< 0.01	

β SE: β standard error; stand β : standardised β ; affected FCHL = hyperlipidemic FCHL status; *: $p < 0.001$

Discussion

In the present study, one-third of FCHL families was diagnosed with Familial Dyslipidemic Hypertension (FDH) and 27% of FCHL relatives had dyslipidemic hypertension (DH). This prevalence is far in excess of the 12% population prevalence described by Williams et al³ as well as the 14.3% prevalence in spouses in the present study. Quantitative analyses revealed a statistically significant 2.25 higher probability of developing DH in FCHL relatives compared to their spouses. The over-expression of dyslipidemic hypertension in FCHL relatives increased with age, as observed in the 30 to 60 y age interval, and was

dependent on waist-circumference, which is a novel finding. The frequent occurrence of FDH in Dutch FCHL families corresponds well with the prediction of Williams et al³ that FCHL would represent the most common type of hyperlipidemia in FDH families, present in 15% of siblings¹⁵⁻¹⁷. The National Heart, Lung and Blood Institute (NHLBI) twin study reported that subjects with dyslipidemic hypertension have a coronary mortality rate that is approximately four times greater than their other twin halves, who shared only lipid abnormalities, or hypertension¹⁸. This figure is in complete agreement with the 5-10 fold higher risk of CAD in FCHL families. The frequent presence of hypertension in affected FCHL relatives does help to explain the hitherto less well understood occurrence of cerebrovascular accidents in FCHL families⁷. The simultaneous occurrence of FDH and FCHL suggests a common mechanism involved in the expression, or even the pathogenesis, of these disorders, apparently related to a genetic hyperresponse to abdominal fat.

Estimates of the heritability of systolic blood pressure (SBP) in population-based studies revealed a figure of 17% and with diastolic blood pressure of 12%¹⁹. Our model derived for systolic blood pressure in women explained 21.7% of the variability, which is in good agreement with these estimates. In addition to age, waist-circumference and affected FCHL status contributed to the expression of SBP and mean arterial pressure in women.

However, in men we could not detect any difference in blood pressure variables between FCHL relatives and spouses. FCHL is expressed more frequently in men (60.6%) than in women (40.7%) in Dutch FCHL families. One explanation is that women are partly protected from FCHL, and that women may suffer from a more severe form of FCHL (dyslipidemia and hypertension) when it develops. Another possibility to explain this men / women difference is an increase in background risk factors for hypertension in male spouses compared to female spouses. In that case, a smaller difference in average blood pressure variables between the group of FCHL men and the group of male spouses is expected, and a smaller difference is more difficult to detect given the sample size.

The Framingham Offspring Study contributed statistical evidence that systolic blood pressure is associated with dyslipidemia through a pathogenic link with obesity²⁰. It was shown that hyperinsulinemia did not represent the pathogenic link between hypertension and dyslipidemia, but that genetic or environment-induced abnormalities of adipose tissue are required for dyslipidemic subjects to become hypertensive²⁰. In the present study, waist-circumference, a measure of abdominal obesity and visceral fat, was identified as the (obesity) link between clinical expression of dyslipidemia and systolic hypertension in FCHL, in agreement with the above-mentioned Framingham model²⁰. Other studies have also shown a relationship between obesity, dyslipidemia and hypertension^{21,22}. Increased Free Fatty Acid flux (FFA) from visceral fat to the liver, resulting in hyperactivity of the sympathetic nervous system, has been proposed as an important mechanism in the pathogenesis of obesity-related hypertension^{23,24}. Moreover, an increased FFA-flux impairs the function of the hypothalamus-pituitary-adrenal axis, resulting in increased cortisol secretion and sodium retention, both contributing to hypertension^{25,26}. Increased FFA-flux from visceral fat directly to the liver can also contribute to the hypersecretion of triglyceride-rich

very low-density lipoproteins from the liver²⁷, a hallmark of FCHL. Increased VLDL secretion and impaired elimination^{12,28} result in increased plasma concentrations of atherogenic lipoprotein remnants, that can also provide a link between dyslipidemia and hypertension. Impaired nitric oxide-dependent vasodilatation in FCHL subjects has been associated with the plasma concentration of atherogenic triglyceride-rich remnant lipoproteins^{13,29}. Therefore, remnants may contribute to the development of premature atherosclerosis and expression of hypertension.

The present study not only ties FDH and FCHL together as specific hypertensive entities, but also suggests that a common mechanism is responsible for the pathogenesis of dyslipidemia as well as hypertension in FCHL. The mechanism could be FFA-flux from visceral adipose tissue, or an indirect mechanism inducing increased adipose tissue mass, or through lipoprotein abnormalities and insulin resistance. In support of this line of reasoning, we have identified in a recently published genome-wide scan⁸ a locus on chromosome 4, containing the alpha-adducin A gene, that showed a highly significant LOD score of 4.0 with systolic blood pressure and free fatty acids in the same FCHL families³⁰. The alpha-adducin A gene (ADD1) has been implicated as the cause of hypertension in a rat model³¹.

In conclusion, in FCHL we identified age, waist-circumference and hyperlipidemia as predictors of systolic blood pressure. Further genetic and metabolic studies are awaited, as this may have future implications for pathogenesis and treatment^{9,10,32}. The present study indicates that visceral adipose tissue strongly contributes to the high prevalence of dyslipidemic hypertension in FCHL families. Visceral fat, measured indirectly as waist-circumference, may represent the common pathogenic denominator in FCHL and FDH. Reduction of visceral fat versus treatment of hyperlipidemia should be tested as potential therapeutic interventions of the hypertension in FCHL individuals.

References

1. Turner RC, Millns H, Neil HA, Stratton IM, Manley SE, Matthews DR, Holman RR. Risk factors for coronary artery disease in non-insulin dependent diabetes mellitus: United Kingdom Prospective Diabetes Study (UKPDS: 23). *British Medical Journal* 1998;316:823-828.
2. Anderson KM, Odell PM, Wilson PWF, Kannel WB. Cardiovascular disease risk profiles. *American Heart Journal* 1991;121:293-298.
3. Williams RR, Hunt SC, Hopkins PN, Stults BM, Wu LL, Hasstedt SJ, Burlow GK, Stephenson SH, Lalouel JM, Kuida H. Familial Dyslipidemic Hypertension. Evidence from 58 Utah families for a syndrome present in approximately 12% of patients with essential hypertension. *Journal of the American Medical Association* 1988;259:3579-3586.
4. Goldstein JL, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG. Hyperlipidaemia in Coronary Heart Disease. II Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidaemia. *Journal of Clinical Investigation* 1973;52:1544-1568.
5. Rose HG, Kranz P, Weinstock M, Juliano J, Haft JJ. Inheritance of combined hyperlipoproteinaemia: evidence for a new lipoprotein phenotype. *American Journal of Medicine* 1973;54:148-160.
6. Nikkilä EA, Aro A. Family study of serum lipids and apolipoproteins in coronary artery disease. *Lancet* 1973;78:954-959.
7. Castro Cabezas M, de Bruin TWA, Erkelens DW. Familial combined hyperlipidaemia: 1973-1991. *Netherlands Journal of Medicine* 1992;40:83-95.
8. Aouizerat BE, Allayee H, Cantor RM, Davis RC, Lanning CD, Wen PZ, Dallinga-Thie GM, de Bruin TWA, Rotter JJ, Lusis AJ. A genome scan for familial combined hyperlipidemia reveals evidence of linkage with a locus on chromosome 11. *American Journal of Human Genetics* 1999;65:397-412.
9. Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: The sixth report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure. *Archives of Internal Medicine* 1997;157:2413-2446.
10. 1999 World Health Organization-International Society of Hypertension: Guidelines for the Management of Hypertension. Guidelines Subcommittee. *Journal of Hypertension* 1999;17:151-183.
11. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge. *Clinical Chemistry* 1972;18:499-502.
12. Castro Cabezas M, de Bruin TWA, de Valk HW, Shoulders CC, Jansen H, Erkelens DW. Impaired fatty acid metabolism in familial combined hyperlipidemia. A mechanism associating hepatic apolipoprotein B overproduction and insulin resistance. *Journal of Clinical Investigation* 1993;92:160-168.

13. Stroes E, de Bruin TWA, de Valk H, Erkelens DW, Banga JD, van Rijn H, Koomans H, Rabelink T. NO activity in familial combined hyperlipidemia: potential role of cholesterol remnants. *Cardiovascular Research* 1997;36:445-452.
14. Ingelfinger JA, Mosteller F, Thibodeau LA, Ware JH. *Biostatistics in clinical medicine*. New York, USA: McGraw-Hill, Inc, 1994.
15. Williams RR, Hopkins PN, Hunt SC, Wu LL, Hasstedt SJ, Lalouel JM, Ash KO, Stults BM, Kuida H. Population-based frequency of dyslipidemia syndromes in coronary-prone families in Utah. *Archives of Internal Medicine* 1990;150:582-588.
16. Hunt SC, Wu LL, Hopkins PN, Stults BM, Kuida H, Ramirez ME, Lalouel JM, Williams RR. Apolipoprotein, low density lipoprotein subfraction, and insulin associations with familial combined hyperlipidemia. Study of Utah patients with familial dyslipidemic hypertension. *Arteriosclerosis* 1989;9:335-344.
17. Williams RR, Hunt SC, Hopkins PN, Wu LL, Hasstedt SJ, Berry TD, Barlow GK, Stults BM, Schumacher MC, Ludwig EH, Elbein SC, Wilson DE, Lifton RP, Lalouel JM. Genetic basis of familial dyslipidemia and hypertension: 15-year results from Utah. *American Journal of Hypertension* 1993;6:319S-327S.
18. Selby JV, Newman B, Quiroga J, Christian JC, Austin MA, Fabsitz RR. Concordance for dyslipidemic hypertension in male twins. *Journal of the American Medical Association* 1991;265:2079-2084.
19. Schork NJ, Weder AB, Trevisan M, Laurenzi M. The contribution of pleiotropy to blood pressure and body-mass index variation: the Gubbio Study. *American Journal of Human Genetics* 1994;54:361-373.
20. Meigs JB, D'Agostino SRB, Wilson PW, Cupples LA, Nathan DM, Singer DE. Risk variable clustering in the insulin resistance syndrome. The Framingham Offspring Study. *Diabetes* 1997;46:1594-1600.
21. Dyer AR, Elliott P, Shipley M, Stamler R, Stamler J. Body mass index and associations of sodium and potassium with blood pressure in INTERSALT. *Hypertension* 1994;23:729-736.
22. Mark AL, Correia M, Morgan DA, Shaffer RA, Haynes WG. State-of-the-art-lecture: Obesity-induced hypertension: new concepts from the emerging biology of obesity. *Hypertension* 1999;33:537-541.
23. Grekin RJ, Dumont CJ, Vollmer AP, Watts SW, Webb RC. Mechanisms in the pressor effects of hepatic portal venous fatty acid infusion. *American Journal of Physiology* 1997;273:R324-R330.
24. Reaven GM, Lithell H, Landsberg L. Hypertension and associated metabolic abnormalities - the role of insulin resistance and the sympathoadrenal system. *New England Journal of Medicine* 1996;334:374-381.
25. Rosmond R, Dallman MF, Bjorntorp P. Stress-related cortisol secretion in men: relationships with abdominal obesity and endocrine, metabolic and hemodynamic abnormalities. *Journal of Clinical Endocrinology and Metabolism* 1998;83:1853-1859.
26. Kelly JJ, Mangos G, Williamson PM, Whitworth JA. Cortisol and hypertension. *Clinical and Experimental Pharmacology and Physiology* 1998;25:S51-S56.
27. Riches FM, Watts GF, Hua J, Stewart GR, Naoumova RP, Barrett PHR. Reduction in visceral adipose tissue is associated with improvement in apolipoprotein B-100 metabolism in obese men. *Journal of Clinical Endocrinology and Metabolism* 1999;84:2854-2861.

28. Cabezas MC, de Bruin TWA, Jansen H, Kock LA, Kortlandt W, Erkelens DW. Impaired chylomicron remnant clearance in familial combined hyperlipidemia. *Arteriosclerosis and Thrombosis* 1993;13:804-814.
29. Bredie SJH, Tack CJJ, Smits P, Stalenhoef AFH. Non-obese patients with familial combined hyperlipidemia are insulin resistant as compared with their non-affected relatives. *Arteriosclerosis Thrombosis and Vascular Biology* 1997;17:1465-1471.
30. Allayee H, de Bruin TWA, Cantor RM, Aouizerat BE, Lusis AJ, Rotter JJ. Genome scan in Dutch dyslipidemic families reveals novel blood pressure loci. *American Journal of Human Genetics* 1999;65:A16 (abstract)
31. Bianchi G, Tripodi G, Casari G, Salardi S, Barber BR, Garcia R, Leonie P, Torielli L, Cusi D, Ferrandi M, Pinna LA, Baralle FE, Ferrari P. Two point mutations within the adducin genes are involved in blood pressure variation. *Proceedings of the National Academy of Sciences of USA* 1994;91:3999-4003.
32. Lasser NL, Grandits G, Caggiula AW, Cutler JA, Grimm RHJ, Kuller LH, Sherwin RW, Stamler J. Effects of antihypertensive therapy on plasma lipids and lipoproteins in the Multiple Risk Factor Intervention Trial. *American Journal of Medicine* 1984;76:52-66.

Abstract

The objective of the present study was to determine whether FCHL subjects had prolonged heart rate corrected QT (QTc) times relative to spouse controls and to investigate the potential relationship of QTc with hyperlipidemia and surrogate markers of the insulin resistance syndrome.

The QT interval was measured in all leads from a standard 12-lead electrocardiogram in 76 FCHL-affected subjects and 92 spouses. All individuals were free of previous myocardial infarction, type 2 diabetes or cardiovascular medication. QTc interval was significantly prolonged in FCHL-affected women, but not in men, compared to spouse controls; the odds ratio of QTc >420 msec was 4.93 [95% confidence interval, 1.22-20.03]. Plasma triglyceride, HDL-cholesterol, fasting insulin and calculated insulin resistance (HOMA) were significantly correlated ($p < 0.05$) with QTc. Regression analyses revealed that plasma insulin, systolic blood pressure, HDL-cholesterol, body mass index, and free fatty acids were independent predictors of QTc.

In conclusion, in FCHL, prolonged QTc was specifically found in hyperlipidemic FCHL women and associated with components of the insulin resistance syndrome. QTc prolongation either relates to the genetic susceptibility to FCHL in women, or represents an adaptation mechanism to insulin resistance. QTc prolongation may add to the risk of coronary artery disease in FCHL.

Chapter 5

QT-interval (QTc) is prolonged in Familial Combined Hyperlipidemia: association with Insulin Resistance

ETP Keulen, K Rijkers, I Lutgens,
JMJP van Lin, NC Schaper,
TWA de Bruin

Submitted for publication

Introduction

An association has been observed between the duration of the heart rate corrected QT-interval (QTc) and sudden death, both in patients with a myocardial infarction and patients who had a diagnostic 24-hour electrocardiography¹⁻⁴. Moreover, prolonged duration of QTc raises the risk of coronary heart disease related death in the general population⁵⁻⁸. This greater risk has been attributed to an imbalance of sympathetic innervation at the level of left and right ganglia stellata⁹, predominant sympathetic over parasympathetic activity^{10,11}, or myocardial membrane defects^{12,13}, each separately or in combination leading to electrical instability in situations of high sympathetic activity. Recently, a new observation was made that, in an elderly male population (age >70 years), QTc was positively associated with plasma insulin and glucose concentrations¹⁴. Therefore, QTc prolongation was suggested as an additional feature of the insulin resistance syndrome. A prolonged QTc interval should be distinguished from the long QT syndrome. This genetic syndrome is caused by multiple monogenetic defects and defined by QTc intervals longer than 440 msec among family members.

Familial Combined Hyperlipidemia (FCHL) is the most common genetic dyslipidemia in humans, with a prevalence of 0.5-1.0% in the general population^{15,16}. Ten percent of survivors of myocardial infarction before the age of 60 years have been reported to be affected with FCHL, resulting in a high relative risk ratio of about 10 for early coronary artery disease in index cases¹⁵. Recently, two reports addressed the seriously increased risk of fatal and non-fatal coronary artery disease in the relatives of FCHL probands and found odds ratios of 1.7 and 5.1, respectively^{17,18}. Patients with FCHL are characterised by an overproduction of apolipoprotein B and very low-density lipoproteins^{19,20} in combination with a relative impairment of lipoprotein catabolism²¹. Furthermore, FCHL affected patients are frequently insulin resistant²², and dyslipidemic hypertension has been reported as well²³. However, these classical cardiovascular risk factors alone can not fully explain the high cardiovascular risk in FCHL¹⁸. Because QTc prolongation is associated with increased risk of coronary heart disease death and has been proposed as additional feature of the insulin resistance syndrome, QTc prolongation may also occur in FCHL.

It is the objective of the present study to determine whether FCHL subjects have prolonged QTc times relative to spouse controls and to investigate the potential relationship of QTc with hyperlipidemia and surrogate markers of the insulin resistance syndrome.

Subjects and methods

In total, in the period of 1997 – 1999, 308 subjects from 27 Caucasian FCHL families attended our laboratory to confirm the diagnosis of FCHL in their family. In the framework of metabolic and genetic studies, a standard resting 12-lead electrocardiogram was made in 105 hyperlipidemic relatives and 103 spouses, using a Marquette 12 SL ECG (GE Marquette Medical Systems, Milwaukee, WI, USA).

Hyperlipidemic subjects were characterised by a primary hyperlipidemia, defined as fasting total plasma cholesterol >6.5 mmol/L (250 mg/dL) or fasting plasma triglyceride concentration >2.0 mmol/L (180 mg/dL). Because a prolonged QT-interval can result from a myocardial infarction, subjects with a previous myocardial infarction (11 FCHL, 3 spouses) have been excluded. Furthermore, subjects with a bundle branch block (1 FCHL), subjects using β -blockers (9 FCHL, 4 spouses)²⁴, or subjects with type 2 diabetes mellitus (7 FCHL, 3 spouses)²⁵ were excluded from the analysis, as these conditions are known to potentially prolong the QT-interval.

QT intervals were automatically measured in all 12 leads of the electrocardiogram. The beginning of the QT-interval was defined as the first deflection of the QRS complex, and the end as return of the T wave to the isoelectric line. QTc interval was calculated with a modified Bazett formula, using heart rate instead of RR interval for adjustment. For this reason subjects with sinus arrhythmia (1 FCHL, 1 spouse) were also excluded from the analysis. The remaining study population consisted of 76 FCHL affected subjects and 92 spouses. The study protocol was approved by the Human Investigations Review Committee and performed according to the Helsinki Declaration. All subjects gave written informed consent.

Measurements

The measurements were performed in the morning (8.00 – 11.00) after an overnight fast (12-14 h). Subjects had refrained from smoking and had not drunk coffee or tea in the morning. Subjects had also abstained from alcohol for at least 72 hours. A medical history was obtained from all participating subjects and a standardised questionnaire was filled out. All patients were weighed in underwear (EKS), height was determined by stadiometer and the body mass index was subsequently calculated as weight in kilograms / (height in meters)². The waist-circumference was measured at the level of the umbilicus, the hip-circumference was measured at the level of the trochanter major, and the waist to hip ratio was calculated. Both waist-circumference and hip-circumference were measured with the subject in standing position.

Systolic and diastolic blood pressures were measured twice, with the Omron 705CP (OMRON Health Care GmbH, Hamburg, Germany), in a standardised fashion. Subjects were in a sitting position after 10 minutes of rest. Cuff size was

adjusted to the circumference of the arm, and the arm was placed with the cuff at heart-level. The mean of two measurements was used in the analyses.

Laboratory methods

Any lipid lowering medication had been withdrawn for two weeks before fasting blood samples were collected. After completion of all physical measurements, venous blood was collected in pre-cooled EDTA (1-mg/ml) Vacutainer tubes for measurements of lipids, apolipoproteins, glucose, and insulin. Total cholesterol and fasting triglyceride concentrations were measured in duplicate by a commercially available colorimetric assay (Monotest Cholesterol kit, Boehringer Mannheim #1442350 and GPO-PAP, Boehringer Mannheim, #701912, respectively). High-density Lipoprotein cholesterol (HDL-C) was determined after phosphotungstate-MgCl₂ precipitation of whole plasma. Fasting plasma low-density lipoprotein cholesterol (LDL-C) was calculated according to the Friedewald formula: $LDL-C = Total\ Cholesterol - HDL-C - (TG \times 0.45)^{26}$, if plasma triglyceride concentration was below 390 mg/dL (4.5 mmol/L). Otherwise LDL-C was measured in the density 1.019-1.063 fractions, prepared after equilibrium ultracentrifugation²⁶. Free fatty acids were measured in plasma samples by an enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, Germany).

Fasting blood glucose was measured immediately on a YSI 2300 monitor (Yellow Spring Instruments, USA). Fasting insulin concentration was determined using an Elisa (Mercodia AB, Uppsala, Sweden), with a cross-reactivity to pro-insulin <0.01%. Surrogate insulin resistance was calculated as the proposed formula of the homeostasis model assessment (HOMA): $Resistance = fasting\ insulin / (7 \times 22.5 \times e^{-ln\ glucose})$, which formula can be simplified as $(fasting\ insulin \times fasting\ blood\ glucose) / (7 \times 22.5)^{27}$. This surrogate marker of insulin resistance shows an association of -0.82 with insulin sensitivity determined by hyperinsulinemic euglycemic clamp²⁸.

Plasma apolipoprotein B and apolipoprotein A-1 were determined by commercially available immunonephelometric assays, using calibrated standards according to the International Federation for Clinical Chemistry (Behringwerke, Marburg, Germany).

Statistical analyses

All values are expressed as mean $\pm$ standard deviation. Log transformed values for fasting insulin and triglyceride concentrations were used in statistical analyses because of non-normal distribution; values are non-logarithmically listed in the Table 5.1 and Figure 5.1 for practical reasons. Differences between FCHL-affected subjects and spouse controls were calculated using linear regression analyses with age as co-variable. Prevalence of a QTc interval greater than 420 msec^{5,6} or 440 msec in either study group, FCHL or spouses, was compared by age-adjusted logistic regression analysis. The subsequent odds ratio was calculated²⁹.

Age-corrected Pearson correlation coefficients were determined for the relationship between continuous QTc variable and surrogate insulin resistance

indices. Moreover, age- and body mass index-corrected univariate analysis of variance was used to test whether tertile categories ($n=28$ or 29 subjects in each) of fasting plasma triglyceride concentration, HDL-cholesterol, fasting plasma insulin and HOMA were significantly associated with QTc. One way ANOVA with Bonferroni correction was used for comparisons between the tertile categories. To test whether variables had an independent effect on the QTc interval, the effects of such a variable, adjusted for other independent variables, was analysed by backward stepwise multiple linear regression analyses with QTc as dependent variable²⁹. FCHL affected status was entered as an independent variable. Although in the present study group several individuals per family were present, a backward stepwise multiple linear regression analyses showed no significant contribution of one of the families to our results. In all statistical analyses, the statistical package SPSS 8.0 (SPSS Inc. Chicago, Ill., USA) was used.

Table 5.1 Characteristics of 76 hyperlipidemic FCHL relatives and 92 married-in spouses.

	FCHL women	Control women	FCHL men	Control men
Number	41	46	35	46
Age (years)	$49.3 \pm 14.2^*$	42.9 ± 10.2	43.3 ± 11.8	47.2 ± 12.1
TC (mmol/L)	$6.7 \pm 1.3^\ddagger$	5.2 ± 1.0	$6.9 \pm 1.7^\ddagger$	5.2 ± 0.9
HDL-C (mmol/L)	$0.98 \pm 0.24^\ddagger$	1.13 ± 0.24	$0.81 \pm 0.21^*$	0.91 ± 0.18
TG (mmol/L)	$2.0 \pm 0.9^\ddagger$	1.1 ± 0.4	$3.0 \pm 2.4^\ddagger$	1.3 ± 0.8
Apo B (g/L)	$1.43 \pm 0.34^\ddagger$	0.98 ± 0.24	$1.37 \pm 0.35^\ddagger$	1.07 ± 0.20
FFA (mmol/L)	0.53 ± 0.22	0.51 ± 0.21	0.42 ± 0.15	0.38 ± 0.18
Glucose (mmol/L)	5.0 ± 0.5	4.7 ± 0.5	4.9 ± 0.7	5.0 ± 0.5
Insulin (pmol/L)	$66.7 \pm 40.6^\ddagger$	40.2 ± 26.5	$71.8 \pm 73.7^*$	45.4 ± 26.5
HOMA	$2.17 \pm 1.48^\ddagger$	1.22 ± 0.79	2.27 ± 2.29	1.48 ± 0.95
BMI (kg/m ²)	$27.5 \pm 4.7^\ddagger$	24.0 ± 3.7	26.1 ± 3.2	26.7 ± 3.1
WHR	$0.90 \pm 0.09^\ddagger$	0.83 ± 0.07	0.95 ± 0.06	0.94 ± 0.06
SBP (mmHg)	$144.7 \pm 27.9^\ddagger$	123.1 ± 16.9	140.5 ± 8.9	136.9 ± 15.3
DBP (mmHg)	$89.1 \pm 15.7^\ddagger$	79.3 ± 9.1	89.6 ± 10.9	88.0 ± 11.5

Apo B = apolipoprotein B, BMI = Body Mass Index, DBP = Diastolic Blood Pressure, FFA: Free Fatty Acids, Glucose: for mg/dL, divide by 0.0555, HDL-C = High Density Lipoprotein Cholesterol, HOMA = calculated surrogate marker of insulin resistance, Insulin: for $\mu\text{U/mL}$, divide by 6.945, SBP = Systolic Blood Pressure, TC = Total Cholesterol (for mg/dL, divide by 0.0259), TG = plasma triglyceride (for mg/dL, divide by 0.0113), WHR = Waist-Hip ratio; * = FCHL vs. spouses, $p < 0.05$; ‡ = FCHL vs. spouses, $p < 0.01$; ‡ = FCHL vs. spouses, $p < 0.001$.

Results

Characteristics of the subjects studied are presented in Table 5.1. FCHL women were significantly older and had a significantly higher body mass index, waist to hip ratio and systolic and diastolic blood pressures compared to female spouses. Male and female FCHL subjects had significantly higher total cholesterol, fasting plasma triglyceride, and apolipoprotein B concentrations and a lower HDL-cholesterol level than spouses did. In addition, FCHL women had significantly higher fasting plasma insulin levels (FI: 66.7 ± 40.6 vs. 40.2 ± 26.5 pmol/L;

$p < 0.001$) and higher calculated insulin resistance (HOMA: 2.17 ± 1.48 vs. 1.22 ± 0.79 ; $p < 0.001$) than female controls. Fasting insulin levels were significantly higher in FCHL men relative to male controls (71.8 ± 73.7 vs. 45.4 ± 26.5 pmol/L, $p < 0.05$), as was observed in women, but the difference in calculated insulin resistance (HOMA) was not statistically significant. These abnormalities confirm earlier abnormalities observed in FCHL subjects.

QTc was corrected for age using linear regression analysis because of the significant age difference between the female populations under study, and the reported age-dependency of QTc³⁰. Age-corrected QTc in FCHL women was significantly prolonged compared to spouses (difference of 7.8 msec, age-corrected $p < 0.01$) (Table 5.2). In FCHL women, 10 out of 41 (24.4%) had a QTc-interval greater than 420 msec compared to 3 out of 46 (6.5%) female spouses, ($p < 0.05$). This resulted in a highly significant odds ratio of 4.93 (95% confidence interval, 1.22-20.03) of QTc > 420 msec in FCHL affected females (Table 5.3). With respect to a QTc cut-off value of 440 msec, no significant difference ($p = 0.10$) was found between FCHL women (3/41; 7.3%) and female spouses (0/46; 0%). In FCHL men, a significantly increased heart rate compared to male spouses was observed ($p < 0.05$), but after correction for heart rate and age, no differences in QTc interval were detected.

Earlier reports suggested that QTc might be part of an insulin resistant state. Subsequently, the potential relationship of QTc with components of the insulin resistance syndrome, and hyperlipidemia, were analysed in women. Men were not included in the analyses.

Table 5.2 Electrocardiogram heart rate and heart rate adjusted QT-interval, in FCHL affected subjects and spouses (p-values shown after adjustment for age).

	FCHL women	Control women	FCHL men	Control men
Number	41	46	35	46
HR (beats/min)	66.9 ± 9.0	62.8 ± 11.0	62.0 ± 7.8	$58.0 \pm 9.0^*$
QTc (msec)	$414.2 \pm 15.8^\dagger$	406.6 ± 9.5	403.6 ± 10.1	404.6 ± 14.4

HR = heart rate, QTc = heart rate corrected QT-interval; mean $\pm$ SD;

* = FCHL vs. spouses, age-adjusted $p < 0.05$; † = FCHL vs. spouses, age-adjusted $p < 0.01$;

Table 5.3 Prevalence of heart rate corrected QT-interval (QTc) greater than 420 msec

	FCHL	Spouses	Odds ratio (95% CI)
Women	10 / 41 (24.4%)*	3 / 46 (6.5%)	4.92 (1.22 – 20.03)
Men	3 / 35 (8.6%)	5 / 46 (10.9%)	NS

CI = confidence interval, NS = non significant; * = FCHL vs. spouses, age-adjusted $p < 0.05$

Age-adjusted Pearson correlations between QTc, lipid values and surrogate markers of insulin resistance, all treated as continuous variables, are depicted in Table 5.4. In women, a significantly positive correlation between QTc and log transformed triglyceride concentration, log transformed fasting insulin and HOMA were observed. A significantly negative correlation was found between

QTc and HDL-cholesterol. To visualise these correlations, tertile categories ($n=29$ subjects in each) of log triglyceride, HDL-cholesterol, log insulin and HOMA were identified. As shown in Figure 5.1, with longer duration of QTc, adjusted for age and BMI, higher triglyceride, insulin and HOMA and lower HDL-cholesterol were found.

Table 5.4 Age-adjusted Pearson correlation coefficients between heart rate corrected QT-interval (QTc), plasma lipids and surrogate markers of insulin resistance variables in women ($n=87$).

	QTc	p-value
BMI	-0.16	0.15, NS
WHR	0.18	0.11, NS
Log TG	0.25	<0.05
HDL-C	-0.29	<0.01
FFA	-0.01	0.96, NS
Log insulin	0.30	<0.01
HOMA	0.26	<0.05

BMI = body mass index, WHR = waist to hip ratio, TG = fasting plasma triglyceride, HDL-C = high-density lipoprotein cholesterol, FFA = free fatty acids, HOMA = calculated surrogate marker of insulin resistance, NS = non-significant.

Next, multiple backward linear regression analysis was used to evaluate whether one of these metabolic factors was the most dominant in predicting a prolonged QTc. This analysis suggested that insulin was the most important metabolic indicator of prolonged QTc, apart from systolic blood pressure, and that HDL-cholesterol was protective (Table 5.5). Age was no contributor. The analysis used age, body mass index, waist-hip ratio, systolic and diastolic blood pressures, total cholesterol, HDL-cholesterol, glucose, log insulin, free fatty acids, glycerol, apolipoprotein B, apolipoprotein A1, FCHL affected status, and heart rate⁸ as independent variables.

Table 5.5 Results of backward stepwise regression analysis with heart rate corrected QT-interval (QTc) as dependent variable.

Variable	β	β SE	Stand. β	p	Model r^2
					0.282 *
Intercept	396.427	12.692		<0.001	
Log insulin	16.614	6.216	0.393	<0.01	
SBP	0.196	0.052	0.411	<0.001	
HDL-C	-11.155	5.535	-0.235	<0.05	
BMI	-0.869	0.355	-0.354	<0.05	
FFA	-12.627	5.822	-0.233	<0.05	

SBP = systolic blood pressure, HDL-C = high-density lipoprotein cholesterol, BMI = body mass index, FFA = free fatty acids, β SE: β standard error; stand β : standardised β ;

* = $p < 0.001$

Figure 5.1 QTc values in tertile categories ($n=29$ for each) in women of: A, fasting plasma triglyceride (TG); B, plasma HDL-cholesterol; C, plasma Insulin; D, HOMA value (see Methods; a higher HOMA value indicates less insulin sensitivity; ref 27, 28)

* = age and BMI-adjusted $p < 0.02$, third vs. first tertile; † = age and BMI adjusted $p < 0.05$, third vs. second tertile.

Discussion

Women with FCHL showed a prolonged, age-adjusted, QTc duration compared to healthy female spouses. Quantitative analyses revealed a statistically significant 4.93 higher probability (odds ratio) of having a QTc greater than 420 msec in FCHL women relative to healthy women. A QTc greater than 440 msec is considered directly clinically relevant, especially in the framework of long QT syndrome. We identified 3 of 41 FCHL women, but no female spouses, with a QTc > 440 msec, and 10 of these 41 women had a QTc > 420 msec. It should be emphasised that even moderate QTc prolongation (QTc between 420-440 msec) has been associated with increased mortality during a 15 years follow-up

study in a healthy Dutch population⁵. In addition, an increased QTc interval has been associated with an increased risk of sudden cardiac death in Caucasians^{3,5-8} as well as in American Indians³¹. The data show that FCHL predisposes to prolonged QTc, specifically in women. In male subjects we could not detect any difference in QTc interval between FCHL relatives and spouses. One possibility is that FCHL-affected men with an increased QTc interval already suffered from cardiovascular disease or even died. The 6 FCHL men who had been excluded from the analyses because of previous myocardial infarction did show some evidence of an increased QTc interval (median QTc 411.5 msec), compared to male spouses (QTc 404.6 msec) and FCHL men without a previous MI (QTc 403.6 msec); (Table 5.2). A second explanation is that women have delayed expression of FCHL and suffer from subsequent cardiovascular events at a higher age. Therefore, FCHL women may have had more time to develop an increased number of cardiovascular risk factors and insulin resistance that promotes a prolongation of the QTc interval. Recently, a stronger relationship between QTc interval and subclinical atherosclerosis, as assessed by intima media thickness, was reported in female nondiabetic subjects without clinical coronary artery disease than in men³². QTc interval was proposed as indicator of subclinical atherosclerosis³². Therefore, in FCHL women, prolonged duration of QTc can increase the risk of sudden cardiac death by ventricular arrhythmia, but may be associated with increased atherosclerotic burden as well. FCHL is known for its associated high risk of developing cardiovascular events, with an odds ratio of 1.7 for fatal and 5.1 for non-fatal coronary artery disease^{17,18}.

In the present study, components of the insulin resistance syndrome, including higher plasma insulin, high triglyceride levels, low HDL-cholesterol and systolic blood pressure, all well-known metabolic risk indicators of cardiovascular mortality, were associated with a prolongation of the QTc interval. These relationships were previously described in healthy men¹⁴. Moreover, regression analyses revealed that fasting plasma insulin, systolic blood pressure and low HDL-cholesterol contributed independently to prolonged QTc. Increased left ventricular mass, secondary to hypertension, can also lead to QTc prolongation^{33,34}. However, none of our subjects had signs of established left ventricular hypertrophy on electrocardiogram.

Body mass index and free fatty acids were not significantly correlated with QTc in univariate analysis, in agreement with findings in a middle aged Finnish population³⁴. Conversely, higher plasma levels of HDL-cholesterol and lower insulin concentrations were associated with protection against QTc interval prolongation.

It has been shown that FCHL affected subjects are as insulin resistant as subjects with type 2 diabetes mellitus, as measured by hyperinsulinemic euglycemic clamp³⁵. Insulin resistance and concomitant hyperinsulinemia are known to increase sympathetic activity, and this increased sympathetic activity may be a dominant factor leading to QTc prolongation³⁶. The presently found positive association between the QTc interval and blood pressure may be related to an unfavourable balance in sympathetic and parasympathetic activity¹⁰. The elevated risk of cardiovascular death associated with QTc prolongation has been attributed to ventricular electrical instability as a consequence of sympathetic

stimulation unopposed by vagal activity^{10,11} or myocardial membrane defects^{12,13}. In fact, higher systolic and diastolic blood pressures and a trend towards an increased heart rate in FCHL women can be interpreted as signs of increased activity of the sympathetic nerve system, but this was not measured directly in the present study.

The present findings point to the metabolic origin of a prolonged QTc interval by deficient intracellular energy substrate utilisation. This concept requires further study.

In conclusion, the duration of QTc is prolonged in women with FCHL and a specific association with components of the insulin resistance syndrome: hyperinsulinemia, systolic blood pressure and low HDL-cholesterol, was found. In theory, QTc prolongation may relate to the genetic susceptibility to FCHL, but the findings suggest an adaptation mechanism to insulin resistance, more pronounced in women. Future studies will need to address the role of QTc duration in female mortality in type 2 diabetes, hypertension and familial combined hyperlipidemia.

References

1. Peters RW, Byington RP, Barker A, Yusuf S. Prognostic value of prolonged ventricular repolarization following myocardial infarction: the BHAT experience. The BHAT Study Group. *Journal of Clinical Epidemiology* 1990;43:167-172.
2. Algra A, Tijssen JG, Roelandt JR, Pool J, Lubsen J. QT interval variables from 24 hour electrocardiography and the two year risk of sudden death. *British Heart Journal* 1993;70:43-48.
3. Algra A, Tijssen JG, Roelandt JR, Pool J, Lubsen J. QTc prolongation measured by standard 12-lead electrocardiography is an independent risk factor for sudden death due to cardiac arrest. *Circulation* 1991;83:1888-1894.
4. Ahnve S. QT interval prolongation in acute myocardial infarction. *European Heart Journal* 1985;6 Suppl D:85-95.
5. Schouten EG, Dekker JM, Meppelink P, Kok FJ, Vandenbroucke JP, Pool J. QT interval prolongation predicts cardiovascular mortality in an apparently healthy population. *Circulation* 1991;84:1516-1523.
6. Dekker JM, Schouten EG, Klootwijk P, Pool J, Kromhout D. Association between QT interval and coronary heart disease in middle-aged and elderly men. The Zutphen Study. *Circulation* 1994;90:779-785.
7. de Bruyne M, Hoes AW, Kors JA, Hofman A, van Bommel JH, Grobbee DE. Prolonged QT interval predicts cardiac and all-cause mortality in the elderly. The Rotterdam Study. *European Heart Journal* 1999;20:278-284.
8. Elming H, Holm E, Jun L, Torp Pedersen C, Kober L, Kircshoff M, Malik M, Camm J. The prognostic value of the QT interval and QT interval dispersion in all-cause and cardiac mortality and morbidity in a population of Danish citizens. *European Heart Journal* 1998;19:1391-1400.
9. Schwartz PJ, Snebold NG, Brown AM. Effects of unilateral cardiac sympathetic denervation on the ventricular fibrillation threshold. *American Journal of Cardiology* 1976;37:1034-1040.
10. Ben DJ, Zipes DP. Differential response to right and left arteria subclaviae stimulation of early afterdepolarizations and ventricular tachycardia induced by cesium in dogs. *Circulation* 1988;78:1241-1250.
11. Takahashi N, Ito M, Ishida S, Fujino T, Saikawa T, Arita M. Effects of vagal stimulation on cesium-induced early afterdepolarizations and ventricular arrhythmias in rabbits. *Circulation* 1992;86:1987-1992.
12. Vincent GM, Timothy KW, Leppert M, Keating M. The spectrum of symptoms and QT intervals in carriers of the gene for the long-QT syndrome. *New England Journal of Medicine* 1992;327:846-852.
13. Zipes DP. The long QT interval syndrome. A Rosetta stone for sympathetic related ventricular tachyarrhythmias. *Circulation* 1992;84:1414-1419.
14. Dekker JM, Feskens EJ, Schouten EG, Klootwijk P, Pool J, Kromhout D. QTc duration is associated with levels of insulin and glucose intolerance. The Zutphen Elderly Study. *Diabetes* 1996;45:376-380.

15. Goldstein JL, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG. Hyperlipidaemia in Coronary Heart Disease. II Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidaemia. *Journal of Clinical Investigation* 1973;52:1544-1568.
16. Castro Cabezas M, de Bruin TWA, Erkelens DW. Familial combined hyperlipidaemia: 1973-1991. *Netherlands Journal of Medicine* 1992;40:83-95.
17. Austin MA, McKnight B, Edwards KL, Bradley CM, McNeely MJ, Psaty BM, Brunzell JD, Motulsky AG. Cardiovascular disease mortality in familial forms of hypertriglyceridemia: A 20-year prospective study. *Circulation* 2000;101:2777-2782.
18. Voors-Pette C, de Bruin TWA. Excess coronary heart disease in Familial Combined Hyperlipidemia, in relation to genetic factors and central obesity. *Atherosclerosis* 2001;157:481-489.
19. Venkatesan S, Cullen P, Pacy P, Halliday D, Scott J. Stable isotopes show a direct relation between VLDL apoB overproduction and serum triglyceride levels and indicate a metabolically and biochemically coherent basis for familial combined hyperlipidemia. *Arteriosclerosis Thrombosis and Vascular Biology* 1993;13:1110-1118.
20. Castro Cabezas M, de Bruin TWA, de Valk HW, Shoulders CC, Jansen H, Erkelens DW. Impaired fatty acid metabolism in familial combined hyperlipidemia. A mechanism associating hepatic apolipoprotein B overproduction and insulin resistance. *Journal of Clinical Investigation* 1993;92:160-168.
21. Cabezas MC, de Bruin TWA, Jansen H, Kock LA, Kortlandt W, Erkelens DW. Impaired chylomicron remnant clearance in familial combined hyperlipidemia. *Arteriosclerosis and Thrombosis* 1993;13:804-814.
22. Bredie SJH, Tack CJJ, Smits P, Stalenhoef AFH. Non-obese patients with familial combined hyperlipidemia are insulin resistant as compared with their non-affected relatives. *Arteriosclerosis Thrombosis and Vascular Biology* 1997;17:1465-1471.
23. Keulen ETP, Voor-Pette C, de Bruin TWA. Familial Dyslipidaemic Hypertension Syndrome: further delineation in Familial Combined Hyperlipidaemia, and the role of abdominal fat mass. *American Journal of Hypertension* 2001;14:357-363.
24. Ackerman MJ. The long QT syndrome: ion channel diseases of the heart. *Mayo Clinical Proceedings* 1998;73:250-269.
25. Bellavere F, Ferri M, Guarini L, Bax G, Piccoli A, Cardone C, Fedele D. Prolonged QT period in diabetic autonomic neuropathy: a possible role in sudden cardiac death? *British Heart Journal* 1988;59:379-383.
26. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge. *Clinical Chemistry* 1972;18:499-502.
27. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412-419.
28. Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, Monauni T, Muggeo M. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care* 2000;23:57-63.

29. Ingelfinger JA, Mosteller F, Thibodeau LA, Ware JH. Biostatistics in clinical medicine. New York, USA: McGraw-Hill, Inc, 1994.
30. de Bruyne MC, Hoes AW, Kors JA, Dekker JM, Hofman A, van Bommel JH, Grobbee DE, Feskens EJ, Schouten EG, Klootwijk P, Pool J, Kromhout D, Meppelink P, Kok FJ, Vandenbroucke JP. Prolonged QT interval: a tricky diagnosis? *American Journal of Cardiology* 1997;80:1300-1304.
31. Okin PM, Devereux RB, Howard BV, Fabsitz RR, Lee ET, Welty TK. Assessment of QT interval and QT dispersion for prediction of all-cause and cardiovascular mortality in American Indians: The Strong Heart Study. *Circulation* 2000;101:61-66.
32. Festa A, D'Agostino R, Rautaharju P, O'Leary DH, Rewers M, Mykkanen L, Haffner SM. Is QT interval a marker of subclinical atherosclerosis in nondiabetic subjects? The insulin resistance atherosclerosis study (IRAS). *Stroke* 1999;30:1566-1571.
33. Cavallaro V, Petretta M, Betocchi S, Salvatore C, Morgano G, Bianchi V, Breglio R, Bonaduce D. Effects of sustained training on left ventricular structure and function in top level rowers. *European Heart Journal* 1993;14:898-903.
34. Karjalainen J, Reunanen A, Ristola P, Viitasalo M. QT interval as a cardiac risk factor in a middle aged population. *Heart* 1997;77:543-548.
35. Pette C, Janssen M-J, van der Kallen CJH, de Bruin TWA. Specific insulin resistance of adipose tissue in familial combined hyperlipidemia. *Circulation* 1999;100:I-196 (abstract).
36. Rowe JW, Young JB, Minaker KL, Stevens AL, Pallotta J, Landsberg L. Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man. *Diabetes* 1981;30:219-225.

Chapter 6

Pro-coagulant endothelium in Familial Combined Hyperlipidemia

ETP Keulen, K Hamulyak, TWA de Bruin
Submitted for publication

Abstract

The approximately 10-fold increased risk of early cardiovascular disease in Familial Combined Hyperlipidemia (FCHL) is not fully explained by classical risk factors such as hyperlipidemia, insulin resistance and hypertension. Abnormal hemostatic properties of endothelium are potentially relevant to the development of clinical cardiovascular disease in FCHL. In this study we report that PAI-1 and vWf are elevated in FCHL subjects, indicating a pro-coagulant state. Surrogate markers of insulin resistance explained 50% of the variation in plasma PAI-1 levels. The significantly 1.8-fold higher levels of PAI-1 in FCHL represent one of the most dramatic biochemical abnormalities reported in this disorder.

Introduction

Major functions of the vascular endothelium are NO production and regulation of hemostasis, i.e. coagulation and fibrinolysis. The NO-production regulates, in part, vasodilatation and proliferation of cells in the vascular wall. In subjects with Familial Combined Hyperlipidemia (FCHL) impaired endothelial derived NO-dependent vasodilatation has been reported¹, and this abnormality can be almost completely reversed by antihyperlipidemic therapy. In FCHL, classical risk factors, including hyperlipidemia, insulin resistance and hypertension, contribute to the 10-fold elevated risk of early cardiovascular disease, but do not explain all of the observed cardiovascular risk. Abnormal hemostatic properties of endothelium can be important in the occurrence of cardiovascular events in FCHL, as described in other insulin resistant states such as type 2 diabetes mellitus. As far as we know, abnormal hemostatic properties of endothelium have not yet been studied in FCHL.

Endothelium produces pro-coagulant and anti-coagulant substances (pro-coagulant: von Willebrand factor (vWf); anti-coagulant: tissue factor pathway inhibitor (TFPI) and thrombomodulin) and components of the fibrinolytic system that promote or inhibit fibrinolysis (tissue type plasminogen activator (t-PA) and plasminogen activator inhibitor 1 (PAI-1), respectively).

In this study we report on an apparent pro-coagulant state of the endothelium in FCHL, as shown by elevated plasma PAI-1 and vWf concentrations. Surrogate markers of insulin resistance explained 50% of the variation in PAI-1 levels in FCHL, a disorder characterised by insulin resistant, dyslipidemia and hypertension.

Subjects and methods

In the framework of metabolic and genetic studies, venous blood was collected from 70 hyperlipidemic FCHL subjects (fasting plasma total cholesterol (TC) >6.5 mmol/L and/or fasting plasma triglyceride (TG) concentration >2.0 mmol/L) and 57 healthy married-in spouses. Fourteen FCHL-affected subjects identified with a previous cardiovascular history (CVD) had their event at least 6 months prior to the blood collection. The study protocol was approved by the Human Investigations Review Committee and performed according to the Helsinki Declaration. All subjects gave written informed consent.

All measurements were performed in the morning (8.00–11.00h) after an overnight fast (12–14h). Subjects had refrained from smoking and abstained from coffee or tea. Lipid lowering medication had been withdrawn for two weeks prior to the study. Venous blood was collected in pre-cooled tubes containing 3.25% sodium citrate (dilution 1:10) for measurements of vWf, TFPI, soluble thrombomodulin and PAI-1, using commercially available assays. Blood collected in the Biopool®stabilite™ devices was used to measure t-PA levels,

using a commercially available assay. Other measurements were described previously².

Statistical analyses

All values are expressed as mean $\pm$ standard deviation, except as median and range for PAI-1. An independent sample t-test was used to determine differences between the two groups. BMI was used as covariant in t-PA and PAI-1 analyses. For technical reasons, t-PA levels were only available in 49 FCHL affected subjects and 51 healthy controls. Log transformed fasting insulin and TG concentrations were used in statistical analyses because of non-normal distribution. The statistical package SPSS 8.0 was used.

Results

FCHL subjects exhibited their characteristic phenotype with significantly higher BMI, waist-circumference, systolic and diastolic blood pressures, total cholesterol (TC) concentration, fasting plasma triglyceride (TG) and insulin levels than controls (Table 6.1).

Table 6.1 Characteristics of 70 hyperlipidemic FCHL relatives and 57 healthy control subjects.

	FCHL (n=70)	Healthy controls (n=57)
Age (years)	47.3 $\pm$ 12.1	45.5 $\pm$ 12.5
TC (mmol/L)	6.9 $\pm$ 1.4†	5.1 $\pm$ 0.8
HDL-C (mmol/L)	0.88 $\pm$ 0.26‡	1.06 $\pm$ 0.27
TG (mmol/L)	3.1 $\pm$ 2.6‡	1.0 $\pm$ 0.4
Apo B (g/L)	1.4 $\pm$ 0.3‡	1.0 $\pm$ 0.2
Glucose (mmol/L)	5.2 $\pm$ 0.8	4.9 $\pm$ 0.5
Insulin (μ U/mL)	10.0 $\pm$ 8.5‡	4.6 $\pm$ 3.3
BMI (kg/m ²)	27.4 $\pm$ 3.9†	25.3 $\pm$ 3.8
Waist-circumference (cm)	95.3 $\pm$ 10.1‡	87.8 $\pm$ 13.1
SBP (mmHg)	140.3 $\pm$ 18.8†	129.7 $\pm$ 20.2
DBP (mmHg)	88.2 $\pm$ 10.6†	82.5 $\pm$ 11.8

TC = Total Cholesterol (for mg/dL, divide by 0.0259), HDL-C = High Density Lipoprotein Cholesterol, TG = plasma triglyceride (for mg/dL, divide by 0.0113), Apo B = apolipoprotein B, glucose: for mg/dL, divide by 0.0555, insulin: for pmol/mL, multiply by 6.945, BMI = Body Mass Index, SBP = Systolic Blood Pressure, DBP = Diastolic Blood Pressure; † = FCHL vs. healthy controls, $p < 0.01$; ‡ = FCHL vs. healthy controls, $p < 0.001$

Notably, FCHL subjects showed significantly higher PAI-1 levels and higher t-PA levels than healthy controls (PAI-1: 18 (3-38) vs. 10 (1-29) U/mL; $p < 0.001$, and t-PA: 10.8 $\pm$ 3.8 vs. 7.5 $\pm$ 3.9 ng/mL; $p < 0.001$) (Table 6.2). Furthermore, vWf was significantly higher in FCHL subjects compared to healthy controls (110.0 $\pm$

35.4 % vs. 94.3 ± 36.8 %, $p < 0.05$) (Table 6.2) Exclusion of 14 FCHL subjects with previous CVD did not alter these results, nor the statistical significance. Multivariate backward regression analyses, with the variables listed in Table 6.1, revealed that 50.5% of PAI-1 levels were explained by logTG, log insulin, glucose and apolipoprotein B, all FCHL traits (Table 6.3). Of these factors, especially hypertriglyceridemia and hyperinsulinemia were identified as the strongest contributors to the association with PAI-1.

Table 6.2 Endothelium derived factors of the coagulant and fibrinolytic system in 70 FCHL affected subjects and 57 healthy married-in spouses.

	FCHL (n=70)	Healthy controls (n=57)
PAI-1 (U/mL)	18 (3 - 38)‡	10 (1 - 29)
t-PA (ng/mL)	10.8 ± 3.8 ‡	7.5 ± 3.9
vWf (%)	110.0 ± 35.4 *	94.3 ± 36.8
TFPI (ng/mL)	47.1 ± 11.6	43.0 ± 11.7
S. Thrombomodulin (ng/mL)	31.8 ± 7.5	30.1 ± 9.2

PAI-1 = Plasminogen Activator Inhibitor 1 (median and range), t-PA = tissue derived Plasminogen Activator, vWf = von Willebrand factor, TFPI = truncated Tissue Factor Pathway Inhibitor, S. Thrombomodulin = soluble thrombomodulin. * = FCHL vs. spouses, $p < 0.05$; † = FCHL vs. spouses, $p < 0.01$; ‡ = FCHL vs. spouses, $p < 0.001$

Table 6.3 Results of backward stepwise regression analysis with PAI-1 as dependent variable.

PAI-1

Variable	β	β SE	Stand. β	P	Model r^2
					0.505 *
Intercept	-4.853	4.887			
Log TG	13.267	2.68	0.430	< 0.001	
Log Insulin	6.260	2.541	0.218	< 0.05	
Glucose	1.751	0.898	0.135	0.054	
Apo B	3.605	2.097	0.131	0.088	

β SE: β standard error; stand β : standardised β ; log TG = log triglyceride; apo B = apolipoprotein B; *: $p < 0.001$

Discussion

In the present study, FCHL affected subjects had higher PAI-1 and vWf levels, indicating a pro-coagulant, anti-fibrinolytic state of the endothelium. PAI-1 and vWf have shown to be independent risk factors of cardiovascular disease^{3,4}. Therefore, it is plausible that increased PAI-1 and vWf expression can contribute to the 10-fold higher risk of CVD in FCHL. Increased PAI-1 and vWf concentrations have been shown to reflect endothelial dysfunction, in agreement with other evidence in FCHL¹. High PAI-1 levels have been found in subjects with

insulin resistance and hypertension, two clinical features that are frequently observed in FCHL. In the present study, components of the insulin resistance syndrome explained 50.5% of the variation in PAI-1 levels, especially hypertriglyceridemia and hyperinsulinemia. This finding offers possibilities to lower PAI-1 levels by treating the contributing abnormalities, hypertriglyceridemia and hyperinsulinemia. Recently, we reported on the role of tumor necrosis factor α (TNF α) and TNF receptor 2 (TNFRSF2b) in FCHL². It has been shown that TNF α -induced PAI-1 expression in vascular endothelial cells as well as PAI-1 secretion from human adipocytes was inhibited by thiazolidinediones, offering another potential treatment modality in FCHL⁵.

In conclusion, the significantly elevated (1.8-fold higher) levels of PAI-1 in FCHL indicate an anti-fibrinolytic state of the endothelium, that is dependent on insulin resistance, and potentially contributes to the risk of premature cardiovascular disease. Because FCHL is best described as insulin resistant hyperlipidemia and hypertension, further studies to the contribution of PAI-1 to risk of cardiovascular disease, and the potential treatment of elevated PAI-1 and vWf levels are warranted.

References

1. Stroes E, de Bruin TWA, Valk de H, Erkelens DW, Banga JD, van Rijn H, Koomans H, Rabelink T. NO activity in familial combined hyperlipidemia: potential role of cholesterol remnants. *Cardiovascular Research* 1997; 36:445-452.
2. Geurts JMW, Janssen RGJH, van Greevenbroek MMJ, van der Kallen CJH, Cantor RM, Bu XD, Aouizerat BE, Allayee H, Rotter JI, de Bruin TWA. Identification of TNFRSF1B as a novel modifier gene in familial combined hyperlipidemia. *Human Molecular Genetics* 2000; 9:2067-2074.
3. Hamsten A, de Faire U, Walldius G, Dahlen G, Szamosi A, Landou C, Blomback M, Wiman B. Plasminogen activator inhibitor in plasma: risk factor for recurrent myocardial infarction. *Lancet* 1987; 2:3-9.
4. Thogersen AM, Jansson JH, Boman K, Nilsson TK, Weinehall L, Huhtasaari F, Hallmans G. high plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. *Circulation* 1998; 98:2241-2247.
5. Kato K, Satoh H, Endo Y, Yamada D, Midorikawa S, Sato W, Mizuno K, Fujita T, Tsumamoto K, Watanabe T. Thiazolidinediones down-regulate plasminogen activator inhibitor type 1 expression in human vascular endothelial cells: A possible role for PPARgamma in endothelial function. *Biochemical and Biophysical Research Communications* 1999; 258:431-435.

Chapter 7

Reduced structural and functional skin capillaries in Familial Combined Hyperlipidemia: association with increased remnant-like lipoprotein cholesterol levels

ETP Keulen, NC Schaper, AJHM Houben, JMJP van Lin, I Lutgens, K Rijkers, GM Dallinga-Thie, TWA de Bruin

Submitted for publication

Abstract

We determined whether abnormalities in basal (BC) and post-occlusive (POC) number of capillaries are present in Familial Combined Hyperlipidemia (FCHL), and investigated the relationship of POC with plasma lipids, remnant-like lipoprotein particles (RLP-C), blood pressure, and surrogate markers of insulin resistance.

Fifty age-matched subjects: 23 (12 men) hyperlipidemic, normotensive FCHL subjects and 27 (14 men) healthy controls participated in this study. Capillary density was measured just above the finger nailfold, before and after 4 minutes of arterial occlusion. BC and POC were significantly lower in FCHL men compared to healthy men, 113.7 ± 15.1 vs. 132.0 ± 18.0 ($p=0.02$) and 123 ± 19.1 vs. 142.3 ± 18.3 ($p=0.03$), respectively. POC was inversely correlated with total cholesterol ($r=-0.61$, $p<0.005$), logTG ($r=-0.52$, $p<0.05$), logRLP-C ($r=-0.51$, $p<0.05$) and log-insulin ($r=-0.48$, $p<0.05$). Multivariate analyses revealed that logRLP-C was the only significant, independent, contributor to POC.

In conclusion, in FCHL men a reduction in structural and functional skin capillaries was observed, and associated with increased atherogenic lipoprotein levels. This loss of capillary surface may be implicated in the pathophysiology of FCHL or can result from adaptation to the hyperlipidemia in FCHL.

Introduction

Familial Combined Hyperlipidemia (FCHL), the most common genetic dyslipidemia in humans, affects 0.5%-2% of the population and occurs in 10-20% of premature myocardial infarction survivors^{1,2}. Recently, two reports showed that FCHL relatives also have a markedly increased risk of fatal and non-fatal coronary artery disease (CAD), with odds ratios of 1.7 and 5.1, respectively^{3,4}. In addition to genetic factors, metabolic cardiovascular risk factors serve as an explanation for this high risk of CAD^{1,4}. FCHL is a metabolic, hyperlipidemic syndrome associated with apolipoprotein B overproduction^{5,6}, relatively impaired lipoprotein elimination^{6,7}, impaired insulin stimulated glucose uptake^{8,9}, and central obesity¹⁰. Moreover, the prevalence of dyslipidemic hypertension in FCHL is increased two-fold¹⁰.

A common feature of FCHL, hypertension and type 2 diabetes is impaired NO-mediated vasodilatation¹¹⁻¹³. In FCHL, atherogenic lipoprotein remnants have been causally linked to impaired endothelial-derived vasodilatation, because this abnormality partly normalised following antihyperlipidemic treatment that resulted in reduced lipoprotein remnant concentrations¹¹. Furthermore, increased levels of remnant particles have been associated with the presence and progression of cardiovascular disease and play an important role in the macrovascular atherogenic process. Increased levels of atherogenic remnant particles have also been demonstrated as part of the insulin resistance syndrome¹⁴. Recently, a new methodology has been described to measure remnant-like lipoprotein particles by immunoaffinity columns¹⁵⁻¹⁸. This methodology facilitates studies of larger groups and can therefore help to shed light on the interaction between atherogenic lipoproteins and the endothelium, or circulation parameters, in subjects at risk of atherosclerosis, such as in FCHL.

In subjects with hypertension or type 2 diabetes, several abnormalities in the microvasculature have been reported¹⁹⁻²⁵. A reduction in the density of capillaries, also known as rarefaction, is often observed in hypertensive patients¹⁹⁻²³ and, moreover, in subjects at risk of developing hypertension²⁶. Another abnormality in microvasculature function, impaired capillary recruitment, has been proposed as a link between elevated blood pressure and insulin resistance, in hypertensive²⁷ and normotensive subjects²⁸. However, no reduction in capillary density and no association between capillary density and blood pressure values have been demonstrated in subjects with type 2 diabetes or impaired glucose tolerance²⁹, who are both known to be insulin resistant. As a result, the possible relationship between insulin resistance and microvascular abnormalities is not clear. Moreover, the possible role of plasma lipids, lipoproteins and remnant particles in microvascular abnormalities have not yet been taken into account in the above mentioned studies. Furthermore, little is known about microvasculature in hyperlipidemic diseases. It was the aim of the present study to determine whether abnormalities in capillary structure and function are present in FCHL. The abnormalities found in capillary density were subsequently evaluated for their potential association with plasma lipids and

remnant-like lipoprotein particles, blood pressure values, and surrogate markers of insulin resistance.

Subjects and methods

Fifty age-matched subjects participated in the present study: 23 (12 men) hyperlipidemic, normotensive FCHL subjects and 27 (14 men) healthy controls. Hyperlipidemic subjects were characterised by a primary hyperlipidemia, defined as fasting plasma total cholesterol (TC) >6.5 mmol/L (250 mg/dL) and/or fasting plasma triglyceride (TG) concentration >2.3 mmol/L (200 mg/dL), as described before¹⁰. None of our subjects had established cardiovascular disease on clinical examination or ECG. Subjects using cardiovascular medication, or salicylates and NSAID's, as well as subjects with a newly diagnosed hypertension (as defined in the present study as blood pressure $>160/95$ on three consecutive visits) were excluded from this study. The study protocol was approved by the Human Investigations Review Committee and performed according to the Helsinki Declaration. All subjects gave written informed consent.

Measurements

The measurements were performed in the morning (8.00 – 11.00) after an overnight fast (12-14 h). Subjects had refrained from smoking and had not drunk coffee or tea in the morning. Subjects had also abstained from alcohol for at least 72 hours. A medical history was obtained from all participating subjects and a standardised questionnaire was filled out. All patients were weighed in underwear, the height was determined by stadiometer and the body mass index (BMI) was subsequently calculated as weight in kilograms / (height in meters)². The waist-circumference was measured at the level of the umbilicus, the hip-circumference was measured at the level of the trochanter major and the waist to hip ratio (WHR) was calculated. Both waist-circumference and hip-circumference were measured with the subject in standing position.

Systolic (SBP) and diastolic (DBP) blood pressures were measured twice, with the Omron 705CP (OMRON Health Care GmbH, Hamburg, Germany) in a standardised fashion, with the subject in sitting position after 10 minutes of rest. Cuff size was adjusted to the circumference of the arm, and the arm was placed with the cuff at heart-level. The mean of two measurements was used in the statistical analyses.

Evaluation of microcirculation

After an acclimatisation period of at least 20 minutes in a quiet, temperature-controlled (25.5 ± 0.5 °C) room, sufficient to result in a stable skin temperature, the microvascular measurements were performed. The subject was seated in upright position with the right hand in fixed position at heart level. Perfused skin capillaries were measured approximately 6 mm proximal to the nail fold of the dorsum of the right 4th finger, using capillary microscopy equipped with a 4x

objective. Only the tip of the capillary loop was visible in the above mentioned area. A drop of oil was applied to the skin to diminish scattering, and improve skin transparency. In the resting phase, three microscopic images ($1,6 \text{ mm}^2$) were videotaped (Panasonic) during 10 seconds for off-line analysis. The mean of these three images was used for baseline number of capillaries (BC). Subsequently, a wrist-cuff was inflated for four minutes at suprasystolic level to obtain arterial occlusion. Immediately after deflation of the wrist-cuff, two microscopic fields were measured for 8 seconds to obtain total post-occlusive number of capillaries (POC). The two post-occlusive fields overlapped the three baseline fields measured and all erythrocyte perfused capillaries were counted from a free-framed reproduction of the videotape and from the running videotape when it was uncertain whether a capillary was present or not. The mean of two fields was used in the analyses. Post-occlusive hyperaemic capillary recruitment (CR) was defined as $[(\text{POC} - \text{BC}) / \text{BC}] \times 100\%$. The day-to-day coefficient of variation was 8%, as determined in ten subjects on two occasions. Thermoregulatory skin perfusion was simultaneously determined with microscopy, using laser-Doppler fluxmetry (LDF, Periflux PF3, Perimed, Sweden), with the standard probe PF308, filter setting: 12 kHz ('wide band') and time constant: 0.2 s. The probe was placed at the dorsum of the middle phalanx of the right 3rd finger. This probe remained in the same position during the experiment. Flux values are expressed as arbitrary, but calibrated, perfusion units (PU). Perfusion units are determined by red blood cell concentration and red blood cell velocity. During the resting microscopy measurements of the capillaries, basal flux (LDBF) was measured as the mean flux during the last two minutes before the arterial occlusion. During the post-occlusive reactive hyperaemia, peak flux (LDPF), delta peak flux ($\text{delPF} = \text{peak flux} - \text{resting flux}$), percent post-occlusive reactive hyperaemia (LDPRH) $[(\text{delPF} / \text{LDBF}) \times 100\%]$, time to peak flux following release of occlusion and time to 50% recovery, were determined. All flux data are presented with the corresponding biological zero subtracted. The biological zero was defined as the mean laser-Doppler flux at the end of the arterial occlusion period. The day-to-day coefficient of variation of LDPRH was 16%, as determined in ten subjects on two occasions. During these measurements skin temperature was measured continuously by an electronic thermometer (Hewlett Packard, USA).

Laboratory methods

Any lipid lowering medication had been withdrawn for two weeks before blood samples were collected. After all measurement of microvasculature were performed, fasting venous blood was collected in pre-cooled EDTA (1-mg/ml) Vacutainer tubes for measurements of lipids, lipoproteins, glucose and insulin. Total cholesterol (TC) and fasting triglyceride (TG) concentrations were measured in duplicate by a commercially available colorimetric assay (Monotest Cholesterol kit, Boehringer Mannheim #1442350 and GPO-PAP, Boehringer Mannheim, #701912, respectively). HDL-cholesterol (HDL-C) was determined after phosphotungstate- MgCl_2 precipitation of whole plasma. Fasting plasma LDL-cholesterol (LDL-C) was calculated according to the Friedewald formula:

$LDL-C = TC - HDL-C - (TG \times 0.45)^{31}$, if plasma TG concentration was below 4.5 mmol/L (390 mg/dL). If plasma TG was above 4.5 mmol/L, LDL-cholesterol was measured in the density 1.019–1.063 fractions, prepared after equilibrium ultracentrifugation. Nonesterified fatty acids (NEFA) were measured in plasma samples by an enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, Germany).

The Remnant-like Lipoprotein Particles (RLP) fraction was prepared by use of an immunoseparation technique described by several laboratories^{15,16}. Briefly, 5 μ L of plasma was added to 300 μ L of mixed immunoaffinity gel suspension containing monoclonal anti-human apoA1 (H-12) and anti-human apoB-100 (JH-H) antibodies (Japan Immunoresearch Laboratories). The reaction mixture was gently shaken for 120 minutes at room temperature. After the supernatant was left standing for 15 minutes, 200 μ L was withdrawn for the assay of RLP cholesterol. Cholesterol in the RLP fraction (coefficient of variation <3%) was measured by an enzymatic assay with the use of an automatic chemistry analyser (Cobas Mira autoanalyser, ABX). In this RLP fraction, both cholesterol-containing apoB-48 chylomicron remnants and apoE-enriched apoB-100 β -VLDL, and intermediate density lipoprotein (IDL) lipoproteins are detected¹⁷. Both species of atherogenic remnants cannot bind to the anti-apoB monoclonal that is presumed to recognise an apoB epitope which is absent in apoB48, and made inaccessible on apoB100 by apoE.

Glucose (FBG) was measured immediately on a YSI 2300 monitor (Yellow Spring Instruments, USA). Fasting insulin concentration (FI) was determined using an Elisa (Mercodia AB, Uppsala, Sweden), with a cross-reactivity with pro-insulin <0.01%. Insulin resistance was calculated with the HOMA formula: Resistance = $FI / (7 \times 22.5 \times \varepsilon^{-\ln FBG})$, which can be simplified as $(FI \times FBG) / (7 \times 22.5)^{32}$. Plasma apolipoprotein B (apo B) and apolipoprotein A-1 (apo A-1) were determined by commercially available immunonephelometric assays, using calibrated standards according to the International Federation for Clinical Chemistry (Behringwerke, Marburg, Germany).

Statistical analyses

All values are expressed as mean $\pm$ standard deviation. Log transformed values for TG, RLP-C, insulin concentrations and HOMA were used in statistical analyses because of non-normal distribution; values are non-logarithmically listed in the tables for practical reasons. Differences between FCHL relatives and healthy controls were calculated using an independent sample T-test for all measurements. The Mann-Whitney-U test was used to calculate difference in laser Doppler derived parameters between FCHL relatives and healthy controls because of non-normal distribution; data are therefore given as median and range. Because a statistically significant difference existed in several microvascular parameters (delta number of capillaries, capillary recruitment and laser Doppler post-occlusive reactive hyperaemia) between healthy men and healthy women, all analyses were executed by gender. Pearson correlation coefficients were determined for the relationship between basal (BC) and post-occlusive (POC) number of capillaries and lipid levels, surrogate markers of

insulin resistance, and blood pressure values. The independent contribution of different variables to BC and POC was assessed by multiple linear regression analysis³³. Variables with a Pearson correlation coefficient <0.1 and Variance Inflation Factor (VIF) below 10 were entered into the multivariate analyses. Statistical analyses were done with the SPSS statistical package (SPSS Inc., Chicago, Ill., USA).

Results

The characteristics of the subjects studied are presented in Table 7.1. In both sexes, FCHL subjects had significantly higher waist to hip ratio (WHR), fasting total cholesterol concentration (TC), triglyceride levels (TG) and apo B concentrations than healthy subjects.

Table 7.1 Characteristics of the study population: 23 FCHL subjects and 27 healthy controls

	FCHL men	Control men	FCHL women	Control women
Number	12	14	11	13
Age (years)	41.3 $\pm$ 11.8	44.3 $\pm$ 8.3	46.2 $\pm$ 11.3	41.3 $\pm$ 6.5
BMI (kg/m ²)	27.7 $\pm$ 2.7	26.2 $\pm$ 3.4	26.5 $\pm$ 3.1*	23.5 $\pm$ 2.5
WHR	0.97 $\pm$ 0.06*	0.92 $\pm$ 0.07	0.87 $\pm$ 0.07*	0.81 $\pm$ 0.06
SBP (mmHg)	130.7 $\pm$ 10.9	127.3 $\pm$ 10.8	140 $\pm$ 16.5†	116.5 $\pm$ 11.6
DBP (mmHg)	80.4 $\pm$ 8.5	83.5 $\pm$ 7.4	88.6 $\pm$ 7.6†	76.2 $\pm$ 6.6
TC (mmol/L)	7.4 $\pm$ 1.6†	5.3 $\pm$ 0.6	7.4 $\pm$ 1.1†	5.0 $\pm$ 1.0
HDL-C (mmol/L)	0.77 $\pm$ 0.23*	1.00 $\pm$ 0.20	1.16 $\pm$ 0.19	1.02 $\pm$ 0.21
TG (mmol/L)	5.4 $\pm$ 5.2†	1.2 $\pm$ 0.4	2.1 $\pm$ 1.7*	1.1 $\pm$ 0.4
RLP-C (mmol/L)	1.54 $\pm$ 1.84†	0.24 $\pm$ 0.05	0.44 $\pm$ 0.42†	0.17 $\pm$ 0.04
Apo B (g/L)	1.4 $\pm$ 0.4†	1.0 $\pm$ 0.2	1.4 $\pm$ 0.2†	1.0 $\pm$ 0.2
Glucose (mmol/L)	5.0 $\pm$ 0.9	4.9 $\pm$ 0.5	5.3 $\pm$ 0.5*	4.8 $\pm$ 0.5
Insulin (pmol/L)	74.2 $\pm$ 46.6†	32.2 $\pm$ 14.8	66.2 $\pm$ 43.7	41.2 $\pm$ 29.6
HOMA	2.5 $\pm$ 1.7†	1.0 $\pm$ 0.6	2.2 $\pm$ 1.5*	1.3 $\pm$ 0.9

BMI = body mass index, WHR = waist to hip ratio, SBP = systolic blood pressure, DBP = diastolic blood pressure, TC = total cholesterol (for mg/dL divide by 0.0259), HDL-C = high-density lipoprotein cholesterol, TG = plasma triglyceride (for mg/dL divide by 0.0113), Apo B = apolipoprotein B, RLP-C = remnant-like lipoprotein particles cholesterol, insulin = for μ U/mL divide by 6.945, HOMA = calculated measure for insulin sensitivity;

* = FCHL vs. healthy controls, $p < 0.05$; † = FCHL vs. healthy controls, $p < 0.01$;

In the fasting state, FCHL men had a 6-fold higher remnant-like lipoprotein cholesterol (RLP-C) concentration than control men, whereas the RLP-C concentrations was only 2-fold higher in FCHL women compared to control women (Table 7.1). FCHL women had a significantly higher BMI compared to healthy women. Systolic (SBP) and diastolic (DBP) blood pressures were also higher in FCHL women than in control women (140.0 $\pm$ 16.5 vs. 116.5 $\pm$ 11.6 mmHg, $p < 0.01$ and 88.6 $\pm$ 7.6 vs. 76.2 $\pm$ 6.6 mmHg, $p < 0.01$, respectively).

Calculated insulin resistance (HOMA) was higher in FCHL men and FCHL women than in control subjects (men: 2.5 ± 1.7 vs. 1.0 ± 0.6 , $p < 0.01$ and in women: 2.2 ± 1.5 vs. 1.3 ± 0.9 , $p < 0.05$) (Table 7.1).

Capillary density

The results of the microscopic analyses of skin capillary density are depicted in Table 7.2. For technical reasons, no data on capillary density were available in two FCHL men, two control men and one FCHL woman. The basal number of capillaries (BC) was significantly lower in FCHL men compared to healthy men, 113.7 ± 15.1 vs. 132.0 ± 18.0 ($p = 0.02$). Moreover, the post-occlusive number of capillaries (POC) was significantly reduced in FCHL men relative to healthy controls, 123.0 ± 19.1 vs. 142.3 ± 18.3 ($p = 0.03$). In contrast between FCHL women and healthy women no differences in BC or POC were observed ($p > 0.1$ for both).

Table 7.2 Capillary Density (CD) indices per 1.6 mm^2 in FCHL subjects and healthy controls, under baseline and post-occlusive condition.

CD-indices	FCHL men	Control men	FCHL women	Control women
Number	10	12	10	13
BC (n)	$113.7 \pm 15.1^*$	132.0 ± 18.0	142.4 ± 29.9	129.3 ± 18.1
POC (n)	$123.0 \pm 19.1^*$	142.3 ± 18.3	160.3 ± 31.8	150.1 ± 22.0
δC (n)	9.3 ± 9.0	$10.3 \pm 6.9\ddagger$	17.9 ± 8.7	20.8 ± 8.1
CR (%)	8.0 ± 8.0	$8.0 \pm 5.6\ddagger$	13.1 ± 8.1	16.1 ± 6.3

BC = basal number of capillaries, POC = post-occlusive number of capillaries, $\delta C = \text{POC} - \text{BC}$, CR = capillary recruitment;

* = FCHL vs. healthy controls, $p < 0.05$; $\ddagger$ = control men vs. control women, $p < 0.01$

Subsequently, we studied whether FCHL specific factors explained the difference in BC and POC. In men ($n=22$; FCHL plus control men), BC was significant inversely correlated with TC ($r = -0.58$; $p < 0.01$), logTG ($r = -0.47$; $p < 0.05$) and log RLP-C ($r = -0.46$, $p < 0.05$) (Table 7.3). Furthermore, in these 22 men, POC was inversely correlated with TC ($r = -0.61$; $p < 0.005$), logTG ($r = -0.52$; $p < 0.05$), log RLP-C ($r = -0.51$; $p < 0.05$) and log insulin ($r = -0.48$; $p < 0.05$) (Table 7.3). No significant correlations with blood pressure indices or other variables were observed. In women, no significant correlations were observed.

Table 7.3 Pearson correlation coefficients between Capillary Density (CD) indices (BC and POC), and plasma lipids, surrogate markers of insulin resistance and blood pressure variables in men (n=22).

	BC	p-value	POC	p-value
TC	-0.578	<0.01	-0.608	<0.005
Log TG	-0.466	<0.05	-0.517	<0.05
Log RLP	-0.461	<0.05	-0.513	<0.05
Log insulin	-0.374	0.10, NS	-0.480	<0.05
HOMA	--	--	-0.384	0.09, NS
SBP	--	--	--	--
DBP	--	--	--	--

TC = total cholesterol, TG = plasma triglyceride, RLP-C = remnant-like lipoprotein particles cholesterol, HOMA = calculated measure for insulin sensitivity; SBP = systolic blood pressure, DBP = diastolic blood pressure, BC = basal number of capillaries, POC = post-occlusive number of capillaries, -- = NS = not significant.

Subsequently, the data were evaluated to identify the most important single contributing factor(s) to the lower number of basal and post-occlusive capillaries. In men, stepwise backward multivariate regression analyses (Table 7.4) showed that plasma total cholesterol significantly contributed to a lower basal number of capillaries. The variable log RLP-C was excluded at the last step of the regression analysis only. With respect to the lower post-occlusive number of capillaries, the analysis revealed that log RLP-C was the only significant contributing variable.

Table 7.4 Results of backward stepwise multivariate regression analysis in men (n=22) with either basal number of capillaries (BC) or post-occlusive number of capillaries (POC) as dependent variables.

BC

Variable	β	β SE	Stand. β	P	r^2
Intercept	169.4	16.4		< 0.001	0.253 *
TC	-7.347	2.636	-0.539	< 0.05	

POC

Variable	β	β SE	Stand. β	P	r^2
Intercept	123.2	5.5		<0.001	0.277 ‡
Log RLP-C	-26.5	9.0	-0.560	<0.01	

β SE: β standard error; stand β : standardised β ; RLP-C = Remnant-like Lipoprotein Particle cholesterol; * = $p < 0.05$, ‡ = $p < 0.01$.

Laser Doppler fluxmetry

No differences were observed in basal laser Doppler flux (LDBF) between FCHL and healthy subjects: FCHL men vs. healthy men: 10.0 (2 – 27.5) perfusion units (PU, see methods) vs. 8.5 (2.5 – 67.0) PU ($p>0.4$); and FCHL women vs. healthy women: 13.5 (5.0 – 23.0) PU vs. 16.5 (6.0 – 69.0) PU ($p>0.2$). Post-occlusive reactive hyperaemia (LDPRH) was higher in FCHL women compared to the healthy females: 537 (239 – 800) % vs. 327 (39 – 565) % ($p<0.05$). In FCHL men, LDPRH was not different from healthy controls: 577 (138 – 2500) % vs. 632 (61 – 2000) % ($p>0.6$). In men and women, the time to peak and time to 50% recovery were not different between FCHL and healthy subjects.

Discussion

In the present study, FCHL men had a reduced number of basal and post-occlusive capillaries compared to healthy control men. This is in line with a structural or anatomical loss of capillaries described in subjects with hypertension²¹. In FCHL men, total cholesterol and atherogenic remnant-like lipoprotein particles, but not blood pressure values or surrogate markers of insulin resistance, were associated with the observed impaired capillary density. Subjects with FCHL are characterised by an overproduction of apolipoprotein B containing lipoproteins, which results in increased Very-Low-Density Lipoproteins⁵, Low-Density Lipoprotein (LDL)³⁴, and presence of small dense LDL particles, all carriers of cholesterol-esters. Furthermore, in FCHL a relative impairment of lipoprotein catabolism has been documented, that contributes to increased lipoprotein remnant concentrations⁷, as confirmed in this study. In the fasting state, FCHL men had a 6-fold higher remnant-like lipoprotein cholesterol (RLP-C) concentration than control men, whereas RLP-C concentrations were only 2-fold higher in FCHL women compared to control women. This observation is a plausible explanation for the observed reduction in post-occlusive number of capillaries in FCHL men, associated with high RLP-C in univariate and multivariate analyses. Additional explanations for the reduction in POC are that FCHL subjects are insulin resistant^{8,9} and hypertensive¹⁰. Several of these metabolic abnormalities might play an additional role in the observed loss of capillaries in FCHL, by yet unknown mechanisms.

A reduced number of skin capillaries has been observed previously in patients with essential hypertension and normotensive relatives^{19,21,23}. However, in the present study no correlations between blood pressure values and capillary density have been observed. Furthermore, no differences in capillary density were found between FCHL women and control women, despite significantly higher blood pressures in FCHL women than controls.

Impaired capillary recruitment has been proposed as a link between elevated blood pressure and insulin resistance, both in hypertensive²⁷ and normotensive subjects²⁸. However, no relationship between surrogate markers of insulin resistance and capillary density or capillary recruitment were observed in the present study, although elevated blood pressure and insulin resistance are part of

FCHL, and are clearly present in our study population. Moreover, no reductions in capillary density, or a link between capillary density and blood pressure, have been observed in insulin resistant subjects with type 2 diabetes or impaired glucose tolerance²⁹.

In the present study, lipid variables were inversely associated with basal and post-occlusive number of capillaries, and hence explained a significant part - up to 25% - of the capillary loss observed in FCHL men. The observation that the reduction in capillary density is specifically found in FCHL men, characterised by 6-fold higher RLP-C concentrations, but not in FCHL women, suggests a mechanism joining both observations. Atherogenic lipoprotein remnants have also been causally linked to impaired endothelial-derived vasodilatation in FCHL¹¹. To our knowledge, up till now, there is no known mechanism that explains how hyperlipidemia results in a reduced number of capillaries, for instance as a consequence of adaptation. It is conceivable that hyperlipidemia affects the activity of transcription factors or the cell cycle, subsequently affecting development and growth of endothelial cells and hence capillaries in FCHL. Such mechanisms may be similar to the putative, abnormal adipocyte differentiation in type 2 diabetes³⁵. In hypertensive subjects, reduced skin capillary density is associated with a reduction in muscle and adipose tissue capillaries²³. If such an association is also present in FCHL, it may have important metabolic implications and affect risk of cardiovascular events. Loss of diffusion surface can potentially impair clearance of circulating lipoproteins, such as RLP-C. Loss of endothelial surface may result in reduced lipoprotein lipase activity, reported in approximately one-third of FCHL-affected subjects³⁶, further aggravating the metabolic lipid abnormalities in FCHL and the risk of cardiovascular events.

The increase in post-occlusive laser Doppler hyperaemia in FCHL women was an unexpected finding. Earlier studies found impaired hyperaemia in subjects with type 2 diabetes²⁴ and impaired glucose tolerance²⁵, and it was suggested that this reduced maximal vasodilatation could be a hallmark of insulin resistance. The present data are clearly not in line with this hypothesis, despite the observed insulin resistance, calculated as high HOMA values, in FCHL women. The increased hyperaemia in FCHL women is at present difficult to explain. It should be noted however, that the increased hyperaemic response in FCHL women was associated with normal capillary density, but that in FCHL men, the normal hyperaemic response was associated with reduced capillary density. As skin laser Doppler flux is determined by both capillary and shunt blood flow, these data suggest that arteriovenous shunt blood flow is increased in FCHL.

In conclusion, in FCHL men a loss of structural and functional skin capillaries was observed, probably leading to a reduction in capillary endothelial surface, and shown to be associated with increased atherogenic lipoprotein levels. Whether this reduction in surface of structural and functional capillaries can be implicated in the pathophysiology of FCHL and its high risk of cardiovascular events, or is the result of adaptation to hyperlipidemia in FCHL, remains to be elucidated.

References

1. Goldstein JL SH, Hazzard WR, Bierman EL, Motulsky AG. Hyperlipidemia in Coronary Heart Disease. II Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. *Journal of Clinical Investigation* 1973;52:1544-1568.
2. Nikkilä EA, Aro A. Family study of serum lipids and apolipoproteins in coronary artery disease. *Lancet* 1973;78:954-959.
3. Austin MA, McKnight B, Edwards KL, Bradley CM, McNeely MJ, Psaty BM, Brunzell JD, Motulsky AG. Cardiovascular disease mortality in familial forms of hypertriglyceridemia: A 20-year prospective study. *Circulation* 2000;101:2777-2782.
4. Voors-Pette C, de Bruin TWA. Excess coronary heart disease in Familial Combined Hyperlipidemia, in relation to genetic factors and central obesity. *Atherosclerosis* 2001;157:481-489.
5. Venkatesan S, Cullen P, Pacy P, Halliday D, Scott J. Stable isotopes show a direct relation between VLDL apoB overproduction and serum triglyceride levels and indicate a metabolically and biochemically coherent basis for familial combined hyperlipidemia. *Arteriosclerosis Thrombosis and Vascular Biology* 1993;13:1110-1118.
6. Castro Cabezas M, de Bruin TWA, Valk de HW, Shoulders CC, Jansen H, Erkelens DW. Impaired fatty acid metabolism in familial combined hyperlipidemia. A mechanism associating hepatic apolipoprotein B overproduction and insulin resistance. *Journal of Clinical Investigation* 1993;92:160-168.
7. Cabezas MC, de Bruin TWA, Jansen H, Kock LA, Kortlandt W, Erkelens DW. Impaired chylomicron remnant clearance in familial combined hyperlipidemia. *Arteriosclerosis and Thrombosis* 1993;13:804-814.
8. Bredie SJH, Tack CJJ, Smits P, Stalenhoef AFH. Non-obese patients with familial combined hyperlipidemia are insulin resistant as compared with their non-affected relatives. *Arteriosclerosis Thrombosis and Vascular Biology* 1997;17:1465-1471.
9. Hunt SC, Wu LL, Hopkins PN, Stults BM, Kuida H, Ramirex ME, Lalouel JM, Williams RR. Apolipoprotein, low density lipoprotein subfraction, and insulin associations with familial combined hyperlipidemia. Study of Utah patients with familial dyslipidemic hypertension. *Arteriosclerosis* 1989;9:335-344.
10. Keulen ETP, Voors-Pette C, de Bruin TWA. Familial Dyslipidaemic Hypertension Syndrome: further delineation in Familial Combined Hyperlipidaemia, and the role of abdominal fat mass. *American Journal of Hypertension* 2001;14(4): 357-363.
11. Stroes E, de Bruin TWA, de Valk H, Erkelens DW, Banga JD, van Rijn H, Hoomans H, Rabelink T. NO activity in familial combined hyperlipidemia: potential role of cholesterol remnants. *Cardiovascular Research* 1997;36:445-452.
12. Cardillo C, Kilcoyne CM, Quyyumi AA, Cannon RO, Panza JA. Selective defect in nitric oxide synthesis may explain the impaired endothelium-dependent vasodilation in patients with essential hypertension. *Circulation* 1998;97:851-856.

13. Williams SB, Cusco JA, Roddy MA, Johnstone MT, Creager MA. Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. *Journal of the American College of Cardiology* 1996;27:567-574.
14. Timar O, Sestier F, Levy E. Metabolic syndrome X: a review. *Canadian Journal of Cardiology* 2000;16:779-789.
15. Nakajima K, Saito T, Tamura A, Suzuki M, Nakano T, Adachi M, Tanaka A, Tada N, Nakamura H, Campos E. Cholesterol in remnant-like lipoproteins in human serum using monoclonal anti apo B-100 and anti apo A-I immunoaffinity mixed gels. *Clinica Chimica Acta, International Journal of Clinical Chemistry* 1993;223:53-71.
16. Campos E, Nakajima K, Tanaka A, Havel RJ. Properties of an apolipoprotein E-enriched fraction of triglyceride-rich lipoproteins isolated from human blood plasma with a monoclonal antibody to apolipoprotein B-100. *Journal of Lipid Research* 1992;33:369-380.
17. Leary ET, Wang T, Baker DJ, Cilla DD, Zhong J, Wannich GR, Nakajima K, Havel RJ. Evaluation of an immunoseparation method for quantitative measurement of remnant-like particle-cholesterol in serum and plasma. *Clinical Chemistry* 1998;44:2490-2498.
18. Schreuder P, Twickler T, Wang T, Nakajima K, Erkelens DW, Dallinga-Thie G. Isolation of remnant particles by immunoseparation: a new approach for investigation of postprandial lipoprotein metabolism in normolipidemic subjects. *Atherosclerosis* 2001;157:145-151.
19. Prasad A, Dunnill GS, Mortimer PS, MacGregor GA. Capillary rarefaction in the forearm skin in essential hypertension. *Journal of Hypertension* 1995;13:265-268.
20. Houben AJ, Canoy MC, Paling HA, Derhaag PJ, de Leeuw PW. Quantitative analysis of retinal vascular changes in essential and renovascular hypertension. *Journal of Hypertension* 1995;13:1729-1733.
21. Antonios TF, Singer DR, Markandu ND, Mortimer PS, MacGregor GA. Structural skin capillary rarefaction in essential hypertension. *Hypertension* 1999;33:998-1001.
22. Antonios TF, Singer DR, Markandu ND, Mortimer PS, MacGregor GA. Rarefaction of skin capillaries in borderline essential hypertension suggests an early structural abnormality. *Hypertension* 1999;34:655-658.
23. Shore AC, Tooke JE. Microvascular function in human essential hypertension. *Journal of Hypertension* 1994;12:717-728.
24. Tur E, Yosipovitch G, Bar OY. Skin reactive hyperemia in diabetic patients. A study by laser Doppler flowmetry. *Diabetes Care* 1991;14:958-962.
25. Jaap AJ, Hammersley MS, Shore AC, Tooke JE. Reduced microvascular hyperaemia in subjects at risk of developing type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 1994;37:214-216.
26. Noon JP, Walker BR, Webb DJ, Shore AC, Holton DW, Edwards HV, Watt GC. Impaired microvascular dilatation and capillary rarefaction in young adults with a predisposition to high blood pressure. *Journal of Clinical Investigation* 1997;99:1873-1879.
27. Serne EH, Gans ROB, ter Maaten JC, ter Wee PM, Donker AM, Stehouwer CDA. Capillary recruitment is impaired in essential hypertension and relates to insulin's metabolic and vascular actions. *Cardiovascular Research* 2001;49:161-168.

28. Serne EH, Stehouwer CDA, ter Maaten JC, ter Wee PM, Rauwerda JA, Donker AM, Gans ROB. Microvascular function relates to insulin sensitivity and blood pressure in normal subjects. *Circulation* 1999;99:896-902.
29. Jaap AJ, Shore AC, Stockman AJ, Tooke JE. Skin capillary density in subjects with impaired glucose tolerance and patients with type 2 diabetes. *Diabetic Medicine* 1996;13:160-164.
30. Houben AJ, Krekels MM, Schaper NC, Fuss LM, Rodriguez SA, de Leeuw PW. Microvascular effects of atrial natriuretic peptide (ANP) in man: studies during high and low salt diet. *Cardiovascular Research* 1998;39:442-450.
31. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge. *Clinical Chemistry* 1972;18:499-502.
32. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412-419.
33. Ingelfinger JA, Mosteller F, Thibodeau LA, Ware JH. *Biostatistics in clinical medicine*. New York, USA: McGraw-Hill, Inc, 1994.
34. Kissebah AH, Alfarsi S, Evans DJ. Low density lipoprotein metabolism in familial combined hyperlipidemia. Mechanism of the multiple lipoprotein phenotypic expression. *Arteriosclerosis* 1984;4:614-624.
35. Danforth E. Failure of adipocyte differentiation causes type II diabetes mellitus? *Nature Genetics* 2000;26:13.
36. Babirak S, Brown B, Brunzell J. Familial combined hyperlipidemia and abnormal lipoprotein lipase. *Arteriosclerosis and Thrombosis* 1992;12:1176-1183.

Chapter 8

Increased intima media
thickness in Familial
Combined
Hyperlipidemia:
association with
apolipoprotein B

ETP Keulen, M Kruijschoop, NC Schaper,
AGP Hoeks, TWA de Bruin

*Accepted for publication in:
Arteriosclerosis Thrombosis and Vascular
Biology*

Abstract

The aim of the present study was to quantify intima media thickness (IMT) in Familial Combined Hyperlipidemia (FCHL) and to evaluate the relationship of IMT with lipids and apolipoproteins, blood pressure values and surrogate markers of insulin resistance.

IMT was measured by ultrasound at the left and right common carotid artery in 46 FCHL affected subjects, free of clinical manifestations of atherosclerosis, and 55 age- and gender-matched healthy controls. FCHL affected subjects had significantly increased IMT compared to healthy controls, with a difference of $57\mu\text{m}$ (age- and gender-corrected $p<0.01$). Significantly positive, age- and gender-corrected, univariate correlations were observed between IMT and total cholesterol, non-HDL cholesterol, fasting plasma triglyceride, apolipoprotein B, fasting insulin, calculated insulin resistance (HOMA), body mass index (BMI) and waist-circumference. Multivariate regression analyses revealed that age, gender, apolipoprotein B and BMI were significant and independent predictors of IMT, explaining 50% of the observed variation ($p<0.001$).

The increased IMT observed in FCHL corresponds to approximately 7 years of physiological IMT increase in excess of the average IMT in age and gender matched controls. The present findings show the important contribution of lipoprotein particles, marked by increased apolipoprotein B concentrations, to the development of increased IMT in FCHL. The increased IMT in FCHL affected subjects is in agreement with the known high risk of cardiovascular disease in FCHL.

Introduction

Familial Combined Hyperlipidemia (FCHL), the most common genetic dyslipidemia in humans, affects 0.5% - 2% of the population and 10% - 20% of premature myocardial infarction survivors exhibit FCHL¹⁻³. Recently, two reports showed that FCHL relatives also have a markedly increased risk of fatal and non-fatal coronary artery disease (CAD), with odds ratios of 1.7 and 5.1, respectively^{4,5}. However, these clinical sequelae are usually preceded by silent atherosclerotic changes in the vessel wall. Ultrasound measurement of intima media thickness (IMT) in the carotid wall is currently used as marker of silent atherosclerosis. Cross-sectional studies have shown an association between carotid IMT and various cardiovascular risk factors⁶⁻⁸ and the prevalence of CAD^{9,10}. Moreover, carotid IMT has been validated as a predictor of future CAD in prospective studies, independent of other cardiovascular risk factors^{11,12}.

FCHL is a hyperlipidemic, metabolic syndrome associated with apolipoprotein B overproduction¹³, relatively impaired lipoprotein elimination¹⁴, impaired insulin stimulated glucose uptake¹⁵, and central obesity¹⁶. Moreover, the prevalence of dyslipidemic hypertension in FCHL families is increased two-fold¹⁶. Clearly, the aggregate of these abnormalities results in an unfavourable, atherogenic risk profile in FCHL and increases the risk of enhanced atherosclerosis and subsequent cardiovascular events.

It is the objective of the present study to assess the amount of silent atherosclerosis, by ultrasound carotid IMT measurement, in FCHL affected subjects in comparison to healthy controls and to investigate the potential relationship of IMT with lipids and lipoproteins, blood pressure values, and surrogate markers of insulin resistance.

Subjects and methods

One hundred and thirteen subjects participated in the present study: 58 (31 men) hyperlipidemic FCHL subjects and 55 (29 men) age- en gender- matched healthy controls. FCHL was diagnosed in a family according to the following criteria: first, a primary hyperlipidemia including fasting total plasma cholesterol (TC) >6.5 mmol/L (250 mg/dL) and/or fasting plasma triglyceride (TG) concentration >2.0 mmol/L (180 mg/dL); second, multiple lipoprotein phenotypes within a family (Fredrickson Classification IIa, IIb or IV); third, a positive family history of premature cardiovascular disease (CVD)^{5,14,16}. Premature CVD was defined as the occurrence of a myocardial infarction (MI) or cerebrovascular accident (CVA) before the age of 60 y. Secondary causes of hyperlipidemia (renal or hepatic insufficiency, hypothyroidism and medication), presence of the apo E2/E2 genotype, and subjects with tendon xanthomas or a diagnosis matching familial hypercholesterolemia were excluded.

Hyperlipidemic FCHL subjects (n=58) included in the present study were characterised by a primary hyperlipidemia, defined as fasting total plasma cholesterol (TC) >6.5 mmol/L (250 mg/dL) and/or fasting plasma triglyceride (TG) concentration >2.0 mmol/L (180 mg/dL)¹⁶. Eleven FCHL subjects had a history of previous cardiovascular disease (CVD): 2 had a MI, 3 had angina pectoris, 1 underwent a coronary artery bypass graft, 3 had a CVA and 2 showed peripheral atherosclerotic disease. All healthy control subjects (n=55) had no clinical signs of CVD. The study protocol was approved by the Human Investigations Review Committee and performed according to the Helsinki Declaration. All subjects gave written informed consent.

Measurements

The measurements were performed in the morning (8.00 – 11.00) after an overnight fast (12-14 h). Subjects had refrained from smoking and did not drink coffee or tea in the morning. Subjects had also abstained from alcohol for at least 72 hours. A medical history was obtained from all participating subjects and a standardised questionnaire was filled out. All patients were weighed in underwear, the height was determined by stadiometer and the body mass index (BMI) was subsequently calculated as weight in kilograms / (height in meters)². The waist circumference was measured at the level of the umbilicus, the hip circumference was measured at the level of the trochanter major and the waist to hip ratio (WHR) was calculated. Both waist-circumference and hip-circumference were measured with the subject in standing position.

Systolic (SBP) and diastolic (DBP) blood pressures were measured twice, with the Omron 705CP (OMRON Health Care GmbH, Hamburg, Germany) in a standardised fashion, with the subject in sitting position after 10 minutes of rest. Cuff size was adjusted to the circumference of the arm, and the arm was placed with the cuff at heart-level. The mean of two measurements was used in the statistical analyses.

IMT-measurement

The IMT measurements were done with an Ultramark 4+ (Ultramark 4+, ATL, Bothel, Washington, USA) with a linear array transducer of 7.5 MHz. All subjects were measured in a supine position in a quiet room. During the measurements the head was tilted to the contra-lateral side at an angle of 45°. The automated radiofrequency (RF) method used to assess IMT has been described in detail before¹⁷. Briefly, a B-mode image was obtained from the common carotid artery (CCA) in a longitudinal section just before the widening of the bulb in the bifurcation. An M-line was positioned perpendicular to the posterior wall approximately 10-20 mm proximal to the carotid bulb, showing a clear intima-media complex during the whole measurement. Subsequently, the ultrasound system was switched to M-mode and ECG-triggered RF data collection was started at a sample frequency of 18.4 MHz, an M-line update frequency of 800 Hz, for a time period of 5 seconds corresponding to 4-6 heartbeats. For each heartbeat, the amplitude distribution was evaluated to automatically detect the positions of the walls. Subsequently, the locations of the intima and the media-

adventitia edge of the posterior wall were determined and IMT was defined as the distance between both edges. The maximum ratio of intima to adventitia amplitude was set at 1.0 to suppress detection of false edges. The resolution for wall thickness detection was approximately $40\ \mu\text{m}$ ¹⁷. Only the posterior wall was investigated, because here the reflections from the blood-intima and media-adventitia transition are distinctly visible, whereas at the anterior wall the trailing edge of the adventitia may obscure the media and intima signals. Each heartbeat within a recording resulted in an estimate of IMT. Measurements were repeated seven times at both left and right CCA. The average of the median values for each measurement obtained on both sides was taken as a representative IMT value. Ultrasound M-mode and RF signals method to measure IMT instead of off-line B-mode measurement has been validated previously¹⁸. The intra-observer variabilities were 5.9% (EK) and 6.3% (MK) as determined in 10 subjects on two occasions. The inter-observer variability was 4.6 % as determined in 10 subjects.

Laboratory methods

Any lipid lowering medication had been withdrawn for two weeks before blood samples were collected. After the completion of the IMT-measurement fasting venous blood was collected in pre-cooled EDTA (1-mg/ml) Vacutainer tubes for measurements of lipids, lipoproteins, glucose and insulin. Total cholesterol (TC) and fasting triglyceride (TG) concentrations were measured in duplicate by a commercially available colorimetric assay (Monotest Cholesterol kit, Boehringer Mannheim #1442350 and GPO-PAP, Boehringer Mannheim, #701912, respectively). HDL-cholesterol (HDL-C) was determined after phosphotungstate-MgCl₂ precipitation of whole plasma. Fasting plasma LDL-cholesterol (LDL-C) was calculated according to the Friedewald formula: $\text{LDL-C} = \text{TC} - \text{HDL-C} - (\text{TG} \times 0.45)$, if plasma TG concentration was below 4.5 mmol/L (390 mg/dL)¹⁹. If plasma TG was above 4.5 mmol/L, LDL-C was measured in the density 1.019-1.063 fractions, prepared after equilibrium ultracentrifugation. Nonesterified fatty acids (NEFA) were measured in plasma samples by an enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, Germany).

Glucose (FBG) was measured immediately on a YSI 2300 monitor (Yellow Spring Instruments, USA). Fasting insulin concentration (FI) was determined using an Elisa (Mercodia AB, Uppsala, Sweden), with a cross-reactivity with pro-insulin <0.01%. Insulin resistance was calculated with the HOMA formula: $\text{Resistance} = \text{FI} / (7 \times 22.5 \times \varepsilon^{-\ln \text{FBG}})$, which can be simplified as $(\text{FI} \times \text{FBG}) / (7 \times 22.5)$ ²⁰. Plasma apolipoprotein B (apo B) and apolipoprotein A-1 (apo A-1) were determined by commercially available immunonephelometric assays, using calibrated standards according to the International Federation for Clinical Chemistry (Behringwerke, Marburg, Germany)²¹.

Statistical analyses

All values are expressed as mean $\pm$ standard deviation. To obtain normal distribution, log transformed values for fasting insulin and triglyceride concentrations were used in statistical analyses; values are non-logarithmically listed in Table 8.1 for practical reasons. Differences between FCHL affected

subjects and healthy controls were calculated using linear regression analyses with age and gender as co-variables²².

Age- and gender-corrected Pearson correlation coefficients were determined for the relationship between continuous IMT variable, plasma lipids and lipoproteins, blood pressure values, and surrogate markers of insulin resistance. To test whether variables had an independent effect on the IMT, the effects of such a variable, adjusted for other independent variables, was analysed by backward stepwise multiple linear regression analyses with IMT as dependent variable²². FCHL affected status was entered as an independent variable. In all statistical analyses, the statistical package SPSS 8.0 (SPSS Inc. Chicago, Ill., USA) was used.

Table 8.1 Characteristics of 58 hyperlipidemic FCHL relatives and 55 healthy control subjects.

	FCHL (n=58)	Controls (n=55)
M / F	31 / 27	29 / 26
Age (y.)	48.5 ± 12.9	47.4 ± 12.9
BMI (kg/m ²)	26.5 ± 3.3	25.8 ± 3.7
Waist (cm)	92.9 ± 10.0	90.4 ± 11.3
SBP (mmHg)	141.2 ± 21.1†	132.0 ± 15.7
DBP (mmHg)	84.3 ± 11.9	80.4 ± 9.9
TC (mmol/L)	6.2 ± 1.4*	4.9 ± 0.8
TG (mmol/L)	2.6 ± 2.1*	1.1 ± 0.4
HDL-C (mmol/L)	0.85 ± 0.24*	0.98 ± 0.25
LDL-C (mmol/L)	4.2 ± 1.4*	3.4 ± 0.8
NonHDL-C (mmol/L)	5.4 ± 1.3*	3.9 ± 0.8
Apo B (g/L)	1.35 ± 0.29*	0.99 ± 0.22
Glucose (mmol/L)	4.9 ± 0.7	4.9 ± 0.5
Insulin (pmol/L)	67.8 ± 34.5*	50.6 ± 21.4
HOMA	2.1 ± 1.2*	1.6 ± 0.7

BMI = body mass index, WHR = waist to hip ratio, SBP = systolic blood pressure, DBP = diastolic blood pressure, TC = total cholesterol (for mg/dL divide by 0.0259), TG = plasma triglyceride (for mg/dL divide by 0.0113), HDL-C = high-density lipoprotein-cholesterol, LDL-C = low-density lipoprotein cholesterol, NonHDL-C = TC minus HDL-C, glucose = for mg/dL divide by 0.0555, insulin = for μ U/L divide by 6.945, HOMA: calculated measure for insulin resistance.

* = FCHL vs. controls $p < 0.01$, † = FCHL vs. controls $p < 0.05$

Results

The characteristics of the subjects studied are presented in Table 8.1. FCHL subjects had significantly higher SBP, fasting total cholesterol concentration (TC), fasting triglyceride levels (TG), low-density lipoprotein cholesterol (LDL-C) and apo B concentrations and lower high-density lipoprotein cholesterol (HDL-C) concentrations compared to healthy subjects. Furthermore, fasting insulin levels

and calculated insulin resistance (HOMA) were higher in FCHL subjects than in control subjects, comparable with the known state of insulin resistance in FCHL.

The results of the Intima Media Thickness (IMT) measurements are depicted in Table 8.2. Previous studies have shown that IMT increases with age and that IMT is increased in the male population compared to the female population^{6,7}. Therefore, IMT was corrected for age and gender using linear regression analysis. For technical reasons, no data on IMT were available in one FCHL subject and two control subjects. Linear regression analyses showed that IMT was significantly increased in FCHL subjects compared to healthy controls, with a difference of 63 μm (age- and gender-corrected $p < 0.01$). As stated above, 11 FCHL affected subjects were diagnosed with previous CVD. All these different cardiovascular manifestations have been associated with a greater IMT in several studies^{9,10,23}. Indeed, in our study population, FCHL subjects with CVD tended to have an increased IMT compared to FCHL subjects without clinical signs of CVD, or healthy controls ($p = 0.06$), respectively (Table 8.2). It is possible that because of the small number of FCHL subjects with CVD and the variation of IMT in these CVD-positive FCHL subjects, the observed differences did not reach the significance level. When FCHL subjects with a history of CVD were excluded from the analyses, a significantly increased IMT in CVD-free FCHL subjects compared to healthy controls was still observed, with a difference of 57 μm (age- and gender-corrected $p < 0.01$).

Table 8.2 Intima-Media Thickness (IMT) in 57 FCHL affected subjects (46 CVD negative subjects and 11 CVD positive subjects) and 53 healthy controls (p-values shown after adjustment for age and gender).

	FCHL total	FCHL / CVD -	FCHL / CVD +	Controls
Number	57	46	11	53
IMT (mm)	$0.72 \pm 0.15^*$	$0.70 \pm 0.13^\dagger$	$0.80 \pm 0.21^\ddagger$	0.65 ± 0.14

CVD = cardiovascular disease, IMT = Intima Media Thickness, mean $\pm$ SD;

* = FCHL total vs. controls; age- and gender adjusted $p < 0.01$,

† = FCHL / CVD - vs. controls; age- and gender adjusted $p < 0.01$,

‡ = FCHL / CVD + vs. controls; age- and gender adjusted $p = 0.06$

The eleven FCHL subjects with previously diagnosed CVD were now excluded from further analyses. Subsequently, we studied whether FCHL specific factors explained the difference in IMT. Age- and gender-adjusted univariate Pearson correlations between IMT, lipid and apolipoproteins, blood pressure values and surrogate markers of insulin resistance, all treated as continuous variables, are depicted in Table 8.3 and partly in Figure 8.1. Significantly positive correlations between IMT and BMI, waist-circumference, TC, logTG, LDL-C, nonHDL-chol, apo B, log insulin and HOMA were observed. However, no significant correlation existed between IMT and blood pressure values, glucose concentrations or family number.

Figure 8.1 Intima media thickness in FCHL affected subjects, free of previous cardiovascular history, and healthy controls versus: A, apolipoprotein B; B, non-HDL-cholesterol; C, low-density lipoprotein (LDL) - cholesterol; and D, body mass index (BMI). Age- and gender-corrected Pearson correlation coefficient: A, $r=0.35$ ($p<0.001$); B, $r=0.31$ ($p<0.01$); C, $r=0.24$ ($p<0.05$); D, $r=0.25$ ($p<0.05$).

Table 8.3 Age- and gender- adjusted Pearson correlation coefficients between intima-media thickness (IMT), plasma lipids and surrogate markers of insulin resistance variables in 46 FCHL-affected subjects without known CVD and 53 healthy controls.

Variable	IMT	p-value
TC	0.35	<0.01
LDL-C	0.24	<0.05
HDL-C	0.02	0.87, NS
Non-HDL-C	0.31	<0.01
Log TG	0.27	0.01
Apo B	0.35	<0.001
Glucose	-0.04	0.74, NS
Log insulin	0.26	<0.05
HOMA	0.29	<0.01
BMI	0.25	<0.05
Waist	0.21	<0.05
SBP	-0.03	0.76, NS
DBP	-0.16	0.14, NS

TC = total cholesterol, LDL-C = low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein-cholesterol, Non-HDL-C = TC minus HDL-C, TG = fasting plasma triglyceride, Apo B = apolipoprotein B, HOMA = calculated measure for insulin resistance BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, NS = non-significant.

Next, multiple backward linear regression analysis was used to evaluate whether one of these significantly correlated metabolic factors was the most dominant in predicting an increased IMT. Age and gender were also included as potentially contributing variables to an increased IMT. TC or nonHDL-C, respectively insulin or HOMA were used in the regression analyses because of close interaction between both of these variables. However, this did not alter the final results. These analyses revealed that apolipoprotein B and BMI, besides age and gender, contributed to an increased IMT and explained more than 50 % of the variance in IMT in our study population (Table 8.4).

Table 8.4 Results of backward stepwise regression analysis in 46 FCHL-affected subjects without known CVD and 53 healthy controls with Intima-Media Thickness (IMT) as dependent variable.

Variable	β	β SE	Stand. β	p	Model r^2
					0.502 *
Intercept	0.168	0.079			
Age	4.860 E-03	0.001	0.479	<0.001	
Gender	-4.811 E-02	0.020	-0.179	<0.05	
Apo B	0.109	0.033	0.255	<0.001	
BMI	6.852 E-03	0.003	0.176	<0.05	

Apo B = apolipoprotein B, BMI = body mass index, β SE = β standard error, stand β = standardised β . * = $p < 0.001$

Discussion

In the present study, FCHL affected subjects had an increased IMT compared to healthy controls. FCHL subjects with a known history of CVD tended to have an even more increased IMT as expected from reported associations between higher IMT with higher risk of clinical manifestations of cardiovascular disease^{9,10,23}. The increased IMT observed in FCHL is in line with their known high risk of fatal and non-fatal cardiovascular disease^{4,5}. The annual progression rate of IMT has been estimated to be around 6-10 μm ²⁴⁻²⁶. Therefore, the observed increase of 57 μm in IMT in FCHL affected subjects without previous CVD (mean age 48.5 years) corresponds to approximately 5-9 years of IMT increase, in excess of the average IMT found in age- and gender-matched controls.

In the FCHL study group, 24 out of 58 (41.3 %) subjects were currently treated with a statin, an HGM-CoA-reductase inhibitor. Thus, the observed IMT in FCHL is probably even underestimated, because antihyperlipidemic treatment with statins has been shown to slow progression of IMT or even reverse the intima-media thickening²⁴⁻²⁷. The presently observed higher IMT is also in line with the occurrence of dyslipidemia approximately 15-20 years earlier in FCHL subjects compared to the general population²⁸.

In the present study, well-known metabolic risk factors of atherosclerosis development, including lipids and apolipoproteins, surrogate markers of obesity and surrogate insulin resistance markers, were univariately associated with an increased IMT. Multivariate regression analyses revealed that age, gender, apolipoprotein B and BMI contributed independently to increased IMT. The effects of age and gender on IMT have been described extensively before⁶, and are expected to be operational in all Western populations. The relationship between IMT and increased apo B concentrations is clinically more important, as all apo B containing lipoproteins are potentially atherogenic. Moreover, in cross-sectional^{29,30} and prospective studies^{31,32}, apo B has been shown to be a good risk indicator of CVD compared to other lipid levels or smoking, diabetes mellitus and hypertension. In addition, apo B lowering has been shown to result in regression of coronary artery disease³³.

Because each lipoprotein particle, i.e. very low-density lipoprotein (VLDL), intermediate density lipoprotein, LDL, chylomicrons and chylomicron remnants, contains one apo B molecule, an increased apo B concentration is an indicator of increased numbers of circulating lipoproteins. FCHL is characterised by an overproduction of apo B containing lipoproteins, which results in increased VLDL¹³ and LDL concentrations³⁴, and the presence of small dense LDL particles^{35,36}. In addition, in FCHL, a relative impairment in lipoprotein catabolism¹⁴ and an increased residence time of VLDL particles, both contributing to higher lipoprotein remnant concentrations, have been documented. Increased flux of atherogenic lipoproteins through the arteries, with in addition increased residence time, is a plausible mechanism for increased exposure of the endothelium to atherogenic lipoproteins in FCHL affected subjects. The present data indicate that increased apo B concentrations, reflecting increased delivery of

atherogenic lipoproteins to the endothelium and the vessel wall, result in increased IMT and, with time, may be associated with clinical cardiovascular sequelae.

In the present study, increased BMI has been associated with higher IMT. Increased BMI is potentially pro-atherogenic in humans through several pathophysiological mechanisms, including associated hyper-lipidemia, hypertension and insulin resistance. Increased BMI has been associated with increased risk of developing CVD, where co-segregation with associated metabolic disorders further enhances cardiovascular risk³⁷. In FCHL, abdominal obesity has been associated with increased risk of non-fatal CAD⁵ and hypertension¹⁶. Furthermore, increased free fatty acids flux from visceral fat directly to the liver can contribute to a hypersecretion of apo B containing triglyceride-rich VLDL from the liver³⁸. Moreover, BMI reduction has been associated with reduced secretion of apo B containing particles from the liver^{38,39}. Therefore, BMI reduction is a potentially important therapeutic goal in FCHL to slow IMT progression or even promote IMT regression, reduce apo B secretion, and lower blood pressure¹⁶.

In conclusion, common carotid artery IMT is increased in FCHL affected subjects with a mean age of 48.5 years. In the present study this increase in IMT corresponded to an acceleration of silent atherosclerosis by approximately 5-9 years. The present findings show the important contribution of an increased number of lipoprotein particles to the development of increased IMT in FCHL. Reduction of abdominal obesity should be tested as a potential therapeutic intervention in FCHL to reduce the secretion of apo B containing lipoproteins and potentially counteract IMT progression. Moreover, early detection of FCHL affected subjects, with subsequent dietary and/or drug treatment, is warranted as the development of subclinical atherosclerosis, associated with future cardiovascular events, is seriously enhanced.

References

1. Goldstein JL, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG. Hyperlipidaemia in Coronary Heart Disease. II Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidaemia. *Journal of Clinical Investigation* 1973;52:1544-1568.
2. Nikkilä EA, Aro A. Family study of serum lipids and apolipoproteins in coronary artery disease. *Lancet* 1973;78:954-959.
3. Genest-Jr JJ, Martin MSS, McNamara JR, Ordovas JM, Jenner J, Myers RH, Silberman SR, Wilson PW, Salem DN, Schaefer EJ. Familial lipoprotein disorders in patients with premature coronary artery disease. *Circulation* 1992;85:2025-2033.
4. Austin MA, McKnight B, Edwards KL, Bradley CM, McNeely MJ, Psaty BM, Brunzell JD, Motulsky AG. Cardiovascular disease mortality in familial forms of hypertriglyceridemia: A 20-year prospective study. *Circulation* 2000;101:2777-2782.
5. Voors-Pette C, de Bruin TWA. Excess coronary heart disease in Familial Combined Hyperlipidemia, in relation to genetic factors and central obesity. *Atherosclerosis* 2001;157:481-489.
6. Salonen R, Salonen JT. Determinants of carotid intima-media thickness: a population-based ultrasonography study in Eastern Finnish men. *Journal of Internal Medicine* 1991;229:225-231.
7. Bonithon-Kopp C, Scarabin PY, Taquet A, Touboul PJ, Malmejac A, Guize L. Risk factors for early carotid atherosclerosis in middle-aged French women. *Arteriosclerosis Thrombosis and Vascular Biology* 1991;11:966-972.
8. Skoglund-Andersson C, Tang R, Bond MG, de-Faire U, Hamsten A, Karpe F. LDL particle size distribution is associated with carotid intima-media thickness in healthy 50-year-old men. *Arteriosclerosis Thrombosis and Vascular Biology* 1999;19:2422-2430.
9. Burke GL, Evans GW, Riley WA, Sharrett AR, Howard J, Barnes RW, Rosamond W, Crow RS, Rautaharju PM, Heiss G, for the ARIC study group. Arterial wall thickness is associated with prevalent cardiovascular disease in middle-aged adults. The Atherosclerosis Risk in Communities (ARIC) Study. *Stroke* 1995;26:3862-391.
10. Hulthe J, Wikstrand J, Emanuelsson H, Wiklund O, deFeyter PJ, Wendelhag I. Atherosclerotic changes in the carotid artery bulb as measured by B-mode ultrasound are associated with the extent of coronary atherosclerosis. *Stroke* 1997;28:1189-1194.
11. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction. The Rotterdam Study. *Circulation* 1997;96:1432-1437.
12. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. *New England Journal of Medicine* 1999;340:14-22.

13. Venkatesan S, Cullen P, Pacy P, Halliday D, Scott J. Stable isotopes show a direct relation between VLDL apoB overproduction and serum triglyceride levels and indicate a metabolically and biochemically coherent basis for familial combined hyperlipidemia. *Arteriosclerosis Thrombosis and Vascular Biology* 1993;13:1110-1118.
14. Cabezas MC, de Bruin TWA, Jansen H, Kock LA, Kortlandt W, Erkelens DW. Impaired chylomicron remnant clearance in familial combined hyperlipidemia. *Arteriosclerosis and Thrombosis* 1993;13:804-814.
15. Bredie SJH, Tack CJJ, Smits P, Stalenhoef AFH. Non-obese patients with familial combined hyperlipidemia are insulin resistant as compared with their non-affected relatives. *Arteriosclerosis Thrombosis and Vascular Biology* 1997;17:1465-1471.
16. Keulen ETP, Voors-Pette C, de Bruin TWA. Familial dyslipidemic hypertension syndrome: Familial combined hyperlipidemia, and the role of abdominal fat mass. *American Journal of Hypertension* 2001;14:357-363.
17. Hoeks AG, Willekes C, Boutouyrie P, Brands PJ, Willigers JM, Reneman RS. Automated detection of local artery wall thickness based on M-line signal processing. *Ultrasound in Medicine and Biology* 1997;23:1017-1023.
18. Willekes C, Hoeks A, Bots M, Brands P, Willigers J, Reneman R. Evaluation of off-line automated intima-media thickness detection of the common carotid artery based on M-line signal processing. *Ultrasound in Medicine and Biology* 1999;25:57-64.
19. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge. *Clinical Chemistry* 1972;18:499-502.
20. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412-419.
21. Albers JJ, Marcovina SM, Kennedy H. International Federation of Clinical Chemistry standardization project for measurements of apolipoproteins A-I and B. II. Evaluation and selection of candidate reference materials. *Clinical Chemistry* 1992;38:658-662.
22. Ingelfinger JA, Mosteller F, Thibodeau LA, Ware JH. *Biostatistics in clinical medicine*. New York, USA: McGraw-Hill, Inc, 1994.
23. Bots ML, Hofman A, Grobbee DE. Common carotid intima-media thickness and lower extremity arterial atherosclerosis. The Rotterdam Study. *Arteriosclerosis Thrombosis and Vascular Biology* 1994;14:1885-1891.
24. Furberg CD, Adams HP, Applegate WB, Byington RP, Espeland MA, Hartwell T, Hunninghake DB, Lefkowitz DS, Probstfield J, Riley WA, Young B, for the Asymptomatic Carotid Artery Progression Study (ACAPS) research group. Effect of lovastatin on early carotid atherosclerosis and cardiovascular events. *Circulation* 1994;1994:1679-1687.
25. Wendelhag I, Wiklund O, Wikstrand J. Intima-media thickness after cholesterol lowering in familial hypercholesterolemia. A three-year ultrasound study of common carotid and femoral arteries. *Atherosclerosis* 1995;117:225-236.
26. Hodis HN, Mack WJ, LaBree L, Selzer RH, Liu C, Alaupovic P, Kwong-Fu H, Azen SP. Reduction in carotid arterial wall thickness using lovastatin and dietary therapy: a randomized controlled clinical trial. *Annals of Internal Medicine* 1996;124:548-556.
27. Smilde TJ, van Wissen S, Wollersheim H, Trip MD, Kastelein JP, Stalenhoef AFH. Effect of aggressive versus conventional lipid lowering on atherosclerosis progression

- in familial hypercholesterolaemia (ASAP): a prospective, randomised, double-blind trial. *Lancet* 2001;357:577-581.
28. Keulen ETP, Lutgens I, Rijkers K, van Lin JMJP, de Bruin TWA. The epidemiology of cardiovascular risk factors in familial combined hyperlipidemia. *Circulation* 2001;2/2:61 (Abstract).
 29. Kwiterovich-Jr PO, Coresh J, Smith HH, Bachorik PS, Derby CA, Pearson TA. Comparison of the plasma levels of apolipoproteins B and A-1, and other risk factors in men and women with premature coronary artery disease. *American Journal of Cardiology* 1992;69:1015-1021.
 30. Westerveld HT, van-Lennep JE, van-Lennep HW, Liem AH, de Boo JA, van der Schouw YT, Erkelens DW. Apolipoprotein B and coronary artery disease in women: a cross-sectional study in women undergoing their first coronary angiography. *Arteriosclerosis Thrombosis and Vascular Biology* 1998;18:1101-1107.
 31. Wald NJ, Law M, Watt HC, Wu T, Bailey A, Johnson AM, Craig WY, Ledue TB, Hadlow JE. Apolipoproteins and ischaemic heart disease: implications for screening. *Lancet* 1994;343:75-79.
 32. Lamarche B, Tchernof A, Mauriege P, Cantin B, Dagenais GR, Lupien DJ, Depres JP. Fasting insulin and apolipoprotein B levels and low-density lipoprotein particle size as risk factors for ischemic heart disease. *Journal of the American Medical Association* 1998;279:1955-1961.
 33. Brown G, Albers JJ, Fisher LD, Schaefer SM, Lin JT, Kaplan C, Zhao XQ, Bisson BD, Fitzpatrick VF, Dodge HT. Regression of coronary artery disease as a result of intensive lipid-lowering therapy in men with high levels of apolipoprotein B. *New England Journal of Medicine* 1990;323:1289-1298.
 34. Kissebah AH, Alfarsi S, Evans DJ. Low density lipoprotein metabolism in familial combined hyperlipidemia. Mechanism of the multiple lipoprotein phenotypic expression. *Arteriosclerosis* 1984;4:614-624.
 35. Hokanson JE, Krauss RM, Albers JJ, Austin MA, Brunzell JD. LDL physical and chemical properties in familial combined hyperlipidemia. *Arteriosclerosis Thrombosis and Vascular Biology* 1995;15:452-459.
 36. Bredie SJ, Kiemeny LA, de Haan AF, Demacker PN, Stalenhoef AF. Inherited susceptibility determines the distribution of dense low-density lipoprotein subfraction profiles in familial combined hyperlipidemia. *American Journal of Human Genetics* 1996;58:812-822.
 37. Schulte H, Cullen P, Assmann G. Obesity, mortality and cardiovascular disease in the Munster Heart Study (PROCAM). *Atherosclerosis* 1999;144:199-209.
 38. Riches FM, Watts GF, Hua J, Stewart GR, Naoumova RP, Barrett PHR. Reduction in visceral adipose tissue is associated with improvement in apolipoprotein B-100 metabolism in obese men. *Journal of Clinical Endocrinology and Metabolism* 1999;84:2854-2861.
 39. Purnell JQ, Kahn SE, Albers JJ, Nevin DN, Brunzell JD, Schwartz RS. Effect of weight loss with reduction of intra-abdominal fat on lipid metabolism in older men. *Journal of Clinical Endocrinology and Metabolism* 2000;85:977-982.

Chapter 9

General discussion

- Results of this thesis in perspective
- FCHL: diagnostic problems

9.1 Results of this thesis in perspective

FCHL, first delineated in 1973, is nowadays recognised as the most common genetic dyslipidemia in humans with a substantially increased risk of premature cardiovascular disease (CVD)¹⁻³. Most of the research in FCHL has focussed upon the pathophysiology of the observed lipid abnormalities and insulin resistance. In addition, the role of (abdominal) obesity in the pathophysiology of FCHL has emerged over the past few years. Recently, androgenic obesity has been linked to increased risk of non-fatal CVD in FCHL³. The relationship between the observed cardiovascular abnormalities described in this thesis and lipid- and apolipoprotein concentrations, insulin resistance and obesity will be discussed below.

In **chapter 4**, the co-segregation of hypertension with dyslipidemia in FCHL families was described. One-third of the FCHL families studied also met the diagnostic criteria matching Familial Dyslipidemic Hypertension (FDH). This percentage may be even underestimated as some FCHL families had too few relatives in the requested age-range (30-59 years) to match the diagnosis of FDH. Increased waist-circumference, and hence abdominal fat mass, was associated with higher blood pressure values observed in FCHL-affected and non-affected female relatives. As blood pressure values were already elevated in non-affected female relatives, it is plausible that a common denominator influences the occurrence of dyslipidemia as well as hypertension in FCHL. Increased free fatty acids flux from visceral adipose tissue may result in overproduction of apo B containing lipoproteins in the liver⁴ as well as hyperactivity of the sympathetic nervous system^{5,6}, thus providing a common link between dyslipidemia and hypertension in FCHL. Obesity has been associated with increased risk of cardiovascular disease in the general population^{7,8}, as well as in FCHL³. In FCHL, obesity was linked to subclinical atherosclerosis, as measured by intima media thickness (IMT) (**chapter 8**).

In conclusion, in FCHL, (abdominal) obesity plays an important role in lipoprotein overproduction, development of insulin resistance, hypertension and increase in IMT. Therefore, reduction of body weight, especially visceral and subcutaneous abdominal fat mass, is a potentially important therapeutic goal in FCHL to reduce apo B containing lipoprotein secretion by the liver, to improve insulin resistance, to lower blood pressure values, and to slow IMT progression, and perhaps even promote IMT regression.

Insulin resistance is often observed in FCHL-affected as well as non-affected relatives^{9,10}. As insulin plays a pivotal role in lipid metabolism and insulin resistance has already been observed in non-affected relatives, insulin resistance may play a role in the development of some features of FCHL. Insulin resistance has been associated with several clinical and metabolic abnormalities including diabetes mellitus, obesity, hypertension, dyslipidemia, impaired fibrinolytic

capacity and a pro-coagulant state¹¹⁻¹³. Increased heart rate corrected QT-interval (QTc) was suggested as an additional feature of the insulin resistance syndrome¹⁴. A prolonged QTc, observed in FCHL affected women, has been associated with some components of the insulin resistance syndrome (**chapter 5**). Whether this QTc prolongation in FCHL is an adaptation mechanism to insulin resistance remains to be elucidated. However, as an increased QTc has been associated with increased risk of cardiac disease related death¹⁵⁻¹⁷, QTc prolongation in FCHL may add to the high risk of CVD. **Chapter 6** describes the increase of endothelium derived pro-coagulant (vWf) and anti-fibrinolytic proteins in FCHL affected subjects, which potentially contributes to the risk of premature CVD found in FCHL. Insulin resistance partly explained the observed increase of plasminogen activator inhibitor 1 (PAI-1) in FCHL affected subjects (**chapter 6**). Improvement of insulin resistance, by weight reduction or perhaps thiazolidinediones, might have beneficial effects on QTc as well as PAI-1, and may potentially lower the cardiovascular risk burden in FCHL.

As mentioned before, the most important metabolic abnormalities found in FCHL concern the overproduction of apo B containing lipoprotein particles¹⁸ and impaired lipoprotein clearance with increased residence time. In the fasting state, FCHL men had a 6-fold higher remnant-like lipoprotein cholesterol (RLP-C) concentration than control men, whereas RLP-C concentrations were 2-fold higher in FCHL women compared to control women (**chapter 7**). The observed reduction in post-occlusive number of capillaries in FCHL men has been associated with higher RLP-C in univariate and multivariate analyses (**chapter 7**). Loss of capillary density and thus diffusion surface can potentially impair clearance of circulating lipoproteins, such as RLP-C. Furthermore, loss of endothelial surface can result in reduced lipoprotein lipase activity, reported in approximately one-third of FCHL-affected subjects¹⁹, further aggravating the metabolic lipid abnormalities in FCHL and the risk of cardiovascular events.

The contribution of lipoprotein particles to vascular abnormalities in FCHL was further underlined by the association between increased intima media thickness (IMT) and higher lipid- and apolipoprotein concentrations (**chapter 8**). Multivariate regression analyses revealed that age, gender, apo B and BMI contributed independently to increased IMT (**chapter 8**). The relationship between IMT and BMI has already been discussed above. The relationship between IMT and increased apo B concentrations is clinically more important, as all apo B containing lipoprotein particles are potentially atherogenic. Furthermore, apo B concentrations reflect total number of lipoprotein particles in the circulation. Increased flux of atherogenic lipoprotein particles through the arteries, with in addition increased residence time, is a plausible mechanism for the increased exposure of endothelium to atherogenic lipoprotein particles and hence increased risk of developing atherosclerosis.

In conclusion, hypertension, QTc prolongation and elevated concentrations of vWf and PAI-1 have been identified as cardiovascular risk factors that potentially contribute to the high risk of cardiovascular disease observed in FCHL. Furthermore, endothelial dysfunction, loss of capillary density and enhanced

subclinical atherosclerosis have been observed in FCHL-affected subjects (mean age 47 years). Early detection of FCHL-affected subjects, with subsequent dietary and/or drug treatment, is needed as the development of endothelial dysfunction and subclinical atherosclerosis, both associated with future cardiovascular events, is seriously enhanced and present at a relatively young age. Antihyperlipidemic therapy can potentially restore endothelial dysfunction in FCHL²⁰. In addition, antihyperlipidemic therapy with HMG-CoA-reductase inhibitors (e.g. statins) has been shown to slow IMT progression and even promote IMT regression^{21,22}. The same results may apply to FCHL as well, although further research is needed. The role of antihyperlipidemic therapy in blood pressure lowering and reversal of QTc prolongation has to be established in future studies as well. Reduction of abdominal obesity, via dietary intervention and promotion of exercise should be tested as a potential therapeutic intervention in FCHL, in order to reduce secretion of apo B containing lipoproteins, to improve insulin resistance, to lower blood pressure and potentially to counteract IMT progression.

9.2 FCHL: diagnostic problems

Presently, FCHL can be diagnosed in families with combined hyperlipidemia, multiple lipoprotein phenotype and associated premature cardiovascular disease. No biochemical or genetic marker is available yet to establish FCHL in one individual. Unavailability of a unique, sensitive and specific marker of the disease, FCHL, presents us with a clinical problem: how to diagnose FCHL in one individual?, and another: how should we define FCHL in clinical practice? As outlined in **chapter 2**, the criteria presented by Goldstein and colleagues in 1973 are used to diagnose FCHL presently, i.e. primary hyperlipidemia, with multiple hyperlipidemic lipoprotein phenotypes and premature cardiovascular disease (CVD) in a family¹. Hypercholesterolemia and hypertriglyceridemia are nowadays defined as levels exceeding the 90th or 95th percentile cut-off values in the population. This potentially leads to a more extreme hyperlipidemia to diagnose FCHL in those countries with established higher lipid concentrations in the population compared to countries with lower mean population cholesterol and triglyceride concentrations²³. Moreover, a decline in mean lipid levels in a population over time, e.g. by public information about healthy dietary habits, will also change the 90th or 95th percentile to a lower level, as is presently the case in Finland (Taskinen MR, FCHL workshop, ESCI 2001, Barcelona, Spain). Both mechanisms render comparisons between FCHL populations and within one FCHL population over time (follow-up) difficult, as different hyperlipidemic population cut-off values will be used.

A multiple lipoprotein phenotype within one family is required to establish the diagnosis of FCHL. In addition, the multiple lipoprotein phenotype can be observed over time within one individual as well. Therefore, a presently normolipidemic subject may, for instance by variation in plasma TG levels, have shown elevated lipid levels in the past. Also, in a cross-sectional survey, a

normolipidemic subject may develop hyperlipidemia later in life, for instance by gaining weight or developing central obesity. In contrast, changes from hyperlipidemia to normolipidemia or from one hyperlipidemic to another hyperlipidemic phenotype might also occur within one individual²⁴. One must bear these difficulties in classification in mind with every genetic, metabolic or clinical evaluation of FCHL.

Clustering of FCHL with other hyperlipidemic states, like hyperapobetalipoproteinemia and familial dyslipidemic hypertension (see **chapter 4**), have been reported within families or within individuals. Hyperapobetalipoproteinemia, characterised by normal LDL-cholesterol levels, increased concentrations of small dense LDL (sdLDL), and elevated apo B concentrations, merely differs from FCHL by definition^{25,26}. Sniderman and colleagues proposed to use hyper apo B and hypertriglyceridemia as FCHL criteria to identify those subjects with high risk of developing cardiovascular events. Austin and colleagues have shown that an increase in sdLDL is associated with TG levels above 1.5 mmol/L²⁷. Furthermore, especially the interaction between apo B and sdLDL leads to the highest risk of cardiovascular events in a general population^{28,29}. Although using the proposed criteria by Sniderman may identify those FCHL subjects with the highest cardiovascular risk, not all subjects identified as FCHL-affected at present, will be identified. However, these latter subjects are still at risk of developing cardiovascular events. Moreover, the familial association, which has been identified as important contributor to non-fatal risk of CVD³, may be neglected using the hyper apo B / hyper TG approach. In addition, the clustering of hyper apo B / hyper TG is not unique to FCHL but is also observed in other disorders, including polycystic ovarian syndrome³⁰, AIDS³¹, insulin resistance syndrome³²⁻³⁴ and the use of steroids³⁵. In FCHL, other associated traits, like reduced HDL-cholesterol and insulin resistance have been observed as well^{36,37}. However, these traits are currently not used to diagnose FCHL. Finally, the role of obesity and increased amount of adipose tissue in FCHL that have been described over the past years, are not incorporated in the diagnostic criteria for FCHL yet.

Another problem in specific diagnostic criteria for FCHL is the clustering observed with other clinical entities, like obesity and perhaps type 2 diabetes mellitus. Patients with these disorders may show VLDL overproduction, insulin resistance, enhanced post-prandial lipidemia, increased free fatty acid flux to the liver, sdLDL and increased cardiovascular risk as well¹¹. Despite differential diagnostic problems, this overlap gives us the opportunity to study the effect of contributing genes in FCHL and type 2 diabetes mellitus, and vice versa.

During the third FCHL workshop held at the ESCI meeting in May 2001 in Barcelona, Spain, the above mentioned diagnostic problems have been discussed extensively. The important role of FCHL in cardiovascular medicine has been stressed as well. World-wide co-operation is warranted to come to a better definition of FCHL for clinical as well as research purposes and to combine efforts to unravel the genetic abnormalities underlying FCHL. In daily clinical

practice, a primary combined hyperlipidemia in a subject with a known family history of hyperlipidemia and premature CVD should focus the physician's thoughts on FCHL. In the current national and international guidelines on hyperlipidemia, initiation of treatment depends on absolute risk of a cardiovascular event during the next 10 years³⁸⁻⁴⁰. However, an established familial hyperlipidemia warrants antihyperlipidemic treatment in any case, despite the absolute risk calculated from tables³⁸⁻⁴⁰. Screening of all family members on hyperlipidemia and other features of FCHL should be conducted as soon as FCHL is suggested as possible diagnosis in a subject, because of the strong association of FCHL with cardiovascular events. Whether this family survey is conducted in a clinical or research setting is of less important to the individual subject, as long as his / hers risk profile is established correctly and proper treatment is initiated whenever needed.

In conclusion, despite the current problems in diagnosing FCHL in daily clinical practice, FCHL is an important disease in cardiovascular biology. Furthermore, current criteria used in clinical science result in the ascertainment of FCHL families with similar characteristics over the world. In the end, FCHL may be considered more like a syndrome, it might turn out that patients manifest a mixture of disease entities, with a variety of metabolic defects and possible genetic markers⁴¹. Global uniformity to establish diagnosis of FCHL and world-wide co-operation to unravel the genetic and metabolic pathophysiology of FCHL should be established and expanded as soon as possible.

References

1. Goldstein JL, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG. Hyperlipidaemia in Coronary Heart Disease. II Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidaemia. *Journal of Clinical Investigation* 1973;52:1544-1568.
2. Austin MA, McKnight B, Edwards KL, Bradley CM, McNeely MJ, Psaty BM, Brunzell JD, Motulsky AJ. Cardiovascular disease mortality in familial forms of hypertriglyceridemia: A 20-year prospective study. *Circulation* 2000;101:2777-2782.
3. Voors-Pette C, de Bruin TWA. Excess coronary heart disease in Familial Combined Hyperlipidemia, in relation to genetic factors and central obesity. *Atherosclerosis* 2001;157:481-489.
4. Riches FM, Watts GF, Hua J, Stewart GR, Naoumova RP, Barrett PH. Reduction in visceral adipose tissue is associated with improvement in apolipoprotein B-100 metabolism in obese men. *Journal of Clinical Endocrinology and Metabolism* 1999;84:2854-2861.
5. Reaven GM, Lithell H, Landsberg L. Hypertension and associated metabolic abnormalities - the role of insulin resistance and the sympathoadrenal system. *New England Journal of Medicine* 1996;334:374-381.
6. Grekin RJ, Dumont CJ, Vollmer AP, Watts SW, Webb RC. Mechanisms in the pressor effects of hepatic portal venous fatty acid infusion. *American journal of physiology* 1997;273:R324-R330.
7. Seidell JC, Andres R, Sorkin JD, Muller DC. The sagittal waist diameter and mortality in men: the Baltimore Longitudinal Study on Aging. *Internal Journal of Obesity Related Metabolic Disorders* 1994;18:61-67.
8. Schulte H, Cullen P, Assmann G. Obesity, mortality and cardiovascular disease in the Munster Heart Study (PROCAM). *Atherosclerosis* 1999;144:199-209.
9. Hunt SC, Wu LL, Hopkins PN, Stults BM, Kuida H, Ramirez ME, Lalouel JM, Williams RR. Apolipoprotein, low density lipoprotein subfraction, and insulin associations with familial combined hyperlipidemia. Study of Utah patients with familial dyslipidemic hypertension. *Arteriosclerosis* 1989;9:335-344.
10. Ascaso JF, Merchante A, Lorente RI, Real JT, Martinez VJ, Carmena R. A study of insulin resistance using the minimal model in nondiabetic familial combined hyperlipidemic patients. *Metabolism* 1998;47:508-513.
11. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 1988;37:1595-1607.
12. Hjermann Ingvar. The Metabolic Cardiovascular Syndrome: Syndrome X, Reaven's Syndrome, Insulin Resistance Syndrome, Atherothrombogenic Syndrome. *Journal of Cardiovascular Pharmacology* 1992;20:S5-S10.
13. Andersen P. Hypercoagulability and Reduced Fibrinolysis in Hyperlipidemia: Relationship to the Metabolic Cardiovascular Syndrome. *Journal of Cardiovascular Pharmacology* 1992;20:S29-S31.

14. Dekker JM, Feskens EJ, Schouten EG, Klootwijk P, Pool J, Kromhout D. QTc duration is associated with levels of insulin and glucose intolerance. The Zutphen Elderly Study. *Diabetes* 1996;45:376-380.
15. Dekker JM, Schouten EG, Klootwijk P, Pool J, Kromhout D. Association between QT interval and coronary heart disease in middle-aged and elderly men. The Zutphen Study. *Circulation* 1994;90:779-785.
16. Elming H, Holm E, Jun L, Torp Pedersen C, Kober L, Kircshoff M, Malik M, Camm J. The prognostic value of the QT interval and QT interval dispersion in all-cause and cardiac mortality and morbidity in a population of Danish citizens. *European Heart Journal* 1998;19:1391-1400.
17. de Bruyne M, Hoes AW, Kors JA, Hofman A, van Bommel JH, Grobbee DE. Prolonged QT interval predicts cardiac and all-cause mortality in the elderly. The Rotterdam Study. *European Heart Journal* 1999;20:278-284.
18. Venkatesan S, Cullen P, Pacy P, Halliday D, Scott J. Stable isotopes show a direct relation between VLDL apoB overproduction and serum triglyceride levels and indicate a metabolically and biochemically coherent basis for familial combined hyperlipidemia. *Arteriosclerosis Thrombosis and Vascular Biology* 1993;13:1110-1118.
19. Babirak S, Brown B, Brunzell J. Familial combined hyperlipidemia and abnormal lipoprotein lipase. *Arteriosclerosis and Thrombosis* 1992;12:1176-1183.
20. Stroes E, de Bruin TWA, de Valk H, Erkelens DW, Banga JD, van Rijn H, Koomans H, Rabelink T. NO activity in familial combined hyperlipidemia: potential role of cholesterol remnants. *Cardiovascular Research* 1997;36:445-452.
21. Hodis HN, Mack WJ, LaBree L, Selzer RH, Liu C, Alaupovic P, Kwong-Fu H, Azen SP. Reduction in carotid arterial wall thickness using lovastatin and dietary therapy: a randomized controlled clinical trial. *Annals of Internal Medicine* 1996;124:548-556.
22. Smilde TJ, van Wissen S, Wollersheim H, Trip MD, Kastelein JJP, Stalenhoef AFH. Effect of aggressive versus conventional lipid lowering on atherosclerosis progression in familial hypercholesterolaemia (ASAP): a prospective, randomised, double-blind trial. *Lancet* 2001;357:577-581.
23. Verschuren WM, Jacobs DR, Bloemberg BP, Kromhout D, Menotti A, Aravanis C, Blackburn H, Buzina R, Dontas AS, Fidanza F. Serum total cholesterol and long-term coronary heart disease mortality in different cultures. Twenty-five-year follow-up of the seven countries study. *Journal of the American Medical Association* 1995;274:131-136.
24. Veerkamp MJ, de Graaf J, Bredie SJH, Hendriks JCM, Stalenhoef AFH. Diagnosis of familial combined hyperlipidaemia (FCH) based on lipid phenotype expression in 32 families: results of a 5-year follow-up study. *European Journal of Clinical Investigation* 2001;31:20 (Abstract).
25. Sniderman AD, Wolfson C, Teng B, Franklin FA, Bachorik PS, Kwiterovich Jr PO. Association of hyperapobetalipoproteinemia with endogenous hypertriglyceridemia and atherosclerosis. *Annals of Internal Medicine* 1982;97:833-839.
26. Sniderman A, Teng B, Genest J, Cianflone K, Wacholder S, Kwiterovich P. Familial aggregation and early expression of hyperapobetalipoproteinemia. *American Journal of Cardiology* 1985;55:291-295.

27. Austin MA, King MC, Vranizan KM, Krauss RM. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. *Circulation* 1990;82:495-506.
28. Lamarche B, Tchernof A, Moorjani S, Cantin B, Dagenais GR, Lupien PJ, Depres JP. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. *Circulation* 1997;95:69-75.
29. Lamarche B, Tchernof A, Mauriege P, Cantin B, Dagenais GR, Lupien PJ, Depres JP. Fasting insulin and apolipoprotein B levels and low-density lipoprotein particle size as risk factors for ischemic heart disease. *Journal of the American Medical Association* 1998;279:1955-1961.
30. Lobo RA, Carmina E. The importance of diagnosing the polycystic ovary syndrome. *Annals of Internal Medicine* 2000;132:989-993.
31. Crook MA, Mir N. Abnormal lipids and the acquired immunodeficiency syndrome: is there a problem and what should we do about it? *International Journal of STD and AIDS* 1999;10:353-356.
32. Lamarche B, Lewis GF. Atherosclerosis prevention for the next decade: risk assessment beyond low density lipoprotein cholesterol. *Canadian Journal of Cardiology* 1998;14:841-851.
33. Timar O, Sestier F, Levy E. Metabolic syndrome X: a review. *Canadian Journal of Cardiology* 2000;16:779-789.
34. Laws A. Free fatty acids, insulin resistance and lipoprotein metabolism. *Current Opinion in Lipidology* 1996;7:172-177.
35. Lajarin F, Zaragoza R, Tovar I, Martinez-Hernandez P. Evolution of serum lipids in two male bodybuilders using anabolic steroids. *Clinical Chemistry* 1996;42:970-972.
36. Bredie SJH, Tack CJJ, Smits P, Stalenhoef AFH. Non-obese patients with familial combined hyperlipidemia are insulin resistant as compared with their non-affected relatives. *Arteriosclerosis Thrombosis and Vascular Biology* 1997;17:1465-1471.
37. Ascaso JF, Lorente R, Merchante A, Real JT, Priego A, Carmena R. Insulin resistance in patients with familial combined hyperlipidemia and coronary artery disease. *American Journal of Cardiology* 1997;80:1484-1487.
38. Simoons ML, Casparie AF. Behandeling en preventie van coronaire hartziekten door verlaging van de serumcholesterolconcentratie; derde consensus 'Cholesterol'. [Therapy and prevention of coronary heart diseases through lowering of the serum cholesterol levels; third consensus 'Cholesterol'. Consensus Working Group, CBO]. *Nederlands Tijdschrift der Geneeskunde* 1998;142:2096-2101.
39. Assmann G, Cullen P, Fruchart C, Lewis B, Mancini M, Carmena R. Coronary heart disease prevention task force. *European Heart Journal* 1999;20:841-844.
40. Wood D. European and American recommendations for coronary heart disease prevention. *European Heart Journal* 1998;19 Suppl AA12-AA19.
41. de Graaf J, Stalenhoef AF. Defects of lipoprotein metabolism in familial combined hyperlipidaemia. *Current Opinion in Lipidology* 1998;9:189-196.

Chapter 10

Summary

Summary

Chapter 1 describes the background and aims of the research described in this thesis. Cardiovascular disease (CVD) is a main cause of death in our Western society. Several epidemiological studies have identified many risk factors that are convincingly linked to the development of CVD. Multiple combinations of these cardiovascular risk factors will lead to an exponentially increased risk of early CVD¹⁻³. In Familial Combined Hyperlipidemia (FCHL), an increased risk of premature (i.e. before age 60 years) CVD is present. Although FCHL is now known for more than 25 years, most of the research has focussed upon the pathophysiology of lipid abnormalities and insulin resistance observed in FCHL. However, little is known about early manifestations of vascular abnormalities in FCHL, and about the natural history of such vascular abnormalities. Furthermore, there is the consideration that the observed lipid abnormalities and insulin resistant state can not solely explain the known high risk of developing CVD in FCHL.

Chapter 2 addresses the collection of Dutch FCHL families and the criteria used to diagnose and ascertain a family with FCHL. Furthermore, several historic, environmental, anthropometric and clinical data obtained in 35 Maastricht FCHL families are described. In these Dutch FCHL families, 39% (143 out of 362 relatives) were diagnosed with hyperlipidemia. Moreover, the prevalence of hyperlipidemia among FCHL relatives steeply increased from 15% (13 out of 86 subjects) under age 30 years, to 71.4% (25 out of 35 subjects) in the age-category 60 to 69 years ($p < 0.01$ for trend). A striking observation was that in FCHL subjects, hyperlipidemia occurred almost 20 years earlier than in the spouse population. In addition, a significant clustering of the main cardiovascular risk factors (hyperlipidemia, hypertension, obesity and current smoking) in FCHL family members was observed compared to spouses ($p < 0.05$ for trend). The above-mentioned abnormalities together clearly indicate an unfavourable atherogenic risk profile in FCHL. This is underlined by the high prevalence of CVD found in hyperlipidemic relatives compared to spouses.

Chapter 3 reviews our current pathophysiological and genetic understanding of FCHL. Although FCHL was delineated about 25 years ago, the complex FCHL phenotype is not fully understood at present. Several studies have shown abnormalities in lipoprotein metabolism, including hepatic hypersecretion of apolipoprotein B (apo B) containing lipoproteins^{4,5} and delayed clearance of atherogenic lipoprotein remnants⁶. Except for the above-mentioned abnormalities in lipid metabolism, insulin resistance of adipose and muscular tissue has been documented in FCHL^{7,8}. Furthermore, FCHL affected subjects often report some increase in body weight, body mass index, waist to hip ratio and waist-circumference, all together representing an increased adipose tissue mass, and, perhaps, reduced muscle mass. Increased free fatty acid (FFA) flux from visceral fat to the liver can contribute to the hypersecretion of triglyceride-

rich very low-density lipoproteins from the liver, a hallmark of FCHL. The observed abnormalities in liver, adipose tissue and muscle in FCHL provide us with interesting target tissues for differential gene expression studies, as will be reviewed in the near future⁹. It is generally believed that the complex FCHL phenotypes result from a defect in one or more major genes in combination with multiple modifier genes and gene-environment interaction. Several modifier genes are subsequently described at the end of **chapter 3**.

Chapter 4 reports on the simultaneous occurrence of hypertension and hyperlipidemia in FCHL. One-third of 27 FCHL-families was diagnosed with Familial Dyslipidemic Hypertension. Significantly more relatives (27.2%) were diagnosed with dyslipidemic hypertension, compared to spouses (14.2%). Furthermore, in FCHL women (affected as well as non-affected relatives, without antihypertensive medication) systolic and diastolic blood pressures were higher compared to spouses. Multiple linear regression analysis showed that age, FCHL-affected status and waist-circumference significantly contributed to systolic blood pressure in females. The present study indicates that visceral adipose tissue strongly contributes to the high prevalence of dyslipidemic hypertension in FCHL families. Reduction of visceral fat versus treatment of hyperlipidemia and hypertension should be tested as potential therapeutic interventions in FCHL-affected individuals.

Chapter 5 describes the heart rate corrected QT-interval (QTc) as measured in all leads of a 12-lead electrocardiogram in 76 FCHL-affected subjects and 92 healthy controls. QTc interval was significantly prolonged in FCHL-affected women, but not in men, compared to healthy controls (difference of 7.8 msec, age-corrected $p < 0.01$). The observed significantly positive correlations in women between QTc and logTG, log insulin and HOMA, and the negative correlation with HDL-cholesterol were in line with the previously suggested relationship between QTc and the insulin resistance syndrome (IRS)¹⁰. Multivariate regression analyses revealed that plasma insulin, systolic blood pressure, HDL-cholesterol, body mass index and FFA were independent predictors of QTc. In conclusion, in FCHL, prolonged QTc was specifically found in hyperlipidemic FCHL women and associated with components of IRS. QTc prolongation may either relate to the genetic susceptibility to FCHL in women, or represent an adaptation mechanism to insulin resistance. QTc prolongation may also add to the risk of developing cardiovascular events in FCHL.

Chapter 6 reports on several endothelium-derived proteins of the coagulation and fibrinolytic systems in FCHL. In 70 hyperlipidemic FCHL subjects and 57 healthy controls, fasting plasma concentrations of von Willebrand factor (vWf: pro-coagulant), Tissue Factor Pathway Inhibitor (TFPI) and thrombomodulin (both anti-coagulant), tissue type plasminogen activator (t-PA; pro-fibrinolytic) and plasminogen activator inhibitor 1 (PAI-1; anti-fibrinolytic) were determined. FCHL affected subjects showed significantly higher PAI-1 and t-PA levels than healthy controls (PAI-1: 18 (3-38) vs. 10 (1-29) U/mL; $p < 0.001$, and t-PA: 10.8 ± 3.8 vs. 7.5 ± 3.9 ng/mL; $p < 0.001$). In addition, vWf was significantly higher in

FCHL subjects compared to healthy controls (110.0 ± 35.4 % vs. 94.3 ± 36.8 %, $p > 0.05$). Surrogate markers of insulin resistance explained 50% of the variation in plasma PAI-1 levels. The observed findings indicate a pro-coagulant, anti-fibrinolytic state of the endothelium, that is dependent on insulin resistance, and potentially contributes to the risk of premature cardiovascular disease found in FCHL. The increased plasma concentrations of the studied endothelium-derived proteins also indicate a dysfunctional endothelium. Further studies to the contribution of PAI-1 and vWf to the risk of cardiovascular disease in FCHL, and the potential treatment of these elevated levels, are warranted.

Chapter 7 describes abnormalities in capillary structure and function observed in FCHL-affected subjects and the possible role of remnant-like lipoprotein cholesterol (RLP-C) concentrations. In 23 (12 men) FCHL-affected subjects and 27 (14 men) healthy controls, capillary density was measured just above the finger nailfold, before and after 4 minutes of arterial occlusion. During the same test, skin blood flow was also measured using laser Doppler fluxmetry. Basal (BC) and post-occlusive (POC) number of capillaries were significantly lower in FCHL men compared to healthy men, 113.7 ± 15.1 vs. 132.0 ± 18.0 ; $p = 0.02$, and 123.0 ± 19.1 vs. 142.3 ± 18.3 ; $p = 0.03$, respectively. Multivariate regression analyses revealed that log RLP-C was the only significant, independent contributor to POC. Laser Doppler post-occlusive reactive hyperaemia (LDPRH) was higher in FCHL women compared to healthy females: 537 (239-800) % vs. 327 (39-365) %; $p < 0.05$. In men no differences in LDPRH were observed. In conclusion, in FCHL men a reduction in structural and functional skin capillaries was observed, and associated with increased atherogenic lipoprotein concentrations. Whether this reduction in capillary surface can be implicated in the pathophysiology of FCHL and its high risk of cardiovascular events, or is the result of adaptation to hyperlipidemia in FCHL, remains to be elucidated.

The increase in LDPRH in FCHL women was an unexpected finding. Previous studies have suggested a reduced LDPRH could be a hallmark of insulin resistance^{11,12}, a metabolic state that is also frequently observed in FCHL. It should be noted however, that the increased hyperaemic response in FCHL women was associated with normal capillary density, but that in FCHL men, the normal hyperaemic response was associated with reduced capillary density. These data combined might suggest that arteriovenous shunt flow be increased in FCHL.

Chapter 8 reports on non-invasive ultrasound measurements of intima media thickness (IMT) in 58 FCHL-affected subjects and 55 age- and gender-matched healthy controls. IMT was measured at the left and right common carotid artery approximately 10-20 mm proximal to the carotid bulb. FCHL-affected subjects without a previous history of cardiovascular disease ($n = 46$) had a significantly increased IMT compared to healthy controls, with a difference of $57 \mu\text{m}$ (age- and gender-corrected $p < 0.01$). A significant positive, age- and gender-corrected, Pearson correlation coefficient was observed between IMT and total cholesterol, non-HDL-cholesterol, LDL-cholesterol, logTG, apo B, fasting insulin, calculated insulin resistance (HOMA), BMI and waist-circumference, respectively.

Multivariate regression analyses revealed that age, gender, apo B, and BMI were independent positive predictors of IMT, explaining a large proportion, 50%, of the observed variation ($p < 0.001$). In conclusion, IMT of the common carotid artery is increased in FCHL-affected subjects (mean age of 48.5 years) and in the present study it corresponded to an acceleration of silent atherosclerosis by approximately 5-9 years. The present finding show the important contribution of atherogenic lipoprotein particles to the development of increased IMT in FCHL.

Finally, **chapter 9** sets the results of this thesis in perspective and addresses some points of attention in further research.

References

1. Castelli WP. Epidemiology of coronary heart disease: the Framingham study. *American Journal of Medicine* 1984;76:4-12.
2. Assmann G, Schulte H. Relation of high-density lipoprotein cholesterol and triglycerides to incidence of atherosclerotic coronary artery disease (the PROCAM experience). Prospective Cardiovascular Munster study. *American Journal of Cardiology* 1992;70:733-737.
3. Yusuf HR, Giles WH, Croft JB, Anda RF, Casper ML. Impact of multiple risk factor profiles on determining cardiovascular disease risk. *Preventive Medicine* 1998;27:1-9.
4. Venkatesan S, Cullen P, Pacy P, Halliday D, Scott J. Stable isotopes show a direct relation between VLDL apoB overproduction and serum triglyceride levels and indicate a metabolically and biochemically coherent basis for familial combined hyperlipidemia. *Arteriosclerosis Thrombosis and Vascular Biology* 1993;13:1110-1118.
5. Bredie SJ, van Drongelen J., Kiemeny LA, Demacker PN, Beatty TH, Stalenhoef AF. Segregation analysis of plasma apolipoprotein B levels in familial combined hyperlipidemia. *Arteriosclerosis Thrombosis and Vascular Biology* 1997;17:834-840.
6. Cabezas MC, de Bruin TWA, Jansen H, Kock LA, Kortlandt W, Erkelens DW. Impaired chylomicron remnant clearance in familial combined hyperlipidemia. *Arteriosclerosis and Thrombosis* 1993;13:804-814.
7. Bredie SJH, Tack CJJ, Smits P, Stalenhoef AFH. Non-obese patients with familial combined hyperlipidemia are insulin resistant as compared with their non-affected relatives. *Arteriosclerosis Thrombosis and Vascular Biology* 1997;17:1465-1471.
8. Hunt SC, Wu LL, Hopkins PN, Stults BM, Kuida H, Ramirez ME, Lalouel JM, Williams RR. Apolipoprotein, low-density lipoprotein subfraction, and insulin associations with familial combined hyperlipidemia. Study of Utah patients with familial dyslipidemic hypertension. *Arteriosclerosis* 1989;9:335-344.
9. Eurlings PMH, van der Kallen CJH, Geurts JMW, van Greevenbroek MMJ, de Bruin TWA. Genetic dissection of familial combined hyperlipidemia. *Molecular Genetics and Metabolism* 2001; in press.
10. Dekker JM, Feskens EJ, Schouten EG, Klootwijk P, Pool J, Kromhout D. QTc duration is associated with levels of insulin and glucose intolerance. The Zutphen Elderly Study. *Diabetes* 1996;45:376-380.
11. Tur E, Yosipovitch G, Bar OY. Skin reactive hyperemia in diabetic patients. A study by laser Doppler flowmetry. *Diabetes Care* 1991;14:958-962.
12. Jaap AJ, Hammersley MS, Shore AC, Tooke JE. Reduced microvascular hyperaemia in subjects at risk of developing type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 1994;37:214-216.

Chapter 11

Samenvatting

Samenvatting

Risicofactoren voor hart- en vaatziekten en vaatafwijkingen in patiënten met Familiäre Gecombineerde Hyperlipidemie.

Hart- en vaatziekten (HVZ) zijn heden ten dage de belangrijkste doodsoorzaak in de Westerse samenleving. Gedurende de afgelopen decennia is er een scala aan cardiovasculaire risicofactoren beschreven. Een combinatie van deze risicofactoren leidt tot een sterkere verhoging van het cardiovasculaire risico dan uit het risico van een enkele factor zou kunnen worden afgelezen. Bij een aantal ziektebeelden vindt er tevens een clustering van cardiovasculaire risico factoren binnen een familie plaats. Een belaste familie anamnese betreffende HVZ is eveneens een erkende risico factor.

Familiair Gecombineerde Hyperlipidemie (FGH) is een familiale aandoening die gekenmerkt wordt door wisselend verhoogde cholesterol en triglyceride waarden in het bloed. Aangedane personen uit deze families hebben een sterk verhoogd risico op het ontwikkelen van cardiovasculaire aandoeningen, zoals een myocard infarct of een herseninfarct, voor hun 60^e levensjaar. Binnen de FGH onderzoekswereld is gedurende de afgelopen 25 jaar met name veel aandacht uitgegaan naar de pathofysiologische oorzaken die leiden tot de beschreven afwijkingen in de lipidenstofwisseling en de rol van insuline resistentie daarin. In **hoofdstuk 3** worden de huidige opvattingen en inzichten omtrent deze pathofysiologische oorzaken samengevat. Bij FGH is er sprake van een overproductie van apolipoproteïne B (apo B) bevattende lipoproteïnen door de lever, met daarbij een vertraagde klaring van deze lipoproteïnen. Een toename in vrije vetzuur flux van het viscerele vet naar de lever wordt als belangrijkste oorzaak gezien voor de overproductie van lipoproteïnen door de lever bij personen met FGH. Daarnaast zijn personen met FGH vaak insuline resistent op zowel spier- als vetniveau. Een toename in vetmassa, met name abdominaal gelokaliseerd, wordt eveneens vaak waargenomen bij individuen met FGH.

Echter, de beschreven pathofysiologische afwijkingen in de lipidenstofwisseling en insuline resistentie binnen het FGH ziektebeeld zijn niet in staat het hoge cardiovasculaire risico volledig te verklaren. Daarnaast zijn er weinig gegevens bekend over vroege vaatafwijkingen bij personen met FGH en de eventuele rol die lipiden hierin kunnen spelen. In het onderzoek beschreven in dit proefschrift is aan beide vragen aandacht besteed.

Op dit moment is er nog geen unieke marker beschikbaar om FGH binnen een individu te kunnen aantonen. Daarom is een uitgebreid familie onderzoek nog steeds noodzakelijk. **Hoofdstuk 2** beschrijft de huidige strategie en de criteria die gebruikt zijn om de diagnose FGH in een familie vast te stellen. Daarnaast worden enkele epidemiologische gegevens beschreven betreffende 35 FGH families uit Maastricht en omgeving. Uit onze gegevens bleek dat bij 39% (143 van de 362) van de familieleden sprake was van een hyperlipidemie. Verder bleek de prevalentie van de hyperlipidemie sterk toe te nemen met de leeftijd:

van 15% (13 van de 86) bij familieleden onder de 30 jaar tot ruim 71% (25 van de 35) bij familieleden met een leeftijd tussen de 60 en 70 jaar ($p < 0.01$ voor trend). De hyperlipidemie trad ongeveer 20 jaar eerder op in FGH familieleden vergeleken met onze controle populatie. Daarnaast werd er een significante clustering van cardiovasculaire risicofactoren (hyperlipidemie, hypertensie, obesitas en roken) gevonden bij familieleden met FGH. De hoge prevalentie van HVZ bij familieleden met FGH onderstreepte nogmaals het hoge cardiovasculaire risico binnen deze patiëntenpopulatie.

In de **hoofdstukken 4 tot en met 6** staan een aantal mogelijke cardiovasculaire risicofactoren beschreven die, indien afwijkend, zouden kunnen bijdragen aan het hoge risico op HVZ binnen de FGH populatie. **Hoofdstuk 4** beschrijft het gezamenlijk voorkomen van hyperlipidemie en hypertensie in een FGH populatie. Eenderde van de 27 onderzochte FGH families voldeed naast de criteria voor FGH tevens aan de criteria voor de diagnose Familiaire Dyslipidemische Hypertensie. Tevens werd de clustering van hypertensie en hyperlipidemie significant vaker waargenomen bij FGH familieleden dan in de controle populatie (27.2% vs. 14.2%, $p < 0.01$). Zowel aangedane als niet-aangedane vrouwelijke familieleden hadden een hogere systolische en diastolische bloeddruk ten opzichte van vrouwelijke controle personen. Multipole lineaire regressie analyse bij vrouwen toonde aan dat de hoogte van de systolische bloeddruk met name afhankelijk was van leeftijd, aanwezigheid van hyperlipidemie and taille omtrek. Hieruit werd geconcludeerd dat visceraal vetweefsel een belangrijke bijdrage kan leveren aan de prevalentie van hyperlipidemie en hypertensie in FGH families. Nader onderzoek dient uit te wijzen of reductie in de viscerale vetmassa een mogelijke therapeutische interventie is ter behandeling van hyperlipidemie en hypertensie bij personen met FGH.

Een verlenging van het hartfrequentie gecorrigeerde QT-interval (QTc) is geassocieerd met plotselinge hartdood, zowel in patiënten die al een myocard infarct hebben doorgemaakt als in de algemene bevolking. In **hoofdstuk 5** werd daarom onderzocht of QTc eventueel verlengd was in een FGH populatie en zo mogelijk zou kunnen bijdragen aan het verhoogde cardiovasculaire risico binnen FGH. QTc werd automatisch gedetecteerd in een 12-afleidingen ECG bij 76 personen met FGH en 92 gezonde controle personen. FGH vrouwen hadden een significante verlenging van QTc vergeleken met controle vrouwen (verschil 7.8 msec, leeftijd-gecorrigeerde $p < 0.01$). Er werd geen verschil tussen de mannelijke populaties gevonden.

Eerder onderzoek suggereerde dat QTc verlenging mogelijk onderdeel zou zijn van het insuline resistentie syndroom. De significante positieve correlaties tussen QTc en logTG, log insuline en HOMA (berekende waarde voor insuline resistentie) en de negatieve correlatie met HDL-cholesterol, die in ons onderzoek werden gevonden bij vrouwen, zijn in overeenstemming met bovenstaande suggestie. Multivariate regressie analyse toonde het verband tussen QTc verlenging en componenten van het insuline resistentie syndroom nogmaals aan.

Concluderend lieten FGH vrouwen een verlengd QTc interval zien, dat geassocieerd was met componenten van het insuline resistentie syndroom. Het blijft de vraag of deze QTc verlenging een gevolg is van de genetische gevoeligheid van vrouwen voor FGH of meer een adaptatie mechanisme voor insuline resistentie. QTc verlenging kan verder mogelijk bijdragen aan het verhoogde cardiovasculaire risico binnen personen met FGH, maar toekomstig follow-up onderzoek zal dit moeten bevestigen.

In de literatuur is de associatie tussen een versterkte stolling en/of onderdrukte fibrinolyse en het optreden van hart- en vaatziekten uitvoerig beschreven. Een versterkte stolling en/of onderdrukte fibrinolyse zou ook kunnen bijdragen aan het hoge cardiovasculaire risico bij personen met FGH. Diverse componenten van het stollings- en fibrinolyse systeem werden onderzocht in 70 hyperlipidemische FGH personen en 57 gezonde controle personen (**hoofdstuk 6**). FGH personen hadden significant hogere plasma concentraties van plasminogeen activator inhibitor 1 (PAI-1) en tissue type plasminogeen activator (t-pa) dan de controle populatie (PAI-1: 18 (3-38) vs. 10 (1-29) U/mL; $p < 0.001$, en t-pa: 10.8 ± 3.8 vs. 7.5 ± 3.9 ng/mL; $p < 0.001$). Tevens was de concentratie van de von Willebrand factor (vWf) significant toegenomen bij FGH personen ten opzichte van de controle onderzoeksgroep (110.0 ± 35.4 % vs. 94.3 ± 36.8 %, $p < 0.05$). Een aantal componenten van het insuline resistentie syndroom (log TG, log insuline, glucose en apo B) verklaarde 50% van de variatie in PAI-1. Het pro-coagulante, anti-fibrinolytische karakter van het endotheel kan mogelijk bijdragen aan het hoge cardiovasculaire risico bij personen met FGH. Toekomstige studies zullen de precieze rol van PAI-1 en vWf in het cardiovasculaire risico bij personen met FGH ophelderen.

In **hoofdstuk 7** worden veranderingen in de capillaire dichtheid bij personen met FGH en de potentiële rol van remnant-achtige lipoproteïnen (RLP-C) concentraties beschreven. RLP-C bestaan uit chylomicron remnants, apo E verrijkte very low-density lipoprotein (β -VLDL) en intermediate density lipoprotein (IDL), allen pro-atherogene remnants. Capillaire dichtheden werden gemeten bij 23 (12 mannen) personen met FGH en 27 (14 mannen) gezonde controle personen. Door middel van capillaire microscopie werd het aantal capillairen proximaal van de nagelriem van de rechter ringvinger in beeld gebracht, voor en na 4 minuten van arteriële occlusie. Tijdens deze test werd eveneens de huiddoorbloeding gemeten met laser Doppler fluximetrie. Het aantal capillairen in FGH mannen was zowel in de basale (BC) als in de post-occlusieve (POC) meting verminderd ten opzichte van controle mannen (BC: 113.7 ± 15.1 vs. 132.0 ± 18.0 ; $p = 0.02$, en POC: 123.0 ± 19.1 vs. 142.3 ± 18.3 ; $p = 0.03$). In multivariate regressie analyse bleek logaritmisch getransformeerd RLP-C de enige significante onafhankelijke factor te zijn die bijdroeg aan POC. De conclusie was dat in FGH mannen een afname in structurele en functionele huidcapillairen werd waargenomen, die geassocieerd was met een toename van atherogene lipoproteïnen concentraties. Toekomstig onderzoek dient uit te wijzen of de afname van capillairen een rol speelt in de pathofysiologie van FGH of meer als een adaptatie aan de hyperlipidemie gezien moet worden.

Daarnaast was de laser Doppler post-occlusieve reactieve hyperaemie (LDPRH) significant hoger in FGH vrouwen dan in controle vrouwen: 537 (239-800) % vs. 327 (39-365) %; $p < 0.05$. Deze toename in LDPRH bij FGH vrouwen was een onverwachte bevinding. Eerder onderzoek suggereerde een relatie tussen een afgenomen LDPRH en insuline resistentie. Aangezien insuline resistentie ook frequent gezien wordt bij personen met FGH werd op voorhand een afname in LDPRH verwacht. Een duidelijke verklaring voor onze bevinding is voornamelijk niet voorhanden. De combinatie van beide metingen (capillaire dichtheden en fluxmetrie) suggereert wel dat er mogelijk een toegenomen arterioveneuze shunt flow aanwezig is bij personen met FGH. De toegenomen hyperaemische respons bij FGH vrouwen ging namelijk samen met een normale capillaire dichtheid, terwijl bij FGH mannen een normale hyperaemische respons werd waargenomen bij een afgenomen capillaire dichtheid.

Intima-media dikte (IMT) werd gemeten in 58 personen met FGH en 55 leeftijd- en geslacht-gematchte gezonde controle personen (**hoofdstuk 8**). IMT werd in de arteria carotis communis, 10 tot 20 mm proximaal van de bulbous carotis gemeten middels non-invasieve ultrageluid techniek. FGH personen met een blanco cardiovasculaire voorgeschiedenis ($n=46$) hadden een significante toename van de IMT vergeleken met de controle populatie (verschil $57 \mu\text{m}$, leeftijd en geslacht gecorrigeerde $p < 0.01$). Significante positieve, leeftijd en geslacht gecorrigeerde, Pearson correlaties werden gevonden tussen IMT en totaal cholesterol, non-HDL cholesterol, LDL-cholesterol, logTG, apo B, nuchter insuline, HOMA (maat voor insuline resistentie), BMI en taille-omtrek. Na multivariate regressie analyse bleven leeftijd, geslacht, apo B en BMI over als onafhankelijke positieve voorspellers van IMT. Deze 4 factoren tezamen verklaarden meer dan 50% van de variatie in IMT ($p < 0.001$). De toename in IMT bij personen met FGH komt overeen met een acceleratie van vroege atherosclerose van ongeveer 7 jaar. De huidige bevindingen ondersteunen de belangrijke rol van atherogene lipoproteïnen deeltjes in IMT toename bij personen met FGH.

Hoofdstuk 9 plaatst bovenstaande bevindingen verder in perspectief en draagt mogelijkheden voor verder onderzoek aan. Tevens wordt er nog kort ingegaan op de problemen met het diagnostiseren van FGH in de kliniek, die inherent zijn aan de huidige wetenschappelijke definities.

List of publications

List of publications

Articles

Jacobs MAJM, **Keulen ETP**, Kanc K, Casteleijn S, Scheffer P, Deville W, Heine RJ. Metabolic efficacy of preprandial administration of Lys (B28), Pro (B29) human insulin analogue in IDDM patients. A comparison with human regular insulin during a three-meal test period. *Diabetes Care* 1997;20:1279-1286.

Kanc K, Janssen MM, **Keulen ETP**, Jacobs MAJM, Popp-Snijders C, Snoek FJ, Heine RJ. Substitution of night-time continuous subcutaneous insulin infusion therapy for bedtime NPH insulin in a multiple injection regimen improves counter-regulatory hormonal responses and warning symptoms of hypoglycaemia in IDDM. *Diabetologia* 1998;41:322-329.

Keulen ETP, Voors-Pette C, de Bruin TWA. Familial Dyslipidemic Hypertension syndrome, further delineation in Familial Combined Hyperlipidemia, and the role of abdominal fat mass. *American Journal of Hypertension* 2001;14:357-363.

Keulen ETP, Kruijschoop M, Schaper NC, Hoeks APG, de Bruin TWA. Increased intima media thickness in Familial Combined Hyperlipidemia: association with apolipoprotein B. *Arteriosclerosis Thrombosis and Vascular Biology* 2001; in press.

Allayee H, de Bruin TWA, Dominguez KM, Cheng LSC, Ipp E, Cantor RM, Krass KL, **Keulen ETP**, Aouizerat BE, Lusi AJ, Rotter JJ. Genome-scan for blood pressure in Dutch dyslipidemic families reveals linkage to a locus on chromosome 4p. *Hypertension* 2001;in press.

Beeks E, Janssen RGJH, Kroon AA, **Keulen ETP**, Geurts JMW, de Leeuw PW, de Bruin TWA. Association between the α -adducin Gly460Trp polymorphism and systolic blood pressure in familial combined hyperlipidemia. *American Journal of Hypertension* 2001;in press.

Keulen ETP, Rijkers K, Lutgens I, van Lin JMJP, Schaper NC, de Bruin TWA. QT-interval (QTc) is prolonged in Familial Combined Hyperlipidemia: association with insulin resistance. Submitted.

Keulen ETP, Hamulyak K, de Bruin TWA. Pro-coagulant endothelium in Familial Combined Hyperlipidemia. Submitted.

Keulen ETP, Schaper NC, Houben AJHM, van Lin JMJP, Lutgens I, Rijkers K, Dallinga-Thie GM, de Bruin TWA. Reduced capillary density in Familial Combined Hyperlipidemia, association with remnant-like lipoprotein particles. Submitted.

Van der Kallen CJH, Voors-Pette C, Bouwman FG, Lu JY, van de Hulst RWJ, Janssen MJ, **Keulen ETP**, Boeckx WD, Rotter JI, de Bruin TWA. Evidence of insulin resistant lipid metabolism in adipose tissue in Familial Combined Hyperlipidemia, but not in type 2 Diabetes. Submitted.

Abstracts

Jacobs MAJM, Kanc K, **Keulen ETP**, Casteleijn S, Heine RJ. The metabolic efficacy of Lispro human insulin analogue in a multiple injection (MIT) regimen. *Netherlands Journal of Medicine* 1995;46:A37.

Jacobs MAJM, Kanc K, **Keulen ETP**, Casteleijn S, Heine RJ. The metabolic efficacy of Lispro human insulin analogue in a multiple injection (MIT) regimen. *Hormone and Metabolic Research* 1995;27:56.

Jacobs MAJM, **Keulen ETP**, Kanc K, Casteleijn S, Heine RJ. Metabolic responses and energy expenditure following preprandial administration of Lispro insulin analogue in IDDM patients. *Diabetologia* 1995;38(suppl 1):A189.

Kanc K, **Keulen ETP**, Jacobs MAJM, Popp-Snijders C, Naseri K, Heine RJ. Night-time continuous subcutaneous insulin infusion (CSII) therapy enhances counter-regulatory hormonal responses and warning symptoms of hypoglycaemia in IDDM patients. *Diabetologia* 1995;38(suppl 1):A205.

Nijpels G, **Keulen ETP**, Bouter LM, Heine RJ. HDL-cholesterol predicts the incidence of cardiovascular disease in persons with impaired glucose tolerance. *Diabetologia* 1996;39(suppl 1):A194.

Keulen ETP, Pette C, de Bruin TWA. Familial Dyslipidemic Hypertension syndrome: delineation in Familial Combined Hyperlipidemia. *Atherosclerosis* 2000;suppl 1:S151:252.

Keulen ETP, Rijkers K, Lutgens I, van Lin JMJP, Schaper NC, de Bruin TWA. Heart-rate corrected QT-interval is prolonged in Familial Combined Hyperlipidemia, and associated with insulin resistance. *European Journal of Clinical Investigation* 2001;31(suppl 1):19.

Voors-Pette C, **Keulen ETP**, de Bruin TWA. Hyperlipidemia in relation to central obesity in Familial Combined Hyperlipidemia. *European Journal of Clinical Investigation* 2001;31(suppl 1):19.

Keulen ETP, Schaper NC, van Lin JMJP, Lutgens I, Rijkers K, Houben AJHM, Dallinga-Thie GM, de Bruin TWA. Reduced post-occlusive capillary density in FCHL, associated with lipoprotein remnants. *European Journal of Clinical Investigation* 2001;31(suppl 1):25.

Keulen ETP, Lutgens I, Rijkers K, van Lin JMJP, de Bruin TWA. The epidemiology of cardiovascular risk factors in Familial Combined Hyperlipidemia. *Atherosclerosis* 2001;suppl 2/2:61.

Keulen ETP, Schaper NC, van Lin JMJP, Lutgens I, Rijkers K, Houben AJHM, Dallinga-Thie GM, de Bruin TWA. Structural loss of functional capillaries in Familial Combined Hyperlipidemia, related to lipoprotein remnants. *Atherosclerosis* 2001;suppl 2/2:143.

Keulen ETP, Hamulyak K, de Bruin TWA. Pro-coagulant endothelium in Familial Combined Hyperlipidemia. *Drugs Affecting Lipid Metabolism congress*, September 2001, New York, USA.

Keulen ETP, Kruijschoop M, Schaper NC, Hoeks APG, de Bruin TWA. Increased intima media thickness in Familial Combined Hyperlipidemia is associated with increased non-HDL cholesterol and apolipoprotein B concentrations. *Drugs Affecting Lipid Metabolism congress*, September 2001, New York, USA.

Dankwoord

Dankwoord

Nu ik aan het eind van dit boekje gekomen ben, rest mij nog één taak: eenieder bedanken die heeft bijgedragen aan de totstandkoming ervan. Dankzij de inspanningen en bijdragen van velen kon dit proefschrift worden gerealiseerd. Daarom wil ik op de laatste pagina's van dit boekje een aantal mensen, in willekeurige volgorde, van harte bedanken:

Allereerst een speciaal woord van dank aan alle deelnemers uit de FGH-families. Zonder de voortdurende bereidwilligheid van velen om weer aan een volgend onderzoek deel te nemen, was de totstandkoming van dit boekje niet mogelijk geweest. De diverse verhalen en anekdotes uit de verschillende families die ons ter ore kwamen, verstevigden de band tussen onderzoekers en proefpersonen. De hartelijke ontvangst tijdens diverse huisbezoeken zal ik nooit vergeten.

Mijn promotor, Prof. Dr. T.W.A. de Bruin, beste Tjerk, jouw enthousiasme voor het FGH-onderzoek en jouw verhuizing van Utrecht naar Maastricht, boden mij de mogelijkheid terug te keren naar het Limburgse grondgebied. Ons niet direct realiserend hoeveel tijd het in kaart brengen van nieuwe FGH-families zou kosten, is het, mede door jouw inzet en enthousiaste begeleiding, toch gelukt om diverse vasculaire gebieden bij FGH-personen in kaart te brengen. Met name jouw onnavolgbare manier om diverse mogelijke oorzaken en gevolgen van gevonden afwijkingen te combineren, hebben onze artikelen een extra dimensie gegeven.

Met name wil ik Josephine van Lin noemen. Josephine, zonder jouw onaflatende inzet was het nooit mogelijk geweest alle FGH-families nauwkeurig in kaart te brengen. Vele twijfelaars werden, na grondige uitleg, door jou over de streep getrokken om toch aan de diverse onderzoeken deel te nemen. Met grote precisie bereidde je alle studies voor en een half woord was vaak al genoeg. Met plezier denk ik terug aan onze vele autoritten naar Noord-Limburg. Josephine, ik hoop dat je als paranimf deze promotie ook een beetje als jouw promotie wilt beschouwen.

Bij dit alles mogen de student-assistenten Kim en Isabelle niet vergeten worden. Kim en Isabelle, jullie hebben beiden gedurende ruim anderhalf jaar vele screeningen voor jullie rekening genomen. De vele hulp bij de diverse onderzoeken heb ik zeer op prijs gesteld. De vele verhalen over jullie nieuwe vriendjes zal ik niet vergeten. Wellicht tot ziens als toekomstige collega's.

Alle medewerkers, oud-medewerkers en student-assistenten van het Laboratorium Moleculair Metabolisme en Endocrinologie en Laboratorium Endocrinologie: Carla, Marleen, Vicky, Margee, Jan, Rob, Freek, Ellen, Wim, Paul, Geertje, Gabriëlle, Jeroen, Esther, Marsha, Kirsten, Liesbeth, Sabien,

Angèle en Martijn. Ik wil jullie bedanken voor de gezellige tijd op het lab, de vele honderden bepalingen en mijn inwijdingen in het moleculair en genetisch werk.

Mijn mede-AIO's Petra, Christine, Margriet, Mireille, Cyriel, Marringje, Wilko, Esther, Kim en Jörgen. Vele uren op het lab. Vind ik genoeg proefpersonen? Krijg ik wel goede resultaten? Samen een doel, velen nog hard zwoegend, sommigen al klaar. Het eindpunt is mooi!

Dr. N.C. Schaper, beste Nicolaas, gezien jouw expertise in diabetes mellitus, en met name de diabetisch voet, werden tijdens onze besprekingen waar mogelijk steeds parallellen getrokken tussen FGH en diabetes. Beide dezelfde insuline resistente grondslag, maar toch zo verschillend. Onze verschillende insteek in de insuline resistentie heeft mijn kennis over mogelijke pathofysiologische mechanismen binnen het metabole syndroom sterk verbreid. Met name het microcirculatie onderzoek was één van jouw stokpaardjes, die we tot een goed einde hebben weten te brengen.

Alle leden van de vakgroep Interne Geneeskunde en met name de werkgroep Endocrinologie wil ik van harte bedanken voor het attenderen van mogelijke FGH probands op ons onderzoek en de goede samenwerking.

Claudia, Monique en Dorien van het circulatie-lab wil ik bedanken voor het soepele inplannen van alle onderzoeken en het gebruik van de diverse onderzoekskamers. De ondersteuning van Boy bij het microcirculatie onderzoek wordt zeer op prijs gesteld.

Dr. G.M. Dallinga-Thie, beste Geesje, jouw remnant-like lipoprotein particles (RLP) metingen voegden een extra dimensie toe aan het microcirculatie onderzoek en hield de band tussen Maastricht en Utrecht staande.

Carina van Zandvoort-Pernot, René van Oerle en Dave Hellenbrand, medewerkers van het lab speciële stolling, worden bedankt voor alle 'citraat'-bepalingen waardoor de pro-coagulante, anti-fibrinolytische status van FGH kon worden beschreven. Martin Nyziel wordt bedankt voor het attenderen op de mogelijkheden van stollingsonderzoek bij personen met FGH. Dr. K. Hamalyuk wil ik bedanken voor de hulp en het commentaar bij de opmaak van het stollingsartikel. De link tussen FGH en stolling/fibrinolyse zal in de toekomst meerdere gezamenlijke projecten tot gevolg hebben.

Prof. Dr. A.P.G. Hoeks, beste Arnold, bedankt voor de geboden mogelijkheden intima media dikte metingen te verrichten bij de FGH personen. De technische ondersteuning van Jean Willigers wordt zeer op prijs gesteld.

De goede samenwerking met Dr. Rob van Dijk, Dr. Edith Feskens en Dr. Marga Ocke, van het RIVM, zal in de toekomst hopelijk vervolgd worden. De resultaten van alle voedingsboekjes zullen nog verder geanalyseerd worden.

Prof. Dr. H. Jansen en mevrouw A.J. Zonneveld ben ik erkentelijk voor de LPL en HL bepalingen. De resultaten van de post-heparine belasting zullen nog verder worden uitgewerkt.

Zonder de inzet van Ruud Schmeitz, Jacqueline Pisters, Trudie Zeegers en Gregor Franssen medewerkers van het MEMIC, was de realisatie van een uitgebreide, gekoppelde en centrale FGH database niet mogelijk geweest.

Alle leden van de promotie commissie: Prof. Dr. P.W. de Leeuw, Prof. Dr. H.J.G.M. Crijns, Prof. Dr. M.J.A.P. Daemen, Prof. Dr. D.E. Grobbee en Prof. Dr. C.D.A. Stehouwer wil ik bedanken voor hun commentaar op en het goedkeuren van mijn manuscript.

Prof. Dr. H.F.P. Hillen (Academisch Ziekenhuis Maastricht) en Dr. B.J. Looij (Maasland Ziekenhuis Sittard) wil ik bedanken voor de geboden mogelijkheid mij verder te bekwamen binnen de interne geneeskunde. Prof. Dr. H.F.P. Hillen wordt tevens bedankt voor alle ondersteuning.

Een speciaal woord nog voor Dr. William van Houtum, mijn paranimf. Beste William, in 1990 zijn we samen begonnen aan de studie geneeskunde in Amsterdam. Onze wegen verliepen lange tijd parallel met vele uren gezamenlijk studeren, eten, computeren en snookeren. De eerste stappen in het onderzoek brachten je naar Texas, de mijne begonnen bij jouw latere promotor Prof. Dr. Rob Heine. Na ons artsexamen had jij genoeg artikelen verzameld om al snel te promoveren en dus vervolgde jij je weg via een AGNIO-schap naar de opleiding tot internist in Alkmaar en Leiden. Ik vervolgde mijn weg middels een promotie-onderzoek in Maastricht. Nu, drie jaar na jou, zal ook ik mijn promotieboekje verdedigen en mijn weg tot internist vervolgen. Door de afstand is het contact noodgedwongen wat minder, maar de vriendschap is onveranderd. Het schaken per e-mail moet in de toekomst nog op gang komen. Bedankt dat je mijn paranimf wilt zijn.

Zoals bij zovele voorgangers is Tiny Wouters onmisbaar voor het perfect redigeren van dit proefschrift. Beste Tiny, bedankt voor al je ondersteuning en "De Greef" kan zich opmaken voor een feestje!

Dimitri Delnoye ben ik zeer erkentelijk voor de mooi ontworpen kafft. Een grote neven- en nichtenschare met veel aanhang biedt vele voordelen.

Lieve pa en ma, jullie hebben dit alles mogelijk gemaakt. Ik kon gaan en staan waar ik wilde, kon gaan studeren en op jullie steun kon ik altijd rekenen. Ook nu jullie eerste kleinkind er is, zijn jullie, net zoals mijn schoonouders, altijd bereid waar nodig bij te springen. Bedankt voor alles.

Lieve Bianca, je hebt me al het mooiste gegeven wat ik me had kunnen wensen. Zonder jouw steun en geduld was dit boekje niet mogelijk geweest. Volgens jou was ik sinds de geboorte van Eva vaak thuis aan het werk. Nu de opleiding tot

internist is begonnen, zal dit minder vaak voorkomen en zal er wat meer op jouw schouders terecht gaan komen. Maar al mijn vrije tijd besteed ik aan jullie.

Lieve Eva, papa's meisje: een glimlach van jou verdrijft alle vermoeidheid en stelt onderzoek en werk in een schaduw.

Curriculum Vitae

Curriculum Vitae

Eric Keulen werd op 31 maart 1972 geboren te Geleen. Na het behalen van het Atheneum diploma aan de Albert Schweitzer Scholengemeenschap (thans Graaf Huyn College) te Geleen, begon hij in 1990 aan de studie geneeskunde aan de Vrije Universiteit te Amsterdam. In 1994, tijdens het vierde jaar van de doctoraalfase begon hij zijn wetenschapsstage op de afdeling Diabetologie van het Academisch Ziekenhuis der Vrije Universiteit (AZVU) te Amsterdam o.l.v. prof. Dr. RJ Heine. Deze eerste aanraking met klinisch wetenschappelijk onderzoek resulteerde in anderhalf jaar durende onderzoeksperiode op diverse plaatsen binnen het diabetes onderzoek in het AZVU en in Hoorn. In augustus 1997 werd het artsexamen cum laude behaald.

In oktober 1997 begon hij, o.l.v. prof. Dr. TWA de Bruin als assistent in opleiding aan zijn promotie onderzoek getiteld 'cardiovascular abnormalities in Familial Combined Hyperlipidemia', waarvan de resultaten in dit boekwerk zijn beschreven. In maart 2000 schonk zijn vrouw Bianca hem een wolk van een dochter, Eva genaamd. In oktober 2001 is hij gestart met de opleiding interne geneeskunde in het Maasland ziekenhuis te Sittard, opleider Dr. BJ Looij.