The effect of statin therapy on vessel wall properties in type 2 diabetes without manifest cardiovascular disease

Beishuizen, E.D.

The effect of statin therapy on vessel wall properties in type 2 diabetes without manifest cardiovascular disease

Edith Dorothé Beishuizen

ISBN 978-90-8559-458-1

Omslag en ontwerp: José Beishuizen-Tabak

Omslag, layout en druk: Optima Grafische Communicatie, Rotterdam

De hoofdsponsoren voor de studies beschreven in dit proefschrift zijn:
Bayer BV, Mijdrecht, Nederland
Merck Sharp & Dohme B.V., Haarlem, Nederland
Pfizer bv, Capelle a/d IJssel, Nederland

De druk van dit proefschrift werd gedeeltelijk gefinancierd door:
AstraZeneca BV; Boehringer Ingelheim bv; Eli Lilly Nederland BV; Merck Sharp & Dohme B.V.; Novartis Pharma B.V.; Novo Nordisk B.V.; Pfizer bv; Schering-Plough BV; Servier Nederland Farma B.V.

The effect of statin therapy on vessel wall properties in type 2 diabetes without manifest cardiovascular disease

PROEFSCHRIFT

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van de Rector Magnificus prof.mr.P.F. van der Heijden,
volgens besluit van het College voor Promoties
te verdedigen op donderdag 4 december 2008
klokke 15.00 uur

door

Edith Dorothé Beishuizen

geboren te Amsterdam
in 1966

Promotiecommissie

Promotores:	Prof.dr.A.E. Meinders
	Prof.dr.J.W. Jukema
Co-promotores:	Dr.M.V. Huisman
	Dr.J.T. Tamsma
Referent:	Prof.dr.J.B.L. Hoekstra
Overige leden:	Prof.dr.J.A. Romijn
	Prof.dr.A.J. Rabelink
	Dr.J.C.M. van der Vijver , HAGA Ziekenhuis, locatie Leyenburg, Den Haag

Voor mijn ouders

Contents

ABBREVIATIONS

AECG	Ambulatory Electrocardiogram
AI	Asian Indian
AIX	Augmentation Index
ALT	Alanine Aminotransferase
BMI	Body Mass Index
CAC	Coronary Artery Calcium
CAD	Coronary Artery Disease
CCA	Common Carotid Artery
CE	Coronary Events
CIMT	Carotid Intima-media Thickness
CRP	C-Reactive Protein
CTAD	citrate, theophyline, adenosine, dipyridamol
CVD	Cardiovascular Disease
DM2	type 2 Diabetes Mellitus
EC	Europid Caucasian
EIA	Enzyme Immuno Assay
FMD	Flow Mediated Dilation
HDL	High density lipoprotein
LDL	Low density lipoprotein
IMT	Intima-media Thickness
MPI	Myocardial Perfusion Imaging
MS	Metabolic Syndrome
MSCT	Multi-Slice Computed Tomography
NMD	Nitroglycerin Mediated Dilation
NO	Nitric Oxide
PAI-1	Plasminogen Activator Inhibitor-1
PWV	Pulse Wave Velocity
QTc	QT interval corrected for heart rate
SE	Stress Echocardiography
SMI	Silent Myocardial Ischemia
SPECT	single photon emission computed tomography
Statins	HydroxyMethylGlutaryl coenzyme A reductase inhibitors
sTM	s-Thrombomodulin
tPA	tissue type Plasminogen Activator
VLDL	Very low density lipoprotein
VWF	von Willebrand Factor

Chapter 1

Introduction

INTRODUCTION

Cardiovascular disease (CVD) is the principal cause of mortality in patients with type 2 diabetes mellitus (DM2). CVD was the cause of death in approximately 60 % of the diabetic subjects in the 12 year follow-up in MRFIT[1]. In the 22 year follow-up of NHANES 1, heart disease was the cause of death in 69.5 % of the diabetic subjects[2]. The relative risk of death for diabetic versus non-diabetic subjects is reported to vary between 1.5 and 4 and is highest in women[2,3]. Patients with diabetes but without a prior myocardial infarction have a similar 7- and 18-year incidence of myocardial infarction compared to those with prior myocardial infarction but without diabetes[3-5]. These studies have lead to the expression of diabetes as a "coronary heart disease equivalent". Apart from the higher incidence rates of cardiovascular events, diabetic patients also have a worse outcome after a first myocardial infarction[6].

Before CVD becomes manifest as angina pectoris, myocardial infarction, claudicatio intermittens or stroke, years of progressive atherosclerotic plaque formation may have preceded. Endothelial dysfunction precedes the development of atherosclerotic plaques. The actual cardiovascular event is not always the result of a narrowing arterial lumen caused by progressive plaque formation, but can be the result of an instable plaque rupture where damage to the endothelium triggers a cascade of thrombotic and inflammatory factors. Both endothelial dysfunction, atherosclerotic plaque formation and the process of plaque rupture is enhanced in patients with DM2 and more extensive atherosclerotic lesions are found at a first cardiac event[7]. The underlying mechanisms for the accelerated atherosclerotic process in DM2 are complex and related to hyperglycemia, insulin resistance and coexisting hypertension, dyslipidemia, and obesity. One of the effects of hyperglycemia is increased oxidative stress, hereby impairing endothelial function and beta cell function, so-called "glucose toxicity". Moreover, advanced glycation endproducts are formed with detrimental effects on endothelial function. Visceral obesity results in increased levels of free fatty acids and inhibition of insulin action.This insulin resistance in relation to obesity comprises a complex change towards a more pro-inflammatory and hypercoagulable state. Insulin resistance, high levels of free fatty acids and thereby increased very low density lipoprotein(VLDL) production and impaired VLDL clearance lead to the typical diabetic dyslipidemia: low levels of high density lipoprotein (HDL) cholesterol, high triglycerides and small, dense and therefore atherogenic low density lipoprotein (LDL) particles. LDL cholesterol is an important risk factor for coronary artery disease in DM2[1,8].

With this new insight in the risk factors for the accelerated process of atherosclerosis in DM2, numerous trials have been designed to investigate the effect of risk factor modification on the incidence of CVD in DM2.

In the UKPDS, treatment of hyperglycemia had a modest effect on cardiovascular morbidity and mortality[9,10]. Blood pressure lowering regimens, however, have lead to a 34 % risk

reduction in cardiovascular endpoints in the same UKPDS study[11,12]. Numerous other studies underscore the importance of tight blood pressure control in DM2[13].

In 1994, the first landmark trial with simvastatin, a HydroxyMethylGlutaryl coenzyme A reductase inhibitor (statin), was published[14]. In this study, a 34 % relative risk reduction in major coronary events in patients with a history of myocardial infarction was shown. In the 4S study only 202 patients with diabetes on a total of 4444 were enrolled. In spite of these low numbers, the risk reductions for the diabetic subgroup were even more pronounced compared with non-diabetics with a 55 % versus 32 % relative risk reduction for major coronary events[15]. In the CARE study, an other secondary prevention trial comparing 5 years of pravastatin 40 mg versus placebo, 586 of the 4159 patients had DM2. In this diabetic subgroup, the relative risk reduction in coronary events was 25 % versus 23 % in the non-diabetic group[16]. In the LIPID study [17] the risk reductions in the diabetic subgroup were non-significant.

In two primary prevention studies, the WOSCOPS and the AFCAPS/TexCAPS, the number of diabetic patients was too small to draw conclusions[18,19]; moreover, in AFCAPS/TexCAPS, use of insulin was an exclusion criterion. ALLHAT-LLA and ASCOT-LLA were the lipid lowering arms of primary prevention trials in hypertension. In ALLHAT-LLA no beneficial effect was seen of pravastatin 40 mg in the total and the diabetic subgroup[20], in ASCOT-LLA atorvastatin 10 mg lead to a 36 % risk reduction of major coronary events, leading to a premature termination of this trial arm. There was no significant risk reduction in the subgroup with DM 2[21].

The Heart Protection Study included diabetes as a prespecified subgroup[22]. In this study subjects with coronary artery disease, DM2 or other risk factors were randomized to simvastatin 40 mg or placebo. In the diabetic subgroup the relative risk reduction for first major vascular event was 33 % in primary prevention. The CARDS, published in 2004, has been the only study to investigate the effect of statin therapy in primary prevention in patients with DM2[23]. Included patients had to have at least one additional risk factor (smoking, hypertensiom, albuminuria or retinopathy) for CVD. The trial was prematurely terminated because of a relative risk reduction for major cardiovascular events of 37 %.

The results of these trials have been translated into new guidelines in which strict glycaemic and blood pressure control, and the use of statins is advocated[24]. The LDL target for statin therapy is related to the absolute risk of the diabetic patient. These LDL goals are based on the assumption that the beneficial effect of statin therapy is solely caused by reduction in LDL cholesterol, "lower is better"[25]. The background for these assumptions arises from the PROVE-IT and REVERSAL studies where pravastatin 40 mg was compared to atorvastatin 80 mg in secondary prevention. In both studies, PROVE-IT being a clinical endpoint study and REVERSAL using intravascular ultrasound to measure atheroma volume, atorvastatin 80 mg was superior to the less intensive regimen with pravastatin.

Others question this emphasis on maximal LDL cholesterol reduction as statins also have an effect on markers of inflammation, coagulation, fibrinolysis, immunomodulation and endothelial function independent of lowering of LDL cholesterol[26,27]. These modes of ac-

tions might lead to plaque stabilisation in coronary artery disease, improved left ventricular ejection fraction in nonischemic dilated cardiomyopathy and reduction of stroke incidence shortly after initiation of statin therapy. To date it remains unsolved however, whether these other, nonlipid ("pleiotropic") effects on the vascular wall play an important role in the risk reductions as seen in the clinical trials[28,29].

AIMS AND OUTLINE OF THE THESIS

The present thesis describes a study, designed at a timepoint when no primary prevention trial had yet been performed to investigate the effects of statin therapy on patients with DM2 without manifest CVD. We have performed a randomized, double-blind placebo-controlled trial in 250 DM2 patients without manifest CVD. The aim of the study was to study non-invasively the effect of two year statin therapy on the vessel wall. Chapter 2 describes the technique and the current status of the non-invasive vascular tools used in this thesis, as well as more recent developed techniques.

Our *first* aim was to determine the effect of statin therapy on the progress of atherosclerosis, as measured non-invasively by ultrasonographic measurements of the intima-media thickness (IMT) of the carotid and femoral arteries (Chapter 3)

Our *second* aim was to study the effect of statin therapy on endothelial function as measured non-invasively with flow mediated dilation (FMD) (Chapter 4).

Our *third* aim was to analyse the effect of statin therapy on C-reactive protein (CRP), a marker of the inflammatory process in atherosclerotic plaques (Chapter 5). We further elaborated the role of inflammatory markers in relation to the metabolic syndrome. For this purpose we analyzed the data from the DALI study (Chapter 6). The DALI study was performed to evaluate the efficacy of atorvastatin 10 and 80 mg versus placebo in patients with DM2 and mild dyslipidemia without CVD. Endpoints in the original study were lipid parameters and endothelial function as assessed by FMD[30]. In the present substudy the baseline laboratory parameters for inflammation and hemostasis and the baseline sonographic parameters IMT and FMD were used to assess the impact of the metabolic syndrome and low grade chronic inflammation as assessed by CRP on vascular phenotype in DM2.

As we performed the main study in an area with a large community of Asian Indians from Surinam, we were not surprised that 19% of the included subjects were Asian Indians. As epidemiological data reveal a high and partly unexplained risk of DM2 and CVD in this population[31], we wanted to evaluate conventional risk factors and the above mentioned vascular parameters separately for this population. Therefore, our *fourth* aim was to produce a vascular phenotype of the Asian Indian population (Chapter 7).

Periods of silent myocardial ischemia can precede a cardiac event in DM2, with a possible etiological role for cardiac autonomic neuropathy. Our *fifth* aim was therefore to determine

the prevalence of silent myocardial ischemia in these patients and to evaluate the effect of statin therapy on silent myocardial ischemia (Chapter 8).

In Chapter 9 the results of these studies are discussed and summarized.

REFERENCES

1. Stamler J, Vaccaro O, Neaton JD, Wentworth D: Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care* 16:434-444, 1993
2. Gu K, Cowie CC, Harris MI: Mortality in adults with and without diabetes in a national cohort of the U.S. population, 1971-1993. *Diabetes Care* 21:1138-1145, 1998
3. Hu FB, Stampfer MJ, Solomon CG, Liu S, Willett WC, Speizer FE, Nathan DM, Manson JE: The impact of diabetes mellitus on mortality from all causes and coronary heart disease in women: 20 years of follow-up. *Arch.Intern.Med.* 161:1717-1723, 2001
4. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M: Mortality from coronary heart disease in subjects with type 2 diabetes and in non-diabetic subjects with and without prior myocardial infarction. *N.Engl.J.Med.* 339:229-234, 1998
5. Juutilainen A, Lehto S, Ronnemaa T, Pyorala K, Laakso M: Type 2 diabetes as a "coronary heart disease equivalent": an 18-year prospective population-based study in Finnish subjects. *Diabetes Care* 28:2901-2907, 2005
6. Miettinen H, Lehto S, Salomaa V, Mahonen M, Niemela M, Haffner SM, Pyorala K, Tuomilehto J: Impact of diabetes on mortality after the first myocardial infarction. The FINMONICA Myocardial Infarction Register Study Group. *Diabetes Care* 21:69-75, 1998
7. Influence of diabetes on 5-year mortality and morbidity in a randomized trial comparing CABG and PTCA in patients with multivessel disease: the Bypass Angioplasty Revascularization Investigation (BARI). *Circulation* 96:1761-1769, 1997
8. 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). *BMJ* 316:823-828, 1998
9. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 352:854-865, 1998
10. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 352:837-853, 1998
11. Efficacy of atenolol and captopril in reducing risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 39. UK Prospective Diabetes Study Group. *BMJ* 317:713-720, 1998
12. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. UK Prospective Diabetes Study Group. *BMJ* 317:703-713, 1998
13. Hovens MM, Tamsma JT, Beishuizen ED, Huisman MV: Pharmacological strategies to reduce cardiovascular risk in type 2 diabetes mellitus: an update. *Drugs* 65:433-445, 2005
14. Randomized trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 344:1383-1389, 1994
15. Pyorala K, Pedersen TR, Kjekshus J, Faergeman O, Olsson AG, Thorgeirsson G: Cholesterol lowering with simvastatin improves prognosis of diabetic patients with coronary heart disease. A subgroup analysis of the Scandinavian Simvastatin Survival Study (4S). *Diabetes Care* 20:614-620, 1997
16. Goldberg RB, Mellies MJ, Sacks FM, Moye LA, Howard BV, Howard WJ, Davis BR, Cole TG, Pfeffer MA, Braunwald E: Cardiovascular events and their reduction with pravastatin in diabetic and glucose-intolerant myocardial infarction survivors with average cholesterol levels: subgroup analyses in the cholesterol and recurrent events (CARE) trial. The Care Investigators. *Circulation* 98:2513-2519, 1998

17. Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. The Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group. *N.Engl.J.Med.* 339:1349-1357, 1998

18. Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, MacFarlane PW, McKillop JH, Packard CJ: Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. *N.Engl.J.Med.* 333:1301-1307, 1995

19. Downs JR, Clearfield M, Weis S, Whitney E, Shapiro DR, Beere PA, Langendorfer A, Stein EA, Kruyer W, Gotto AM, Jr.: Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. Air Force/Texas Coronary Atherosclerosis Prevention Study. *JAMA* 279:1615-1622, 1998

20. Major outcomes in moderately hypercholesterolemic, hypertensive patients randomized to pravastatin vs usual care: The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT-LLT). *JAMA* 288:2998-3007, 2002

21. Sever PS, Dahlof B, Poulter NR, Wedel H, Beevers G, Caulfield M, Collins R, Kjeldsen SE, Kristinsson A, McInnes GT, Mehlsen J, Nieminen M, O'Brien E, Ostergren J: Prevention of coronary and stroke events with atorvastatin in hypertensive patients who have average or lower-than-average cholesterol concentrations, in the Anglo-Scandinavian Cardiac Outcomes Trial--Lipid Lowering Arm (ASCOT-LLA): a multicentre randomized controlled trial. *Lancet* 361:1149-1158, 2003

22. Collins R, Armitage J, Parish S, Sleigh P, Peto R: MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomized placebo-controlled trial. *Lancet* 361:2005-2016, 2003

23. Nissen SE, Tuzcu EM, Schoenhagen P, Brown BG, Ganz P, Vogel RA, Crowe T, Howard G, Cooper CJ, Brodie B, Grines CL, DeMaria AN: Effect of intensive compared with moderate lipid-lowering therapy on progression of coronary atherosclerosis: a randomized controlled trial. *JAMA* 291:1071-1080, 2004

24. Buse JB, Ginsberg HN, Bakris GL, Clark NG, Costa F, Eckel R, Fonseca V, Gerstein HC, Grundy S, Nesto RW, Pignone MP, Plutzky J, Porte D, Redberg R, Stitzel KF, Stone NJ: Primary prevention of cardiovascular diseases in people with diabetes mellitus: a scientific statement from the American Heart Association and the American Diabetes Association. *Diabetes Care* 30:162-172, 2007

25. Cannon CP: The IDEAL cholesterol: lower is better. *JAMA* 294:2492-2494, 2005

26. Jasinska M, Owczarek J, Orszulak-Michalak D: Statins: a new insight into their mechanisms of action and consequent pleiotropic effects. *Pharmacol.Rep.* 59:483-499, 2007

27. Davignon J: Beneficial cardiovascular pleiotropic effects of statins. *Circulation* 109:III39-III43, 2004

28. Davidson MH: Clinical significance of statin pleiotropic effects: hypotheses versus evidence. *Circulation* 111:2280-2281, 2005

29. Robinson JG, Smith B, Maheshwari N, Schrott H: Pleiotropic effects of statins: benefit beyond cholesterol reduction? A meta-regression analysis. *J.Am.Coll.Cardiol.* 46:1855-1862, 2005

30. van Venrooij FV, van de Ree MA, Bots ML, Stolk RP, Huisman MV, Banga JD: Aggressive lipid lowering does not improve endothelial function in type 2 diabetes: the Diabetes Atorvastatin Lipid Intervention (DALI) Study: a randomized, double-blind, placebo-controlled trial. *Diabetes Care* 25:1211-1216, 2002

31. Bongers I, Westendorp RG, Stolk B, Huysmans HA, Vandenbroucke JP: [Early coronary heart disease together with type II diabetes mellitus in persons of Hindustani origin]. *Ned.Tijdschr.Geneeskd.* 139:16-18, 1995

Chapter 2

Non-invasive cardiac imaging techniques and vascular tools for the assessment of cardiovascular disease in type 2 Diabetes Mellitus

**R Djaberi[1], **ED Beishuizen[2], AM Pereira[2], AJ Rabelink[3], JW Smit [2], JT Tamsma[2], MV Huisman[2], JW Jukema[1,4].

** Both authors contributed equally.

[1] Department of Cardiology, Leiden University Medical Center

[2] Department of General Internal Medicine and Endocrinology, Leiden University Medical Center

[3] Department of Nephrology, Leiden University Medical Center,

[4] Eindhoven Laboratory of Experimental Vascular Medicine

The Netherlands

Diabetologia 2008; 51:1581-1593

ABSTRACT

Cardiovascular disease is the major cause of mortality in type 2 diabetes mellitus (DM2). The criteria for the selection of those asymptomatic patients with DM2 who should undergo cardiac screening and the therapeutic consequences of screening remain controversial.

Non-invasive techniques as markers of atherosclerosis and myocardial ischemia may aid risk stratification and the implementation of tailored therapy for the individual patient with DM2. In the present article we review the literature on the implementation of non-invasive vascular tools and cardiac imaging techniques in this patient group. The value of these techniques as endpoints in clinical trials and as risk estimators in asymptomatic diabetic patients is discussed.

Carotid intima-media thickness, arterial stiffness and flow mediated dilation are abnormal long before the onset of DM2. These vascular tools are therefore most likely to be useful in identification of 'at risk' patients in early stages of atherosclerotic disease. The additional value of these tools in risk stratification and tailored therapy in DM2 remains to be proven.

Cardiac imaging techniques are more justified in individuals with a strong clinical suspicion of advanced coronary artery disease (CAD). Asymptomatic myocardial ischemia can be detected by stress echocardiography and myocardial perfusion imaging. The more recently developed non-invasive multi-slice computed tomography angiography is recommended for exclusion of CAD, and can therefore be used to screen asymptomatic patients with DM2, but has the associated disadvantages of high radiation exposure and costs. Therefore, we propose an algorithm for the screening of asymptomatic diabetic patients, the first step of which consists of coronary artery calcium score assessment and exercise ECG.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of mortality in DM2[1]. Current guidelines on the treatment of dyslipidemia and hypertension in diabetes recommend rigorous *primary* prevention with target lipid and blood pressure levels similar to those used for secondary prevention in non-diabetic patients[2]. To date, there is much debate as to whether all diabetic patients will benefit from this strategy and whether risk stratification should be attempted.

Non-invasive imaging techniques as markers of atherosclerosis and myocardial ischemia may help risk stratification and the implementation of tailored therapy for the individual patient. However, many of these tools have not been validated in diabetic individuals. In this article we will review the reproducibility and predictive value of the following *surrogate markers of atherosclerosis*: intima-media thickness (IMT), arterial stiffness and flow mediated dilation (FMD). We will discuss the diagnostic accuracy and predictive value of imaging techniques used for *direct anatomic assessment of coronary atherosclerosis***:** coronary artery calcium (CAC) scores and multi-slice Computed Tomography (MSCT) angiography, and *functional tests that detect myocardial ischemia:* ambulatory electrocardiography (AECG), exercise electrocardiography, stress echocardiography (SE) and nuclear myocardial perfusion imaging (MPI) by single photon emission computed tomography (SPECT). Finally, the value of these non-invasive techniques as endpoints in clinical trials and as risk estimators in diabetic patients will be discussed. We will concentrate on methods of risk stratification and the implementation of non-invasive techniques in patients with DM2, as the value of these techniques has scarcely been studied in type 1 diabetes.

SURROGATE MARKERS OF ATHEROSCLEROSIS

Carotid Intima-Media Thickness (CIMT)

Since its introduction in the early 1990s, intima-media thickness (IMT), especially carotid IMT (CIMT), has increasingly been used as a surrogate marker of atherosclerotic disease. IMT can be assessed non-invasively using B-mode ultrasound. Two approaches are used: 1) multiple measurements of CIMT in the near and far walls of the three main segments of carotid arteries (common carotid, bifurcation and internal carotid); and 2) automated computerized measurement of CIMT restricted to the far wall of the distal common carotid artery. Computerized measurement of CIMT is superior in terms of precision and reproducibility, with an approximately 3% difference between two successive measurements [3]. As a result, common CIMT has become a valid tool for large-scale multicenter studies. However, the common carotid artery is less likely to have intrusive plaque than the bifurcation and internal segments of the carotid arteries.

CIMT correlates with prevalent CVD and with risk factors for CVD [4]. In prospective studies, CIMT has proven to be a consistent and independent predictor for coronary events (CE) and stroke in the general population [5-6].

Carotid Intima-Media Thickness in DM2

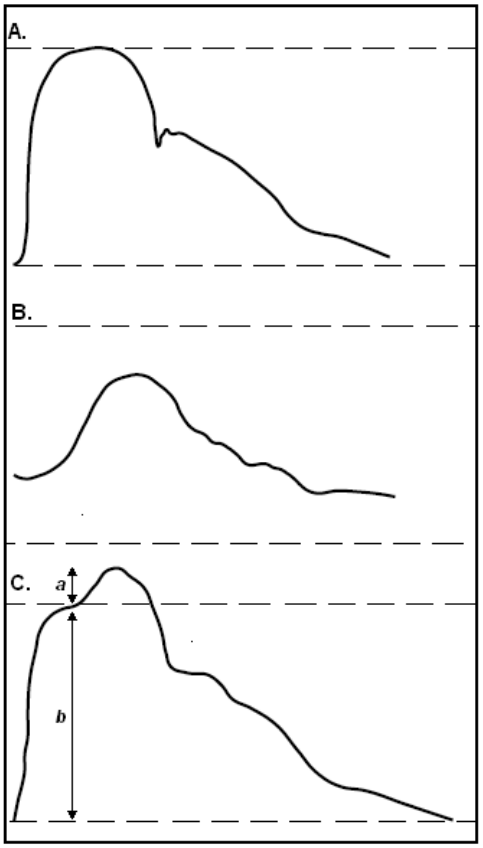
Mean common CIMT in middle aged individuals is reported to range from 0.71-0.98 mm in diabetic patients versus 0.66 – 0.85 mm in control patients [7-9]. In diabetic individuals without a history of myocardial infarction CIMT is similar to that in non-diabetic individuals with a history of myocardial infarction [9]. Progression of maximal CIMT in the IRAS study was twice as high in persons with diabetes versus controls [10], but other studies report lower rates [11]. In DM2, prevalent CVD is associated with higher CIMT [9]. In two prospective studies, baseline CIMT was shown to be an independent predictor of cardiovascular events [12-13]. However, when Folsom and colleagues analyzed CIMT in a large cohort with 1500 diabetic participants, they found that CIMT has predictive value for future CE only in combination with several other novel risk factors [14].

Arterial Stiffness

Whereas IMT is a marker of *structural* vessel wall properties, arterial stiffness reflects *functional* wall properties. Stiffness can be measured in many ways, including distensibility, pulse wave velocity (PWV) and augmentation index (AIx). Distensibility, defined as the change in arterial lumen diameter during the cardiac cycle, can be evaluated by ultrasound imaging using wall-tracking systems based on Doppler shift or using B- or M-Mode. The change in arterial diameter during the cardiac cycle varies by about 5-6% in middle-aged individuals[15]. PWV is the speed with which the arterial pressure wave progresses through the arterial tree and this increases with increasing vascular stiffness. The PWV can be determined either by placing a probe on two sites and recording the waveform simultaneously, or by recording the waveforms independently and comparing the time delay at both sites with a simultaneously measured QRS complex. PWV gradually increases with age, from about 4 m/sec in the third decade to 10 m/sec in the ninth decade. The AIx, which is the augmentation of aortal pressure as a percentage of pulse pressure, has also emerged as a parameter for arterial stiffness (Figure 1) [16-17]. Studies report excellent reproducibility of PWV, with a CV of approximately 3.2 %, which is lower than that for distensibility indices (CV 5.3%) or AIx (CV 10.1%) [17-19].

In cross-sectional studies, arterial stiffness is strongly associated with age and classical risk factors for CVD [15, 20-21], and it has been related to angiographic coronary atherosclerosis [17]. In a cohort of men aged > 70 years, baseline arterial distensibility predicted cardiovascular mortality during a two year follow-up, but added little to clinical risk estimation [22]. However, in a Danish population study, aortic PWV predicted a composite of cardiovascular events outcome above and beyond traditional risk factors [23].

Figure 1. The pulse pressure wave form.



A. The incident wave generated by the left ventricle (in ascending aorta). **B.** Waves reflected back from the peripheral vascular bed (ascending aorta). **C.** Resultant wave in the ascending aorta which is a combination of **A** and **B**. The augmentation index (AIx) is the measure of additional pressure to which the left ventricle is subjected as a result of wave reflection and is calculated as: $AIx = [\boldsymbol{a}/(\boldsymbol{b}+\boldsymbol{a})] \times 100$.

Arterial stiffness in DM2

Diabetic patients have increased arterial stiffness [17,24]. Compromised carotid distensibility and PWV have been demonstrated even before the onset of diabetes, in patients with impaired glucose tolerance. Healthy offspring of DM2 patients have a higher PWV than matched controls [17,25]. Arterial stiffness in DM2 is related to prevalent CVD [16] and has shown to be an independent predictor of CAD [26].

Baseline distensibility did not predict mortality in 140 indiviuals with impaired glucose tolerance during a follow-up period of 6.6 years [18]. Conversely, PWV does seem to have a reasonable predictive value for mortality in patients with impaired glucose tolerance and DM2 [24].

Flow Mediated Dilation (FMD)

Flow Mediated Dilation (FMD) of the brachial artery is a non-invasive technique for measuring endothelial function. FMD is measured with B-Mode ultrasound or a wall-track system. The brachial artery is visualized in the elbow, and by inflating a cuff (mostly distal to the elbow) for 4 minutes, hypoxia is created. After deflation, reactive hyperemia induces shear stress, thereby stimulating nitric oxide (NO) synthesis, resulting in NO dependent dilation [27]. FMD is thus defined as the percentage change in the diameter of the brachial artery after hypoxia, estimated to be 5-10% in healthy individuals. The observed brachial artery dilation has shown to be closely related to coronary vasoreactivity [28].

FMD fluctuates during the day and is influenced by the temperature, stress, diet, glucose levels and the menstrual cycle [29]. Within-subject variability of FMD is therefore often poor with CVs of 14-50% [29-30]. In spite of the biological variation, there is good intra- and interobserver reproducibility for measurements of baseline and maximum post-ischemia brachial artery (diameter variations approximately 4%) [30].

FMD ranges from about 10% in young adults to 0% in patients with established CAD and it has proven to be predictive for the presence of CAD [31] and for future CE in high-risk populations [32]. High sensitivity and high negative predictive values were calculated using cut-off points of 8.1-10% [32]. FMD has not been independently associated with CE in patients at lower risk [33].

Flow Mediated Dilation in DM2

DM2 is associated with endothelial dysfunction. The underlying mechanisms are suspected to be related to hyperglycemia (sorbitol, hexosamine, PKC-, and AGE-pathways) and insulin resistance, which result in mitochondrial superoxide overproduction, and thus decreased NO availability [34-35]. Clustering of risk factors such as dyslipidemia, hypertension and obesity in the metabolic syndrome play an additional role. Insulin-mediated vasodilation is at least in part NO dependent, thus explaining how insulin resistance may cause endothelial dysfunction.

The predictive value of endothelial dysfunction in epicardial coronary arteries of diabetic patients has been established for long-term CE [36]. However, to our knowledge, no studies to date have evaluated the relationship between FMD and prediction of CE in DM2.

DIRECT ANATOMIC ASSESSMENT OF CORONARY ATHEROSCLEROSIS

Coronary Artery Calcium (CAC) scores

Anatomical and intravascular studies have illustrated that the presence of coronary calcium is indicative of coronary atherosclerosis [37]. Coronary calcification can be detected non-invasively by Electron Beam Computed Tomography (EBCT), and more recently by Multi-slice Computed

Tomography (MSCT). Agatston et al. developed a coronary calcium scoring algorithm based on calcification volume and density, that is now widely used in clinical practice [38]. The extent of coronary calcium increases with age, and is, on average, higher in men than in women [39-40].

Coronary Artery Calcium scores in DM2

Diabetic patients without manifest CVD have a higher CAC score than non-diabetic individuals, independent of classical risk factors [41-43]. In addition, CAC scores show significantly more progression over time in patients with DM2 than in non diabetic patients [44].

In a study by Raggi et al. 10,377 patients (903 with diabetes) were followed for a period of 5.0 ± 3.5 years after CAC imaging. Mortality increased with increasing baseline CAC levels for both diabetic and non diabetic individuals. However, despite similar CAC scores, there was a greater increase in mortality in diabetic than non-diabetic patients for every increase in CAC score [45]. The predictive value of CAC scores in diabetes has been questioned by Qu et al. who found no significant relationship between CE and CAC scores during a six year follow-up of 269 diabetic patients [46].

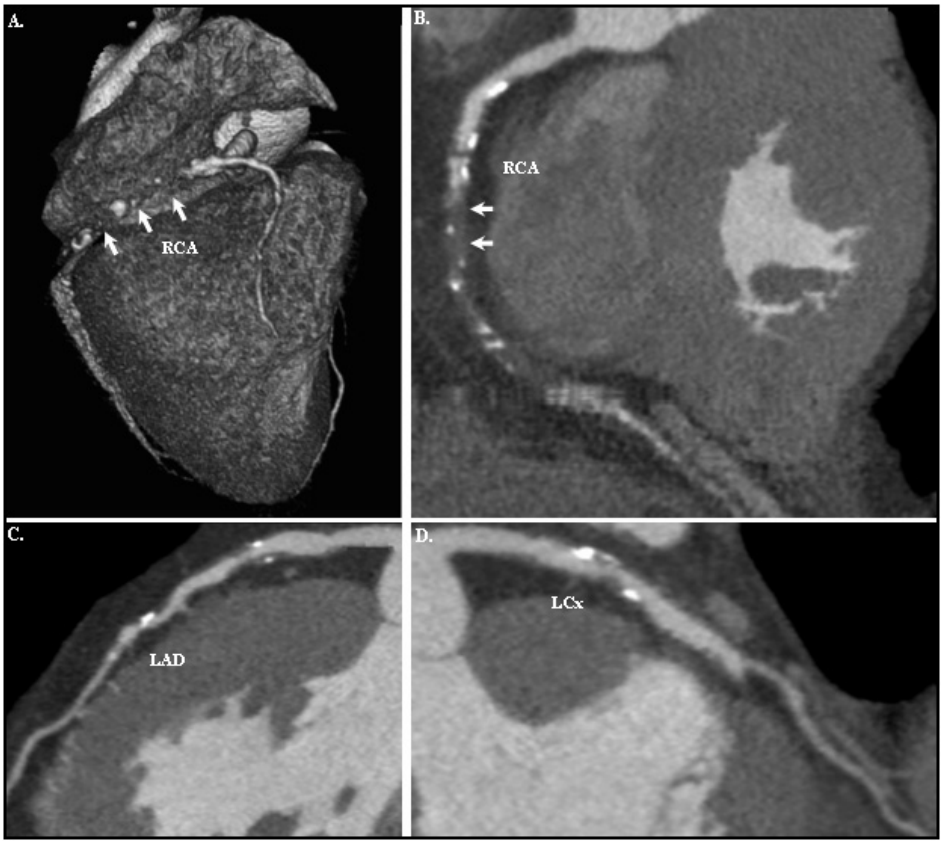
Multi-Slice Computed Tomography (MSCT) Coronary Angiography

The application of MSCT scanners for non-invasive coronary angiography has developed rapidly over recent years. Employment of 16 and 64 slice systems has demonstrated a sensitivity ranging from 83-99% and specificity of 93-98% [47-51]. Several studies have demonstrated that CT angiography has a high negative predictive value of 99% on average [47-51]. Therefore, the technique is currently most suited to exclude CAD.

Besides visualization of the coronary artery lumen (Figure 2), CT angiography allows the identification of non-stenotic atherosclerosis and various types of plaques. In addition, chronic myocardial infarction and left ventricular ejection fraction can be assessed. Non-stenotic atherosclerosis may prove to be a predictor of CE; however, this remains to be determined in prospective long-term clinical studies. Plaques can be classified as non-calcified, mixed or calcified. Initial comparisons have shown that calcification may represent the duration of atherosclerosis, whereas non-calcified and mixed lesions are more frequently observed in patients with an acute coronary syndrome [52].

MSCT is subject to a number of limitations, including exposure to a relatively high dose of radiation, currently in the range of 9-12 mSv [47,51], lower accuracy in the presence of severe calcification and movement artefacts, and limited application possibilities in case of irregular heart rate [49-51]. Taking the radiation exposure and the high negative predictive value of MSCT angiography into consideration, this technique is recommended for excluding CAD in patients of intermediate risk.

Figure 2. An asymptomatic patient with DM2 was screened for CAD using MSCT angiography.



A. the occluded right coronary artery (RCA) is easily visible using the three-dimensional volume rendering technique which provides an overview of coronary anatomy. Arrows indicate occlusion. **B.** Multiplanar reconstruction of the RCA gives a more precise overview of abnormalities. **C.** and **D.** multiplanar reconstruction of the left anterior descending (LAD) and left circumflex (LCx) coronary arteries.

MSCT Coronary Angiography in DM2

MSCT angiography has demonstrated a higher percentage of non-calcified and calcified plaques and a relatively lower percentage of mixed plaques in DM2[53], which can be explained by the rapid progression of atherosclerosis. Schuijf et al. have reported a sensitivity and specificity of 95% for detection of stenosis. Inclusion of uninterpretable segments reduced sensitivity and specificity to 81% and 82%, respectively [54]. In an evaluation on the diagnostic accuracy of 16 slice MSCT angiography, there were no statistically significant differences between the diabetic and non-diabetic individuals in the study population[55]. Importantly, negative predictive value of MSCT angiography in DM2 was found to be 98% and 100% on segmental and patient basis, respectively [55]. The prevalence of CAD has been assessed by

MSCT angiography in 70 asymptomatic patients with DM2. The majority of the patients (80%) had atherosclerosis (obstructive CAD (luminal narrowing ≥ 50%) in 26%, non-obstructive CAD in 54% of the patients) [56]. Thus, results on the use of non-invasive MSCT angiography for CAD screening and as a prognostic indicator in the diabetic population appear promising, but further studies in larger population groups are needed.

FUNCTIONAL TESTS IN ASSESSMENT OF CORONARY ARTERY DISEASE

Functional tests detect myocardial ischemia which is the physiologic consequence of coronary obstruction. These include: ambulatory ECG, exercise ECG, stress echocardiography and nuclear myocardial perfusion imaging.

Ambulatory ECG

It has been postulated that periods of silent myocardial ischemia (SMI), which can be detected with Ambulatory ECG (AECG), precede a first coronary event. AECG monitoring can be performed with a three-channel recording system for a continuous period of 48 hours. Transient myocardial ischemia is defined as the presence of episodes showing more than 0,1 mV (1mm) horizontal or downsloping ST-segment depression. The sensitivity of AECG for detecting CAD is poor, ranging from 19-62% [57-59]. Compared with coronary angiography, the specificity of AECG ranged from 54- 92% [57-60]. Frequent episodes of transient ischemia detected by AECG have shown to be a marker for an increased coronary event rate in asymptomatic middle-aged men and in patients with known CAD [61].

Ambulatory ECG in DM2

The prevalence of SMI as assessed by AECG in DM2 varies between 35-58% [62-64]. Although the prevalence of SMI determined by this method is expected to be higher in diabetic than non-diabetic individuals, findings have been inconsistent. Comparison of diabetic and non-diabetic patients in the ACIP study, illustrated lower rates of asymptomatic ischemia in DM2, despite more extensive and diffuse coronary disease in the latter group [65]. A study comparing exercise ECG with AECG for detection of SMI in DM2 reported that AECG identified ischemia only in diabetic patients with three-vessel disease whereas exercise ECG also revealed ischemia in one- and two-vessel disease [66]. In one study, patients with previously detected silent ischemia had a higher incidence of new CE (87%) than those with no silent ischemia (51%) during a 40 months follow-up period[63]. Further studies are needed to validate the prognostic value of SMI detected by AECG.

Exercise Electrocardiography (ECG)

The exercise ECG is considered positive for myocardial ischemia if horizontal downsloping or upsloping ST-segment depression of ≥ 0.1mV occurs at least 0.08 s after the J point**.** In a pooled meta-analysis of 24,074 patients who had undergone both an exercise ECG and conventional coronary angiography, mean sensitivity and specificity were calculated to be 68% and 77%, respectively [67]. Sensitivity was higher in three-vessel disease [67]. In addition to myocardial ischemia, the exercise ECG provides information on exercise capacity and hemodynamic response, which both have prognostic value [68].

The prognostic significance of exercise-induced myocardial ischemia has been evaluated in prospective studies [69-70]. In a population-based study, an average follow-up period of 10 years was completed in 1,769 asymptomatic men who had undergone an exercise ECG. The risks of acute CE and cardiac death were increased 1.7- and 3.5- fold, respectively, in men with SMI compared with men without SMI, after adjusting for conventional factors [69].

Exercise ECG in DM2

The use of an exercise ECG for diagnosing myocardial ischemia specifically in the setting of DM2 has not been assessed in large studies. In an evaluation of the correlation between the ECG exercise test and coronary angiography for the identification of significant coronary artery stenosis in 59 diabetic patients, the sensitivity and specificity were 75% and 77% respectively [71]. The mean positive predictive value of the exercise ECG for predicting angiographic coronary disease varies between 70% and 90% [72-73]. However, the test is often inconclusive or unfeasible in diabetic patients (approximately 32%) because exercise capability may be impaired by peripheral vascular or neuropathic disease [72]. Furthermore, the specificity of this method is lower for detecting significant CAD in DM2 because of the presence of microvascular disease.

Abnormal ECG stress tests have shown to be independent predictors of CE [74-75]. A 38 month follow-up of 262 asymptomatic diabetic patients who had undergone a maximal ECG stress test showed a good negative predictive value (97%) for major cardiac end points [74]. Gerson et al., showed that exercise ECG successfully identified all diabetic patients who developed clinical CAD within 50 months, but provided little prognostic information after the first 50 months, suggesting the need for serial testing [75].

Stress echocardiography

Stress Echocardiography (SE) is a well-established functional technique for assessing CAD that can be used to demonstrate inducible wall motion abnormalities in the general population. Exercise or a pharmacological form of stress can be used. In the case for the former, echocardiography is performed shortly after exercise. This method provides additional information on exercise capacity, symptoms and hemodynamic response, which are beneficial prognostic factors. A potential hindrance may be rapid resolution of ischemia after exercise,

and therefore normalization of any wall motion abnormality prior to echocardiography. Pharmacologically induced SE is preferred in those with a limited exercise capacity. An additional advantage is that images are obtained during stress. In a meta-analysis of 10,817 patients in which dobutamine was compared with stress testing with adenosine or dipyridamole, dobutamine echocardiography had the highest combination of sensitivity (80%) and specificity (84%) for the diagnosis of CAD[76]. The accuracy of the method is dependent on the degree of stenosis, the amount of myocardium at risk and the degree of induced wall motion abnormality [77]. False negative results are more likely with submaximal exercise (in the case of exercise-induced stress), single-vessel disease and moderate stenosis (50-70%) [78].

The presence of ischemia on SE and the number of ischemic segments predict the likelihood of CE during long-term follow-up in the general population with known or suspected CAD [79-80]. However, in a 10 year follow-up of 1,832 asymptomatic patients who underwent SE, exercise testing and a resting echocardiogram, SE did not offer additional prognostic information in terms of identifying patients at a higher risk of CE [81].

Stress echocardiography in DM2

The diagnostic accuracy of SE for significant CAD in DM2 has been verified in two studies. In one study in which 55 diabetic patients underwent dobutamine SE and invasive angiography, sensitivity and specificity of SE were 81% and 85%, respectively [82]. Another study that compared SE with coronary angiography in 52 DM2 patients reported a similar sensitivity (82%), but a much lower specificity (54%) [83].

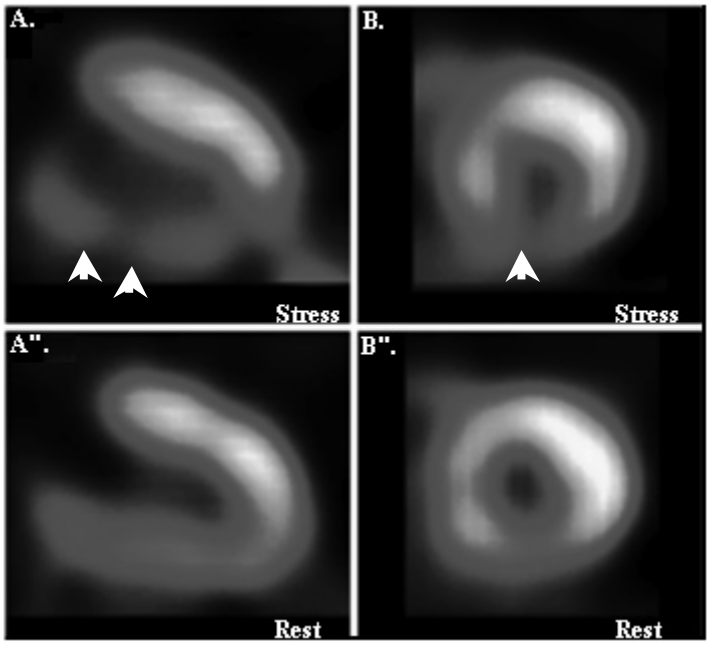
In a prospective study, SE plus an exercise ECG were used to screen 71 DM2 patients with unknown asymptomatic cardiac disease and ≥ 2 cardiovascular risk factors. Those who obtained an abnormal result in one test underwent coronary angiography, and if necessary, revascularization. Compared with patients randomized to the control arm (n=70), CE were significantly reduced in the screening arm during follow-up [84]. The preclinical diagnosis of CAD by SE may therefore be effective. However, more studies are needed to determine the prognostic role of SE in screening for cardiac disease in asymptomatic DM2 patients.

Nuclear SPECT Myocardial Perfusion Imaging (MPI)

The majority of studies on ischemia have used SPECT MPI. This imaging modality reveals the the presence and extent of perfusion defects. Images are taken following exposure to stress (exercise or pharmacological) and at rest, allowing the identification of fixed and reversible defects(Figure 3). The dimensions of the left ventricle and ejection fraction can also be determined. An analysis of the diagnostic accuracy of pharmacologically induced stress MPI in a pooled meta-analysis of 10,817 patients with angiographic data reported a mean sensitivity and specificity of 88% and 77%, respectively [85].

Perfusion defects are significant predictors of CE in patients with known or suspected CAD [86]. However, over a follow-up period of 4,6 years the presence of perfusion defects did

Figure 3. Myocardial perfusion imaging was carried out in the patient described in Figure 2, in whom coronary abnormalities had been observed on MSCT angiography.



A. A perfusion defect was observed in the posterolateral segment (indicated by arrows) during stress, which did not exist during rest as shown in **A"**, indicating ischemia. **B.** Partial ischemia was observed during stress, shown by an increase in size of the defect in the inferior segment (indicated by arrow), in comparison to the rest scan **B".**

not independently predict CE in a purely asymptomatic group of volunteers [87]. Normal MPI results have shown a low CE rate (1%) over a 5 year follow-up period[88]. Significant predictors of future CE after pharmacologically induced stress MPI include large defects, defects in multiple coronary artery territory suggestive of multi-vessel disease, major irreversible defects, left ventricular dilatation and decreased resting left ventricular ejection fraction [86].

Nuclear SPECT MPI Imaging in DM2

To our knowledge, the diagnostic accuracy of MPI in DM2 has only been studied by Kang et al., who performed MPI and conventional coronary angiography in 138 DM2 patients. Mean sensitivity and specificity were 86% and 56%, respectively for ≥ 50% coronary stenosis, and 90% and 50% for ≥ 70% coronary stenosis [89].

In asymptomatic diabetic patients, the rate of SMI diagnosed by stress MPI ranges from 17-59% (Table 1) [90-95]. In general, a higher percentage of perfusion defects has been detected in retrospective studies [90-91]. In the DIAD study, which included 1,123 asymptomatic patients with DM2, the occurrence of perfusion defects was not significantly associated with the traditional risk factors for CVD [92].

Table 1. Comparison of studies which have used SPECT MPI to detect silent ischemia in diabetic patients.

Study Group	No. of patients	Patient characteristics	Study nature	Abnormal results (percentage)	Other details
Rajagopalan et al.[90]	n = 1427	No known cardiac history. Patients with abnormal resting ECG included.	Retrospective	58% abnormal scans 18% high-risk scans (high-risk: SSS ≤ 47)*	High-risk scans were associated with ECG Q waves, PAD*, HbA1c, male gender, age, LDL cholesterol.
Miller et al. [91]	n = 1738	No known cardiac history. Patients with abnormal resting ECG included.	Retrospective	59% abnormal scans	High-risk scans in 19.7%.
Wackers et al. [92] (DIAD study)	n = 522	No known cardiac history. Patients with abnormal resting ECG excluded.	Prospective	22% abnormal results (out of which 73% abnormal scans and 37% other abnormalities)	Abnormal test result was not associated with traditional cardiac risk factors. 50% of patients were incapable of exercise.
Sultan et al. [93]	n = 419	No known cardiac history. ≥ 1 traditional cardiac risk factor besides DM2. Patients with abnormal resting ECG included.	Prospective	17% abnormal scans (abnormal: defect ≥ 3/20 segments)	Male gender, triglycerides, low creatinine clearance, HbA1c > 8%, were independent predictors of abnormal scans.
Zellweger et al. [94]	n = 826	No known cardiac history.	Prospective	39% abnormal scans (abnormal: SSS < 4 or SDS ≥ 2)*	
Valensi et al. [95]	n = 370	No known cardiac history. ≥ 2 traditional cardiac risk factors besides DM2. Patients with abnormal resting ECG excluded.	Prospective	26% abnormal scans	Silent ischemia was associated with higher age, triglycerides and lower HDL levels.

* PAD = peripheral arterial disease; SSS = summed stress score; SDS = summed difference score.

During an intermediate follow-up period, persistent and reversible perfusion defects have shown to be predictors of CE in asymptomatic diabetic patients [93-95]. Rajagopalan et al, categorized diabetic patients according to SPECT imaging scans, as high, intermediate or low risk. The annual mortality rate was 5.9%, 5.0% and 3.6%, respectively, with a significant difference in mortality ($p<0.001$) between the three groups [90]. The long-term prognostic value of MPI in asymptomatic diabetic patients needs to be further analyzed. It is speculated that concurrent abnormalities of perfusion imaging scans in diabetic patients with normal coronary angiograms may be due to microangiopathy or endothelial dysfunction, and therefore represent an increased likelihood of future CE [96].

CONCLUSIONS

CIMT, arterial stiffness and variably FMD are abnormal long before the onset of DM2.

Therefore these measurements are the most likely to be useful for the identification of at risk patients during the early stages of atherosclerotic disease, when functional wall properties are still reversible. However, further studies are necessary to evaluate whether these tools provide any additional prognostic value when used in combination with clinical risk scores (Table 2), before they can be implemented on large scale in clinical practice.

In individuals with a strong clinical suspicion of advanced CAD, cardiac imaging techniques are more warranted. When functional techniques are compared, AECG and exercise ECG are less sensitive and specific than functional cardiac imaging tests for the detection of ischemia in DM2. Head-to-head comparison has revealed that SPECT MPI has a higher sensitivity than SE for the detection of multi-vessel and single-vessel CAD [97]. Furthermore, the predictive value of SPECT MPI in the diabetic population has been studied more extensively than that of SE (Table 2). CAC scoring and the more recently developed MSCT non-invasive coronary angiography allow quantification of atherosclerotic burden. CAC scores have been shown to predict CE [56]. MSCT coronary angiography has good sensitivity for the identification of prevalent CAD and can therefore enable more widespread screening in combination with CAC scores in DM2, but its use is limited by radiation exposure and costs.

We propose an algorithm for the screening of asymptomatic diabetic patients (Figure 4). A selection strategy with a CAC score >100 AU has shown to be an effective way of identifying patients with moderate to large perfusion defects [98]. Nevertheless, recent observations have shown that low CAC scores do not exclude CAD in DM2 [56]. Based on this, the initial step of our algorithm involves the combined use of CAC assessment and exercise ECG to maximize sensitivity for detection of CAD. MPI or MSCT coronary angiography seem to be justified for individuals with a CAC score >100 or a positive exercise ECG. Conventional coronary angiography can then be considered in the presence of ischemia according to stress MPI

Table 2. Comparison of various non-invasive vascular tools and cardiac imaging techniques

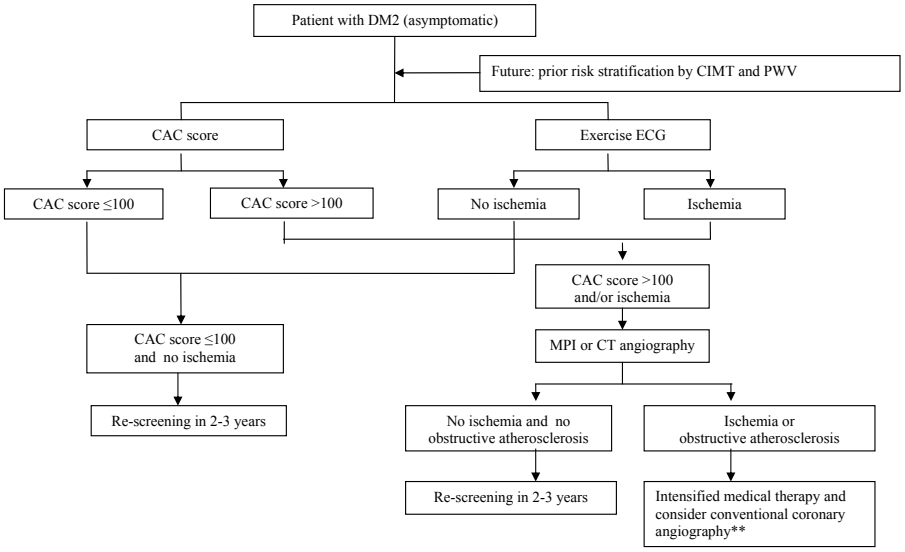
		Reproducibility	Detection of prevalent CAD		Prediction of CAD events		Details
			non-DM2	DM2	non-DM2	DM2	
I	**Vascular Tools**						
	IMT	Good: variability <5%	++[4]	+[9]	++[5,6]	+[12-14]	
	Vascular Stiffness	Mediocre: variability 11-15%	++[17]	+[16,26]	+[22,23]	+[18,24]	
	FMD	Poor: variability up to 50%	++[31]	Unknown	±[32,33]	Unknown	High intersession variability
II	**Anatomical Tests**						
	CAC scores	Good	++[37]	++[56]	++[100]	±[45,46] Limited studies	
	MS-CT angiography	Good	++[47-51]	++[54-60]	Unknown	Unknown	High radiation dosis
III	**Functional Tests**						
	AECG	Unknown	±[57-60] Reasonable Sensitivity Low Specificity	±[65,66] Low Sensitivity	+[61]	±[63] Limited studies	
	Exercise ECG	Unknown	+[67] Reasonable Sensitivity Reasonable Specificity	+[71-73] Reasonable Sensitivity Low Specificity	+[69,70]	+[74,75]	Not feasible in 32% of patients with DM2
	Nuclear MPI	Good	+[85] Good Sensitivity Reasonable Specificity	+[89] Good Sensitivity Low Specificity	++[86-88]	++[90,93-95] Based on intermediate follow-up	More long-term follow-up studies in DM2 are needed
	Stress Echocardiography	Good	+[76-78] Good Sensitivity Good Specificity	+[82,83] Limited studies Good Sensitivity Good Specificity	±[79-81]	±[84] Limited studies	Relative high false-negative rate in single-vessel disease and moderate stenosis

++ strong and consistent association in several studies in multivariate analysis; + association in most studies, or only one available study, in multivariate analysis; ± association in some studies, or association only in univariate analysis.

or obstructive atherosclerosis illustrated by MSCT angiography. Prospective studies may be conducted to evaluate the effectiveness of such a screening approach.

The criteria for the selection of those asymptomatic patients with DM2 who should undergo non-invasive cardiac screening for risk stratification remain controversial. The 'two or more risk factors' criterion for screening, as suggested by the 1998 ADA guidelines, failed to accurately identify a large number of patients with ischemia in the DIAD study [92]. Future studies may prove non-invasive vascular tools such as CIMT, PWV and FMD to be more effective in identification of patients at risk who should be screened for CAD(Figure 4).

Figure 4. Proposed algorithm for screening of asymptomatic diabetic patients.



* Choice of test according to availability and patient characteristics (in patients with severely impaired kidney function or atrial fibrillation, CT angiography should be avoided).
** Conventional coronary angiography can be considered in the presence of obstructive atherosclerosis in a proximal segment of a coronary artery or extensive ischemia.

The future

In DM2 patients , plaque development is not only accelerated, but also distinct, exhibiting more lipid-rich atheroma, macrophage infiltration and a higher thrombogenic potential compared with non-diabetic individuals [99]. This implies that screening tools such as magnetic resonance angiography, which enable assessment of plaque composition, and may reflect the real culprit, i.e. plaque vulnerability, could emerge as more potent risk predictors in DM2. However, the application of magnetic resonance angiography as a screening tool is not feasible in the near future because of high costs and complex methodology involved.

REFERENCES

1. Morrish NJ, Wang SL, Stevens LK, Fuller JH, Keen H (2001) Mortality and causes of death in the WHO Multinational Study of Vascular Disease in Diabetes. Diabetologia 44 [Suppl 2]: S14-21
2. Buse JB, MD, Ginsberg HN, MD, Bakris GL et al. (2007) Primary Prevention of Cardiovascular Diseases in People With Diabetes Mellitus. A scientific statement from the American Heart Association and the American Diabetes Association. Diabetes Care 30:162-172
3. Graf S, Gariepy J, Massonneau M et al. (1999) Experimental and clinical validation of arterial diameter waveform and intimal media thickness obtained from B-mode ultrasound image processing. Ultrasound Med Biol 25: 1353-1363
4. Burke GL, Evans GW, Riley WA et al. (1995) Arterial wall thickness is associated with prevalent cardiovascular disease in middle-aged adults. The Atherosclerosis Risk in Communities (ARIC) Study. Stroke 26: 386-391
5. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr (1999) Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med 340: 14-22
6. Chambless LE, Heiss G, Folsom AR et al. (1997) Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) Study, 1987-1993. Am J Epidemiol 146: 483-494
7. Niskanen L, Rauramaa R, Miettinen H, Haffner SM, Mercuri M, Uusitupa M (1996) Carotid artery intima-media thickness in elderly patients with NIDDM and in non-diabetic subjects. Stroke 27: 1986-1992
8. Bonora E, Kiechl S, Oberhollenzer F et al. (2000) Impaired glucose tolerance, Type II diabetes mellitus and carotid atherosclerosis: prospective results from the Bruneck Study. Diabetologia 43: 156-164
9. Lee CD, Folsom AR, Pankow JS, Brancati FL; Atherosclerosis Risk in Communities (ARIC) Study Investigators (2004) Cardiovascular events in diabetic and non-diabetic adults with or without history of myocardial infarction. Circulation 109: 855-60
10. Wagenknecht LE, Zaccaro D, Espeland MA, Karter AJ, O'Leary DH, Haffner SM (2003) Diabetes and progression of carotid atherosclerosis: the insulin resistance atherosclerosis study. Arterioscler Thromb Vasc Biol 23: 1035-1041
11. van der Meer IM, Iglesias del Sol A, Hak AE, Bots ML, Hofman A, Witteman JC (2003) Risk factors for progression of atherosclerosis measured at multiple sites in the arterial tree: the Rotterdam Study. Stroke 34: 2374-2379
12. Bernard S, Sérusclat A, Targe F et al. (2005) Incremental predictive value of carotid ultrasonography in the assessment of coronary risk in a cohort of asymptomatic type 2 diabetic subjects. Diabetes Care 28: 1158-1162
13. Yamasaki Y, Kodama M, Nishizawa H et al. (2000) Carotid intima-media thickness in Japanese type 2 diabetic subjects: predictors of progression and relationship with incident coronary heart disease. Diabetes Care 23: 1310-1315
14. Folsom AR, Chambless LE, Duncan BB, Gilbert AC, Pankow JS; Atherosclerosis Risk in Communities Study Investigators. (2003) Prediction of coronary heart disease in middle-aged adults with diabetes. Diabetes Care 26: 2777-2784
15. Benetos A, Laurent S, Hoeks AP, Boutouyrie PH, Safar ME (1993) Arterial alterations with aging and high blood pressure. A non-invasive study of carotid and femoral arteries. Arterioscler Thromb 13: 90-97
16. Fukui M, Kitagawa Y, Nakamura N et al. (2003) Augmentation of central arterial pressure as a marker of atherosclerosis in patients with type 2 diabetes. Diabetes Res Clin Pract 59: 153-161

17. Weber T, Auer J, O'Rourke MF et al. (2004) Arterial stiffness, wave reflections, and the risk of coronary artery disease. Circulation 109: 184-189

18. Henry RM, Kostense PJ, Spijkerman AM et al. (2003) Arterial stiffness increases with deteriorating glucose tolerance status: the Hoorn Study. Circulation 107: 2089-2095

19. Wilkinson IB, Fuchs SA, Jansen IM et al. (1998) Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis. J Hypertens 16: 2079-84

20. Lakatta EG, Levy D (2003) Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part I: aging arteries: a "set up" for vascular disease. Circulation 107: 139-146

21. Simons PC, Algra A, Bots ML, Grobbee DE, van der Graaf Y (1999) Common carotid intima-media thickness and arterial stiffness: indicators of cardiovascular risk in high-risk patients. The SMART Study (Second Manifestations of ARTerial disease). Circulation 100: 951-957

22. Störk S, van den Beld AW, von Schacky C et al. (2004) Carotid artery plaque burden, stiffness, and mortality risk in elderly men: a prospective, population-based cohort study. Circulation 110: 344-348

23. Willum-Hansen T, Staessen JA, Torp-Pedersen C et al. (2006) Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. Circulation 113: 664-670

24. Cruickshank K, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG (2002) Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? Circulation 106: 2085-2090

25. Schram MT, Henry RM, van Dijk RA et al. (2004) Increased central artery stiffness in impaired glucose metabolism and type 2 diabetes: the Hoorn Study. Hypertension 43: 176-181

26. Hatsuda S, Shoji T, Shinohara K et al. (2006) Regional arterial stiffness associated with ischemic heart disease in type 2 diabetes mellitus. J Atheroscler Thromb 13: 114-121

27. Joannides R, Haefeli WE, Linder L et al. (1995) Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. Circulation 91: 1314-1319

28. Anderson TJ, Uehata A, Gerhard MD et al. (1995) Close relation of endothelial function in the human coronary and peripheral circulations. J Am Coll Cardiol 26: 1235-1241

29. Hijmering ML, Stroes ES, Pasterkamp G, Sierevogel M, Banga JD, Rabelink TJ (2001) Variability of flow mediated dilation: consequences for clinical application. Atherosclerosis 157: 369-373

30. De Roos NM, Bots ML, Schouten EG, Katan MB (2003) Within-subject variability of flow mediated vasodilation of the brachial artery in healthy men and women: implications for experimental studies. Ultrasound Med Biol 29: 401-406

31. Schroeder S, Enderle MD, Ossen R et al. (1999) Non-invasive determination of endothelium-mediated vasodilation as a screening test for coronary artery disease: pilot study to assess the predictive value in comparison with angina pectoris, exercise electrocardiography, and myocardial perfusion imaging. Am Heart J 138: 731-739

32. Gokce N, Keaney JF Jr, Hunter LM et al. (2003) Predictive value of non-invasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. J Am Coll Cardiol 41: 1769-1775

33. Fathi R, Haluska B, Isbel N, Short L, Marwick TH (2004) The relative importance of vascular structure and function in predicting cardiovascular events. J Am Coll Cardiol 43: 616-623

34. Creager MA, Lüscher TF, Cosentino F, Beckman JA (2003) Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: Part I. Circulation 108: 1527-1532

35. Tan KC, Chow WS, Ai VH, Metz C, Bucala R, Lam KS (2002) Advanced glycation end products and endothelial dysfunction in type 2 diabetes. Diabetes Care 25: 1055-1059

36. Nitenberg A, Pham I, Antony I, Valensi P, Attali JR, Chemla D (2005) Cardiovascular outcome of patients with abnormal coronary vasomotion and normal coronary arteriography is worse in type

2 diabetes mellitus than in arterial hypertension: a 10 year follow-up study. Atherosclerosis 183: 113-120

37. Schmermund A, Baumgart D, Görge G et al. (1998) Measuring the effect of risk factors on coronary atherosclerosis: coronary calcium score versus angiographic disease severity. J Am Coll Cardiol 31: 1267-1273

38. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R (1990) Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol 15: 827-832

39. Allison MA, Wright CM (2005) Age and gender are the strongest clinical correlates of prevalent coronary calcification (R1). Int J Cardiol 98: 325-330

40. Elkeles RS, Feher MD, Flather MD et al. (2004) The association of coronary calcium score and conventional cardiovascular risk factors in Type 2 diabetic subjects asymptomatic for coronary heart disease (The PREDICT Study). Diabet Med 21: 1129-1134

41. Hoff JA, Quinn L, Sevrukov A et al. (2003) The prevalence of coronary artery calcium among diabetic individuals without known coronary artery disease. J Am Coll Cardiol 41: 1008-1012

42. Schurgin S, Rich S, Mazzone T (2001) Increased prevalence of significant coronary artery calcification in patients with diabetes. Diabetes Care 24: 335-338

43. Reaven PD, Sacks J; Investigators for the VADT (2005) Coronary artery and abdominal aortic calcification are associated with cardiovascular disease in type 2 diabetes. Diabetologia 48: 379-385

44. Raggi P, Cooil B, Ratti C, Callister TQ, Budoff M (2005) Progression of coronary artery calcium and occurrence of myocardial infarction in patients with and without diabetes mellitus. Hypertension 46: 238-243

45. Raggi P, Shaw LJ, Berman DS, Callister TQ (2004) Prognostic value of coronary artery calcium screening in subjects with and without diabetes. J Am Coll Cardiol 43: 1663-1669

46. Qu W, Le TT, Azen SP et al. (2003) Value of coronary artery calcium scanning by computed tomography for predicting coronary heart disease in diabetic subjects. Diabetes Care 26: 905-910

47. Mollet NR, CHDemartiri F, Krestin GP et al. (2005) Improved diagnostic accuracy with 16-row multi-slice computed tomography coronary angiography. J Am Coll Cardiol 45: 128-132

48. Hoffmann MH, Shi H, Schmitz BL et al. (2005) Non-invasive coronary angiography with multislice computed tomography. JAMA 293: 2471-2478

49. Raff GL, Gallagher MJ, O'Neill WW, Goldstein JA (2005) Diagnostic accuracy of non-invasive coronary angiography using 64-slice spiral computed tomography. J Am Coll Cardiol 46: 552-557

50. Mollet NR, CHDemartiri F, van Mieghem CA et al. (2005) High-resolution spiral computed tomography coronary angiography in patients referred for diagnostic conventional coronary angiography. Circulation 112: 2318-2323

51. Ropers D, Rixe J, Anders K et al. (2006) Usefulness of multidetector row spiral computed tomography with 64- x 0.6-mm collimation and 330-ms rotation for the non-invasive detection of significant coronary artery stenoses. Am J Cardiol 97: 343-348

52. Hoffmann U, Moselewski F, Nieman K et al. (2006) Non-invasive assessment of plaque morphology and composition in culprit and stable lesions in acute coronary syndrome and stable lesions in stable angina by multidetector computed tomography. J Am Coll Cardiol 47: 1655-1662

53. Pundziute G, Schuijf JD, Jukema JW et al. (2007) Non-invasive assessment of plaque characteristics with multislice computed tomography coronary angiography in symptomatic diabetic patients. Diabetes Care 30: 1113-1119

54. Schuijf JD, Bax JJ, Jukema JW et al. (2004) Non-invasive angiography and assessment of left ventricular function using multislice computed tomography in patients with type 2 diabetes. Diabetes Care 27: 2905-2910

55. Schuijf JD, Mollet NR, CHDemartiri F et al. (2006) Do risk factors influence the diagnostic accuracy of non-invasive coronary angiography with multislice computed tomography? J Nucl Cardiol 13: 635-641

56. Scholte AJ, Schuijf JD, Kharagjitsingh AV et al. (2007) Prevalence of coronary artery disease and plaque morphology assessed by multi-slice computed tomography coronary angiography and calcium scoring in asymptomatic patients with type 2 diabetes. Heart [Epub ahead of print]

57. Crawford MH, Mendoza CA, O'Rourke RA, White DH, Boucher CA, Gorwit J (1978) Limitations of continuous ambulatory electrocardiogram monitoring for detecting coronary artery disease. Ann Intern Med 89: 1-5

58. Ochotny R, Luczak D, Górski L, Błaszczyk K, Jasek S, Koźbiał H (1992) [24-hour ECG monitoring by the Holter system in early diagnosis of coronary disease] Pol Arch Med Wewn 87: 265-270

59. Nair CK, Khan IA, Esterbrooks DJ, Ryschon KL, Hilleman DE (2001) Diagnostic and prognostic value of Holter-detected ST-segment deviation in unselected patients with chest pain referred for coronary angiography: a long-term follow-up analysis. Chest 120: 834-839

60. Quyyumi A, Crake T, Wright C, Mockus L, Fox K (1987) The role of ambulatory ST-segment monitoring in the diagnosis of coronary artery disease: comparison with exercise testing and thallium scintigraphy. Eur Heart J 8: 124-129

61. Sajadieh A, Nielsen OW, Rasmussen V, Hein HO, Hansen JF (2005) Prevalence and prognostic significance of daily-life silent myocardial ischaemia in middle-aged and elderly subjects with no apparent heart disease. Eur Heart J 26: 1402-1409

62. Chiariello M, Indolfi C, Cotecchia MR, Sifola C, Romano M, Condorelli M (1985) Asymptomatic transient ST changes during ambulatory ECG monitoring in diabetic patients. Am Heart J 110: 529-534

63. Aronow WS, Mercando AD, Epstein S (1992) Prevalence of silent myocardial ischemia detected by 24-hour ambulatory electrocardiography, and its association with new coronary events at 40-month follow-up in elderly diabetic and non-diabetic patients with coronary artery disease. Am J Cardiol 69: 555-556

64. Marín Huerta E, Rayo I, Lara JI et al. (1989) Silent myocardial ischemia during Holter monitoring in patients with diabetes mellitus [Article in Spanish] Rev Esp Cardiol 42: 519-529

65. Caracciolo EA, Chaitman BR, Forman SA et al. (1996) Diabetics with coronary disease have a prevalence of asymptomatic ischemia during exercise treadmill testing and ambulatory ischemia monitoring similar to that of non-diabetic patients. An ACIP database study. ACIP Investigators. Asymptomatic Cardiac Ischemia Pilot Investigators. Circulation 93: 2097-2105

66. Ahluwalia G, Jain P, Chugh SK, Wasir HS, Kaul U (1995) Silent myocardial ischemia in diabetics with normal autonomic function. Int J Cardiol 48: 147-153

67. Gianrossi R, Detrano R, Mulvihill D et al. (1989) Exercise-induced ST depression in the diagnosis of coronary artery disease. A meta-analysis. Circulation 80: 87-98

68. Roger VL, Jacobsen SJ, Pellikka PA, Miller TD, Bailey KR, Gersh BJ (1998) Prognostic value of treadmill exercise testing: a population-based study in Olmsted County, Minnesota. Circulation 98: 2836-2841

69. Laukkanen JA, Kurl S, Lakka TA et al. (2001) Exercise-induced silent myocardial ischemia and coronary morbidity and mortality in middle-aged men. J Am Coll Cardiol 38: 72-79

70. Giagnoni E, Secchi MB, Wu SC et al. (1983) Prognostic value of exercise EKG testing in asymptomatic normotensive subjects. A prospective matched study. N Engl J Med 309: 1085-1089

71. Paillole C, Ruiz J, Juliard JM, Leblanc H, Gourgon R, Passa P (1995) Detection of coronary artery disease in diabetic patients. Diabetologia 38: 726-731

72. Bacci S, Villella M, Villella A et al. (2002) Screening for silent myocardial ischaemia in type 2 diabetic patients with additional atherogenic risk factors: applicability and accuracy of the exercise stress test. Eur J Endocrinol 147: 649-654

73. Janand-Delenne B, Savin B, Habib G, Bory M, Vague P, Lassmann-Vague V (1999) Silent myocardial ischemia in patients with diabetes: who to screen. Diabetes Care 22: 1396-1400

74. Cosson E, Paycha F, Paries J et al. (2004) Detecting silent coronary stenoses and stratifying cardiac risk in patients with diabetes: ECG stress test or exercise myocardial scintigraphy? Diabet Med 21: 342-348

75. Gerson MC, Khoury JC, Hertzberg VS, Fischer EE, Scott RC (1988) Prediction of coronary artery disease in a population of insulin-requiring diabetic patients: results of an 8-year follow-up study. Am Heart J 116: 820-826

76. Kim C, Kwok YS, Heagerty P, Redberg R (2001) Pharmacologic stress testing for coronary disease diagnosis: A meta-analysis. Am Heart J 142: 934-944

77. Bartunek J, Marwick TH, Rodrigues AC et al. (1996) Dobutamine-induced wall motion abnormalities: correlations with myocardial fractional flow reserve and quantitative coronary angiography. J Am Coll Cardiol 27: 1429-1436

78. Marwick TH, Nemec JJ, Pashkow FJ, Stewart WJ, Salcedo EE (1992) Accuracy and limitations of exercise echocardiography in a routine clinical setting. J Am Coll Cardiol 19: 74-81

79. Arruda-Olson AM, Juracan EM, Mahoney DW, McCully RB, Roger VL, Pellikka PA (2002) Prognostic value of exercise echocardiography in 5,798 patients: is there a gender difference? J Am Coll Cardiol 39: 625-631

80. McCully RB, Roger VL, Mahoney DW et al. (1998) Outcome after normal exercise echocardiography and predictors of subsequent cardiac events: follow-up of 1,325 patients. J Am Coll Cardiol 31: 144-149

81. Marwick TH, Case C, Short L, Thomas JD (2003) Prediction of mortality in patients without angina: use of an exercise score and exercise echocardiography. Eur Heart J 24: 1223-1230

82. Elhendy A, van Domburg RT, Poldermans D et al. (1998) Safety and feasibility of dobutamine-atropine stress echocardiography for the diagnosis of coronary artery disease in diabetic patients unable to perform an exercise stress test. Diabetes Care 21: 1797-1802

83. Hennessy TG, Codd MB, Kane G, McCarthy C, McCann HA, Sugrue DD (1997) Evaluation of patients with diabetes mellitus for coronary artery disease using dobutamine stress echocardiography. Coron Artery Dis 8: 171-174

84. Faglia E, Manuela M, Antonella Q et al. (2005) Risk reduction of cardiac events by screening of unknown asymptomatic coronary artery disease in subjects with type 2 diabetes mellitus at high cardiovascular risk: an open-label randomized pilot study. Am Heart J 149: e1-6

85. Iskandrian AS, Verani MS (1996) Exercise perfusion imaging in coronary artery disease: Physiology and diagnosis. In: Davis FA, Nuclear Cardiac Imaging: Principles and Applications, Philadelphia, pp 73-143

86. Thomas GS, Miyamoto MI, Morello AP 3rd et al. (2004) Technetium 99m sestamibi myocardial perfusion imaging predicts clinical outcome in the community outpatient setting. The Nuclear Utility in the Community (NUC) Study. J Am Coll Cardiol 43: 213-223

87. Fleg JL, Gerstenblith G, Zonderman AB et al. (1990) Prevalence and prognostic significance of exercise-induced silent myocardial ischemia detected by thallium scintigraphy and electrocardiography in asymptomatic volunteers. Circulation 81: 428-436

88. Elhendy A, Schinkel A, Bax JJ, van Domburg RT, Poldermans D (2003) Long-term prognosis after a normal exercise stress Tc-99m sestamibi SPECT study. J Nucl Cardiol 10: 261-266

89. Kang X, Berman DS, Lewin H et al. (1999) Comparative ability of myocardial perfusion single-photon emission computed tomography to detect coronary artery disease in patients with and without diabetes mellitus. Am Heart J 137: 949-957

90. Rajagopalan N, Miller TD, Hodge DO, Frye RL, Gibbons RJ (2005) Identifying high-risk asymptomatic diabetic patients who are candidates for screening stress single-photon emission computed tomography imaging. J Am Coll Cardiol 45: 43-49

91. Miller TD, Rajagopalan N, Hodge DO, Frye RL, Gibbons RJ (2004) Yield of stress single-photon emission computed tomography in asymptomatic patients with diabetes. Am Heart J 147: 890-896

92. Wackers FJ, Young LH, Inzucchi SE et al. (2004) Detection of silent myocardial ischemia in asymptomatic diabetic subjects: the DIAD study. Diabetes Care 27: 1954-1961

93. Sultan A, Piot C, Mariano-Goulart D et al. (2006) Myocardial perfusion imaging and cardiac events in a cohort of asymptomatic patients with diabetes living in southern France. Diabet Med 23: 410-418

94. Zellweger MJ, Hachamovitch R, Kang X et al. (2004) Prognostic relevance of symptoms versus objective evidence of coronary artery disease in diabetic patients. Eur Heart J 25: 543-550

95. Valensi P, Pariès J, Brulport-Cerisier V et al. (2005) Predictive value of silent myocardial ischemia for cardiac events in diabetic patients: influence of age in a French multicenter study. Diabetes Care 28: 2722-2727

96. Nitenberg A, Ledoux S, Valensi P, Sachs R, Attali JR, Antony I (2001) Impairment of coronary microvascular dilation in response to cold pressor--induced sympathetic stimulation in type 2 diabetic patients with abnormal stress thallium imaging. Diabetes 50: 1180-1185

97. O'Keefe JH Jr, Barnhart CS, Bateman TM (1995) Comparison of stress echocardiography and stress myocardial perfusion scintigraphy for diagnosing coronary artery disease and assessing its severity. Am J Cardiol 75: 25D-34D

98. Anand DV, Lim E, Hopkins D et al. (2006) Risk stratification in uncomplicated type 2 diabetes: prospective evaluation of the combined use of coronary artery calcium imaging and selective myocardial perfusion scintigraphy. Eur Heart J 27:713-721

99. Moreno PR, Murcia AM, Palacios IF et al. (2000) Coronary composition and macrophage infiltration in atherectomy specimens from patients with diabetes mellitus. Circulation 102: 2180-2184

100. Detrano R, Guerci AD, Carr JJ et al. (2008) Coronary calcium as a predictor of coronary events in four racial or ethnic groups. N Engl J Med 358: 1336-1345

Chapter 3

Two year statin therapy does not alter the progression of Intima-Media Thickness in patients with type 2 diabetes mellitus without manifest cardiovascular disease

ED Beishuizen[1], MA van de Ree[2], JW Jukema[3],
JT Tamsma[1], JCM van der Vijver[4]
AE Meinders[1], H Putter[5], MV Huisman[1]

[1] Department of General Internal Medicine and Endocrinology, Leiden University Medical Center
[2] Department of Internal Medicine, Diakonessenhuis, Utrecht
[3] Department of Cardiology, Leiden University Medical Center
[4] Department of Internal Medicine, Leyenburg Hospital, The Hague
[5] Department of Biostatistics, Leiden University Medical Center

The Netherlands

Diabetes Care 2004; 27: 2887-2892

ABSTRACT

Objective

Cardiovascular disease (CVD) is the most important cause of mortality in patients with type 2 diabetes mellitus (DM2). We aimed to determine the effect of statin therapy versus placebo on the progression of carotid Intima-Media Thickness (IMT) in DM2 patients without manifest CVD.

Research Design and Methods

A randomized, placebo-controlled, double-blind clinical trial was performed in 250 patients with DM2. Patients were given either 0.4 mg cerivastatin or placebo daily. In August 2001, when cerivastatin was withdrawn from the market, 0.4 mg cerivastatin was replaced by 20 mg simvastatin, without deblinding the study. The primary endpoint was the change of mean common carotid IMT, as measured by B-mode ultrasound, over 2 years.

Results

Common carotid IMT at baseline was 0.780 mm in the placebo group and 0.763 mm in the statin group and did not change significantly after two years. There was no significant difference in IMT change in any carotid segment between the groups. LDL cholesterol was reduced by 25 % in the statin group and increased by 8% in the placebo group ($p<0.001$). Cardiovascular events occurred in 12 patients in the placebo group and in 2 patients in the statin group ($p=0.006$).

Conclusions

There was no effect of 2 years' statin therapy on carotid IMT in DM2. The natural history of IMT in our patients was milder than anticipated. In contrast, we observed a significantly lower cardiovascular event rate on statin therapy. Prognostic tools other than IMT should be explored in this patient group.

INTRODUCTION

Cardiovascular disease (CVD), including cerebrovascular disease, coronary artery disease (CAD), and peripheral vascular disease, is the most important cause of mortality in patients with type 2 diabetes[1]. The severity and progression of atherosclerosis can be assessed non-invasively by ultrasonographic measurements of the intima-media thickness (IMT) in the carotid and femoral arteries. Ultrasonographic IMT measurements of the far wall relate to histological IMT measurements[2]. Carotid IMT correlates with prevalent CVD[3], angiographically proven coronary atherosclerosis[4], and risk factors for CVD, including LDL cholesterol[5,6]. In prospective studies, carotid IMT has proven to be predictive of CVD[7-9], and as a consequence, IMT is increasingly used as an intermediate end point in clinical trials. Mean common carotid IMT in middle- aged subjects without CAD is reported to range from 0.71 to 0.91 mm in diabetic patients vs. 0.66 to 0.74 mm in control subjects[10,11]. In diabetes, IMT is less consistently correlated to classical risk factors such as LDL cholesterol. Importantly, at the time our study was designed, data on the progression and predictive value of IMT in type 2 diabetes were lacking.

During the last 10 years, large clinical trials have demonstrated that HMG-CoA (hydroxymethylglutaryl coenzyme A) reductase inhibitors (statins) reduce the risk of cardiovascular events in the setting of secondary and primary prevention[12-15]. Subgroup analyses in diabetic patients in several of these studies showed conflicting results[14-16]. However, these trials were not specifically designed for type 2 diabetic patients in the setting of primary prevention. We therefore set out to evaluate the effect of statin therapy versus placebo on the progression of carotid and femoral IMT in type 2 diabetic patients without established CVD.

RESEARCH DESIGN AND METHODS

Patients

Patients were recruited from the departments of internal medicine at two nonacademic teaching hospitals, the Leyenburg Hospital and the Red Cross Hospital, the Hague, the Netherlands. Subjects were eligible for the study if they had been diagnosed with type 2 diabetes for at least 1 year, were aged 30–80 years, and were without a history of CVD (defined as CAD, electrocardiographic criteria for a past myocardial infarction, ischemic stroke, peripheral artery bypass surgery, percutaneous transluminal angioplasty, or amputation because of atherosclerotic disease).

At a screening visit, fasting blood samples were drawn and a resting electrocardiogram performed. Patients with fasting total cholesterol > 6.9 mmol/l or < 4.0 mmol/l, triglycerides > 6.0 mmol/l, creatinine kinase values more than three times and alanine aminotransferase (ALT) more than two times the upper limit of normal, or creatinine clearance < 30 ml/min

were excluded. Any lipid-lowering therapy had to be discontinued 8 weeks before the screening visit. The study was approved by the medical ethics committees of both hospitals and performed in accordance with the Declaration of Helsinki.

Study Objectives

The primary end point of the study was the change in mean IMT of the common carotid artery after 24 months. Secondary end points were the changes in mean and maximum IMT of the carotid bifurcation, internal carotid artery, common femoral artery, and superficial femoral artery and the changes in aggregate carotid IMT (defined as the average of the mean IMT of the three carotid segments), all after 24 months. The change in mean IMT after 12 months was also considered a secondary end point. The following predefined cardiovascular events were evaluated during the study: cardiovascular death, nonfatal myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass graft surgery, nonfatal stroke, peripheral artery bypass graft, percutaneous transluminal angioplasty, or amputation because of atherosclerotic disease.

Study design

After giving written informed consent, 250 patients participated at least 1 week after the screening visit. Patients were randomly assigned to receive 0.4 mg cerivastatin (Bayer, Mijdrecht, the Netherlands) or placebo daily for a period of 2 years. Double-blind study medication was assigned using a predetermined computer- generated randomization scheme with a block size of 10. On 8 August 2001, cerivastatin was withdrawn from the market due to reports of serious morbidity and mortality possibly related to the drug [17]. At that moment, all 250 patients had been included in the study with a mean follow-up of 15.4 months (range 6–23).

All patients were instructed to discontinue the study drug. The study was not unblinded at any time point. No patient had developed myopathy, and creatinine kinase values above five times the upper limit of normal had not been observed. After consultation with independent experts in lipid-lowering treatment studies, it was decided to continue the study. Cerivastatin (0.4 mg) was replaced by simvastatin (20 mg) daily, on the basis of a comparable LDL reduction[18,19]. Both simvastatin and matching placebo tablets (Merck Sharp & Dohme, Haarlem, the Netherlands) were given according to the original allocation. The study was continued 1 month after the discontinuation of cerivastatin. The total use of study medication was kept at 24 months, resulting in the study being prolonged for 1 month.

Follow-up

Patients returned to the study site after a 12-h fast at 3, 6, 12, 18, and 24 months, when blinded lipid and safety measurements (creatinine kinase and ALT) were performed. Carotid IMT was measured at baseline, 12 months, and 24 months. Femoral IMT was performed at baseline and 24 months. Two-year follow-up for clinical events was performed for all 250 patients.

Ultrasound measurements

Ultrasound imaging was performed with an Acuson Aspen scanner with a linear array 7.5-MHz probe. All images were recorded digitally and on a S-VHS videotape for off-line, blinded analysis by an independent core laboratory (Heartcore, Leiden, the Netherlands). During the study, all measurements were performed by the same two certified ultrasonographers.

In the supine position, the left and right carotid arteries, near and far walls, were examined longitudinally at the angle that resulted in an optimal and maximal IMT (while avoiding plaques) for each segment. The segments scanned were the distal 1.0 cm of the common carotid artery, the carotid bifurcation, and the proximal 1.0 cm of the internal carotid artery. The optimal angle was used for follow- up. The same procedure was done for the common femoral artery and superficial femoral artery.

For each segment, three *R* wave– triggered images were stored. Mean and maximal IMT were measured, when possible, over the entire 1 cm of the vessel segment. The three IMT measurements were averaged. To obtain mean and maximal IMT per vessel segment, far and near wall, left and right values were averaged.

During the first year of the study, a reproducibility investigation was performed for the two ultrasonographers in 16 subjects. For the common carotid artery, interobserver variability (expressed as mean difference ± SD) was 0.0082 ± 0.050 mm and intraobserver variability was 0.0067 ± 0.049 and 0.00036 ± 0.058 mm for the two observers. For the common femoral artery, interobserver variability was 0.039 ± 0.11 mm and intraobserver variability was 0.0085 ± 0.10 and 0.060 mm ± 0.078 mm for the two observers.

Laboratory investigations

All laboratory measurements were performed at the Department of Clinical Chemistry and Hematology of the Leyenburg Hospital, according to ISO 15189 standard procedures.

Statistical analysis

When this study was designed, no data were available on IMT progression in type 2 diabetes. From clinical studies in patients with CAD, we assumed a progression rate of 0.03 mm per 2 years for the common carotid artery IMT. The number of patients needed to detect a difference in mean common carotid artery IMT of 0.04 mm after 2 years (expected SD 0.10) with a power of 80% ($\alpha = 0.05$) was 100 patients in each group. To allow for a 20% drop-out rate, the total number of patients randomized would be 250.

The primary treatment comparison is between placebo and statin therapy in patients completing the study (on-treatment analysis). Changes from baseline within each treatment group were analyzed using Student's paired *t* test. Comparisons of the effects between the treatment groups were performed using Student's independent samples *t* test. Mixed-model analysis was used as a sensitivity analysis to assess the influence of missing values on the

results, under the assumption of "missing at random" [20], and to investigate systematic differences between replications, positions, and between far and near wall.

Stepwise regression techniques were used to investigate the effect on baseline IMT and on changes in IMT of sex, age, smoking habits, ethnicity, blood pressure, anthropometric parameters, and duration of cerivastatin versus simvastatin use. To test the equivalence of 0.4 mg cerivastatin and 20 mg simvastatin, LDL levels before and after the switch to simvastatin were compared using Student's paired *t* test. Correlation between changes in IMT and changes in lipid levels were evaluated by calculating Pearson's correlation coefficients. The occurrence of clinical events was expressed as a proportion and evaluated using Chi-squared test or Fisher's exact test as appropriate. All analyses were two sided with a level of significance of $\alpha = 0.05$.

RESULTS

Of a total of 302 patients screened, 52 did not fulfill the entry criteria. The baseline characteristics of the 250 randomized patients did not differ between the groups and are reported in Table 1. None of the patients had recently been on lipid-lowering therapy.

Of the 250 patients randomized, 68 did not complete the study: 46 in the placebo group and 22 in the statin group. In 16 patients in the placebo group and in 8 in the statin group, the only reason for discontinuation was the withdrawal of cerivastatin from the market. Drop-out rates were slightly lower in the Caucasian group than in the Asian-Indian and other ethnic

Table 1 Baseline Characteristics of 250 Randomized Patients

	Placebo (n=125)	**Statin (n=125)**
Male sex	57 (46)	61 (49)
Age (years)	58.2 ± 11.4	58.8 ±11.3
Ethnicity:		
Caucasian	86 (69)	83 (66)
Asian-Indian	20 (16)	28 (22)
other	19 (15)	14 (11)
BMI (kg/m^2)	31.0 ± 6.0	31.0 ± 6.3
Waist-to-hip ratio	0.99 ± 0.09	0.98 ± 0.08
Current smoker	33 (26)	28 (22)
Hypertension	66 (53)	60 (48)
Diabetes duration (years)*	7 ± 8	6 ± 7
Insulin use	69 (55)	62 (50)
HbA1c (%)	7.60 ± 1.48	7.53 ± 1.10
Microalbuminuria†	19 (15)	24 (19)

Data are means ± SD or n (%);
*Median values ± SD
† Men, > 2.5 g/mol creatinine;women > 3.5 g/mol creatinine

groups (22 vs. 35%, respectively, 42%, p = 0.02). The other baseline characteristics of the 182 patients who completed the study did not differ from the 68 drop outs (data not shown).

Overall compliance, as assessed by pill counting, was 97% and was equal in the statin and placebo groups. Compliance was not reduced after the switch to simvastatin.

Lipids

LDL cholesterol was reduced by 25% in the statin group and increased by 8% in the placebo group ($P < 0.001$), and changes in HDL cholesterol and triglycerides were not significantly different between the groups (Table 2). Average LDL cholesterol levels were higher after the switch to simvastatin (2.34 before vs. 2.56 mmol/l after the switch, p < 0.001).

Table 2 Plasma lipid and lipoprotein concentrations

	Placebo (n=79)			Statin(n=103)			
	baseline	**2 year**	**p**	**baseline**	**2 year**	**p**	**p***
TC (mmol/l)	5.60 ± 0.77	5.74 ± 0.93	0.058	5.49 ± 0.72	4.49 ± 1.01	<0.001	<0.001
LDL-c(mmol/l)	3.55 ± 0.71	3.78 ± 0.81	0.003	3.44 ± 0.71	2.58 ± 0.95	<0.001	<0.001
HDL-c(mmol/l)	1.21 ± 0.37	1.22 ± 0.38	0.963	1.23 ± 0.39	1.20 ± 0.36	0.144	0.284
TG (mmol/l)	1.88 ± 0.79	1.72 ± 1.22	0.206	1.82 ± 0.97	1.60 ± 1.38	0.043	0.371
ApoB100 (mg/l)	1.15 ± 0.23	1.11 ± 0.24	0.094	1.10 ± 0.21	0.84 ± 0.26	<0.001	<0.001

Data are means ± SD

* p value for difference in percent change between placebo and statin group

TC = total cholesterol; LDL-c = LDL cholesterol; HDL-c =HDL cholesterol; TG = triglycerides. Apo = Apolipoprotein

To convert to mg/dl: cholesterol: multiply by 38.6; triglycerides: multiply by 88.5

IMT

Baseline mean IMT's were not significantly different between the groups. Common carotid artery IMT in the placebo group was 0.780 ± 0.129 mm at baseline and 0.774 ± 0.124 mm at 2 years (p = 0.50), and in the statin group, it was 0.763 ± 0.124 mm at baseline and 0.765 ± 0.116 mm at 2 years (p = 0.78) (Table 3). There was no significant difference between the change in IMT in the placebo and statin groups (mean difference - 0.0075 mm [95% CI -0.0281 to 0.0132 mm], p = 0.48). After 2 years, the mean changes in IMT of the other segments were also not significantly different between the groups and compared with baseline. This was also observed for the changes in maximal IMT (data not shown). Finally, the changes in mean common carotid artery IMT after 1 year were equal in both groups (-0.0155 mm in the placebo group vs. -0.0166 mm in the statin group, p for difference in change = 0.90). Mixed-model analysis confirmed these results.

Determinants for baseline IMT were age (r = 0.358, p < 0.001) and systolic blood pressure (r = 0.26, p < 0.001). Baseline IMT and changes in IMT were not correlated with LDL cholesterol or any other lipid parameter. Baseline IMT and changes in IMT were also not related to sex, ethnicity, diabetes duration, insulin use, HbA1c, anthropometric parameters, or smoking

Table 3 mean IMT of 182 patients who completed the study

	Baseline		2 years		Mean change		
	IMT	SD	IMT	SD	IMT	95% CI	p*
Placebo (n=79)							
Primary endpoint							
CCA	0.780	0.129	0.774	0.124	-0.006	-0.0223 to 0.0109	0.50
Secondary endpoint							
BIF	0.815	0.148	0.805	0.143	-0.010	-0.0267 to 0.0072	0.25
ICA	0.640	0.136	0.670	0.130	0.031	-0.0104 to 0.0718	0.14
aggrCA	0.757	0.137	0.763	0.120	0.006	-0.0117 to 0.0229	0.52
CFA	0.663	0.149	0.652	0.141	-0.011	-0.0390 to 0.0165	0.42
SFA	0.551	0.111	0.549	0.099	-0.002	-0.0236 to 0.0197	0.86
Statin (n=103)							
Primary endpoint							
CCA	0.763	0.124	0.765	0.116	0.002	-0.0112 to 0.0149	0.78
Secondary endpoint							
BIF	0.823	0.140	0.806	0.122	-0.017	-0.0358 to 0.0017	0.07
ICA	0.684	0.183	0.689	0.181	0.005	-0.0211 to 0.0314	0.69
aggrCA	0.759	0.116	0.762	0.105	0.003	-0.0116 to 0.0175	0.69
CFA	0.630	0.151	0.635	0.148	0.005	-0.0152 to 0.0258	0.61
SFA	0.543	0.092	0.538	0.107	-0.005	-0.0187 to 0.0078	0.42

Values are in millimeters. Mean change: mean change from baseline to 2 years. CCA: common carotid artery; BIF: carotid bifurcation; ICA: internal carotid artery; aggrCA: aggregate carotid artery (mean of all segments); CFA: common femoral artery; SFA: superficial femoral artery

habits. The effect of the two statins used was analyzed by correcting the change in IMT for duration of cerivastatin treatment (range 6–23 months). This did not change the results.

Clinical events

Cardiovascular events occurred in 12 patients in the placebo group and 2 in the statin group ($p = 0.006$). Coronary events occurred in four patients in the placebo group and none in the statin group ($p = 0.122$). Four patients in the placebo group and three in the statin group died. The causes of death were cancer ($n = 4$), sepsis ($n = 1$), and hemorrhagic stroke ($n = 2$). Malignancies occurred in eight patients: four in the placebo group and four in the statin group. Myalgia was reported 18 times in the statin group and 26 times in the placebo group and was never accompanied by an increase in creatinine kinase. In one patient in the statin group, at his 24-month visit, ALT was raised more than three times above the upper limit of normal. This was attributed to steatosis hepatis.

CONCLUSIONS

This is the first prospective study in patients with type 2 diabetes but without overt CVD that investigated the effect of statins versus placebo on carotid and femoral IMT. Despite a mean LDL cholesterol reduction of 25%, we did not find any effect of 2 years' statin therapy on mean common carotid IMT.

In patients with familial hypercholesterolemia and in patients with established CAD, statin therapy has resulted in significantly less progression or even regression of carotid IMT[21-23]. In the only other randomized controlled lipid intervention IMT study in type 2 diabetic patients, 3 years' therapy with bezafibrate did not have any effect on carotid and femoral IMT [24]. Our findings warrant several remarks. First, the mean LDL cholesterol reduction of 25% is fully comparable to statin-induced LDL cholesterol reductions ranging between 22 and 29% in studies of non-diabetic patients, showing a significant effect after 18–24 months on carotid IMT [21,23,25,26]. Second, contrary to the postulated progression of mean common carotid artery IMT of 0.03mm per 2 years, in the present study there was a nonsignificant regression of 0.006 mm per 2 years in the placebo group. It could be argued that our patient population had been low risk. However, we included diabetic patients with a broad range in age and diabetes duration, while their baseline common carotid artery IMT was quite comparable to that of patients in other studies[10,27]. Moreover, the observed rate of first major vascular events (myocardial infarction, strokes, and revascularizations) in our placebo group, which translates to 14% per 5 years, is similar to the incidence rate of 13.5% in the diabetic placebo subgroup without prior CVD in the Heart Protection Study [16]. Thus, it seems unlikely that our results have been influenced by any healthy volunteer effect. Third, in our study, we observed no association between LDL cholesterol reduction and IMT reduction. This is at variance with the effect of statin therapy on IMT in non-diabetic patients [22] but in agreement with the effect of 3 years' bezafibrate treatment (LDL cholesterol reduction 9.6%) on carotid and femoral IMT in type 2 diabetic patients [24]. Equally, baseline IMT in the present study was not correlated to baseline LDL cholesterol or any other lipid level, similar to the results of several previous cross-sectional studies in type 2 diabetes[10,28]. Finally, given the low inter- and intra-observer variability in our IMT measurements compared with other studies[29], we strongly feel that the quality of the assessments has not biased the results.

As our study was designed with the assumption of similar IMT progression rates in diabetic and coronary patients, we conclude from the lack of IMT progression in our placebo group that IMT progression rates in diabetic patients are lower than those of patients with CAD. This is supported by recently published cohort studies in which diabetes was not predictive of IMT progression [30,31]. Moreover, the ARIC (Atherosclerosis Risk in Communities) study[32] and the IRAS (Insulin Resistance Atherosclerosis Study)[33] have recently shown progression rates for diabetic subjects of 0.011 and 0.0072 mm/year, respectively, both of which are lower than those observed in patients with CAD[34].

We observed a statistically significant effect on the incidence of predefined cardiovascular events. This is in agreement with the results of the recently published CARDS (Collaborative Atorvastatin Diabetes Study)[35] and with the results of a meta-analysis on the effects of lipid management for type 2 diabetic patients in primary prevention[36]. As we did not find any IMT regression, we postulate that statin-induced cardiovascular event reduction in diabetic patients is not related to IMT regression. From a pathophysiological point of view, the intimal and medial layers of the vessel wall in type 2 diabetes are most likely irreversibly changed by processes such as extracellular matrix glycosylation and media calcification[37,38]. These changes may resist global regression based on interference with local intravascular cholesterol metabolism. We hypothesize that although statins do not influence the irreversibly changed glycosylated extracellular matrix, it may well have an effect on outcome in type 2 diabetic patients by its beneficial influence on plaque vulnerability. To accurately measure IMT in our study, we avoided eccentric plaques; therefore, we cannot address this hypothesis with the available data.

Our study has its limitations. First, cerivastatin was withdrawn from the market, resulting in a change from cerivastatin to simvastatin. After correcting the change in IMT for duration of cerivastatin treatment, however, the results remained unchanged. Second, as a result of the withdrawal of cerivastatin, we had a higher withdrawal rate than anticipated in our sample size estimation. However, except for ethnicity, which was not a determinant of IMT or IMT progression, baseline characteristics did not differ between the drop outs and the 182 patients who fulfilled the study. Moreover, given the narrow CI of the mean difference in common carotid artery IMT change between placebo and statin (95% CI -0.0281 to 0.0132 mm), we can exclude a type II error.

In conclusion, 2 years of statin therapy in a broad range of type 2 diabetic patients without prior manifest atherosclerotic disease did not have any effect on carotid and femoral IMT. The natural history of atherosclerosis progression, as measured by IMT in type 2 diabetic patients, was milder than previously postulated.

We observed a lower cardiovascular event rate in patients on statin therapy, which is in line with other clinical trials. As this benefit has not been related to IMT regression, other mechanistic explanations, like a beneficial effect on plaque vulnerability, might be of importance. Vessel wall biology in type 2 diabetes is distinct from other high-risk patients, and this implies that prognostic tools other than IMT should be evaluated in this patient group.

REFERENCES

1. Stamler J, Vaccaro O, Neaton JD, Wentworth D: Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care* 16:434– 444, 1993
2. Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R: Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation* 74: 1399–1406, 1986
3. Burke GL, Evans GW, Riley WA, Sharrett AR, Howard G, Barnes RW, Rosamond W, Crow RS, Rautaharju PM, Heiss G: Arterial wall thickness is associated with prevalent cardiovascular disease in middle- aged adults: the Atherosclerosis Risk in Communities (ARIC) study. *Stroke* 26: 386–391, 1995
4. Lekakis JP, Papamichael CM, Cimponeriu AT, Stamatelopoulos KS, Papaioannou TG, Kanakakis J, Alevizaki MK, Papapanagiotou A, Kalofoutis AT, Stamatelopoulos SF: Atherosclerotic changes of extracoronary arteries are associated with the extent of coronary atherosclerosis. *Am J Cardiol* 85:949 –952, 2000
5. Salonen JT, Salonen R: Risk factors for carotid and femoral atherosclerosis in hypercholesterolaemic men. *J Intern Med* 236:561–566, 1994
6. O'Leary DH, Polak JF, Kronmal RA, Savage PJ, Borhani NO, Kittner SJ, Tracy R, Gardin JM, Price TR, Furberg CD: Thickening of the carotid wall: a marker for atherosclerosis in the elderly? Cardiovascular Health Study Collaborative Research Group. *Stroke* 27:224 –231, 1996
7. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr: Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults: Cardiovascular Health Study Collaborative Research Group. *N Engl J Med* 340: 14–22, 1999
8. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE: Common carotid intimamedia thickness and risk of stroke and myocardial infarction: the Rotterdam Study. *Circulation* 96:1432–1437, 1997
9. Chambless LE, Heiss G, Folsom AR, Rosamond W, Szklo M, Sharrett AR, Clegg LX: Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) study, 1987–1993. *Am J Epidemiol* 146: 483–494, 1997
10. Niskanen L, Rauramaa R, Miettinen H, Haffner SM, Mercuri M, Uusitupa M: Carotid artery intima-media thickness in elderly patients with NIDDM and in non-diabetic subjects. *Stroke* 27:1986 – 1992, 1996
11. Goff DC Jr, D'Agostino RB Jr, Haffner SM, Saad MF, Wagenknecht LE: Lipoprotein concentrations and carotid atherosclerosis by diabetes status: results from the Insulin Resistance Atherosclerosis Study. *Diabetes Care* 23:1006–1011, 2000
12. Randomized trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 344:1383–1389, 1994
13. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomized placebo-controlled trial. *Lancet* 360: 7–22, 2002
14. Major outcomes in moderately hypercholesterolemic, hypertensive patients randomized to pravastatin vs usual care: the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT-LLT). *JAMA* 288:2998–3007, 2002
15. Sever PS, Dahlof B, Poulter NR, Wedel H, Beevers G, Caulfield M, Collins R, Kjeldsen SE, Kristinsson A, McInnes GT, Mehlsen J, Nieminen M, O'Brien E, Ostergren J: Prevention of coronary and stroke events with atorvastatin in hypertensive patients who have average or lower-than average cholesterol concentrations, in the Anglo-Scandinavian Cardiac Outcomes Trial–Lipid Lowering Arm (ASCOT-LLA): a multicentre randomized controlled trial. *Lancet* 361:1149–1158, 2003

16. Collins R, Armitage J, Parish S, Sleigh P, Peto R: MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomized placebo-controlled trial. *Lancet* 361: 2005–2016, 2003

17. Staffa JA, Chang J, Green L: Cerivastatin and reports of fatal rhabdomyolysis. *N Engl J Med* 346:539–540, 2002

18. Jones P, Kafonek S, Laurora I, Hunninghake D: Comparative dose efficacy study of atorvastatin versus simvastatin, pravastatin, lovastatin, and fluvastatin in patients with hypercholesterolemia (the CURVES study). *Am J Cardiol* 81:582– 587, 1998

19. Stein E: Cerivastatin in primary hyperlipidemia: a multicenter analysis of efficacy and safety. *Atherosclerosis* 139 (Suppl. 1): S15–S22, 1998

20. Laird NM, Ware JH: Random-effects models for longitudinal data. *Biometrics* 38:963–974, 1982

21. MacMahon S, Sharpe N, Gamble G, Hart H, Scott J, Simes J, White H: Effects of lowering average of below-average cholesterol levels on the progression of carotid atherosclerosis: results of the LIPID Atherosclerosis Substudy: LIPID Trial Research Group. *Circulation* 97:1784–1790, 1998

22. Smilde TJ, van Wissen S, Wollersheim H, Trip MD, Kastelein JJ, Stalenhoef AF: Effect of aggressive versus conventional lipid lowering on atherosclerosis progression in familial hypercholesterolaemia (ASAP): a prospective, randomized, double- blind trial. *Lancet* 357:577–581, 2001

23. de Groot E, Jukema JW, van Boven AJ, Reiber JH, Zwinderman AH, Lie KI, Ackerstaff RA, Bruschke AV: Effect of pravastatin on progression and regression of coronary atherosclerosis and vessel wall changes in carotid and femoral arteries: a report from the Regression Growth Evaluation Statin Study. *Am J Cardiol* 76:40C– 46C, 1995

24. Elkeles RS, Diamond JR, Poulter C, Dhanjil S, Nicolaides AN, Mahmood S, Richmond W, Mather H, Sharp P, Feher MD: Cardiovascular outcomes in type 2 diabetes: a double-blind placebo-controlled study of bezafibrate: the St. Mary's, Ealing, Northwick Park Diabetes Cardiovascular Disease Prevention (SENDCAP) study. *Diabetes Care* 21:641– 648, 1998

25. Furberg CD, Adams HP Jr, Applegate WB, Byington RP, Espeland MA, Hartwell T, Hunninghake DB, Lefkowitz DS, Probstfield J, Riley WA: Effect of lovastatin on early carotid atherosclerosis and cardiovascular events: Asymptomatic Carotid Artery Progression Study (ACAPS) Research Group. *Circulation* 90:1679–1687, 1994

26. Mercuri M, Bond MG, Sirtori CR, Veglia F, Crepaldi G, Feruglio FS, Descovich G, Ricci G, Rubba P, Mancini M, Gallus G, Bianchi G, D'Alo G, Ventura A: Pravastatin reduces carotid intima-media thickness progression in an asymptomatic hypercholesterolemic mediterranean population: the Carotid Atherosclerosis Italian Ultrasound Study. *Am J Med* 101:627–634, 1996

27. Keven K, Gullu S, Cesur V, Gurses MA, Aytac S, Kamel N, Erdogan G: Carotid intima-media thickness in patients with newly diagnosed T2DM and impaired glucose tolerance. *Diabetes Nutr Metab* 12: 49–51, 1999

28. Kanters SD, Algra A, Banga JD: Carotid intima-media thickness in hyperlipidemic type I and type II diabetic patients. *Diabetes Care* 20:276 –280, 1997

29. Kanters SD, Algra A, van Leeuwen MS, Banga JD: Reproducibility of in vivo carotid intima-media thickness measurements: a review. *Stroke* 28:665–671, 1997

30. Lakka TA, Laukkanen JA, Rauramaa R, Salonen R, Lakka HM, Kaplan GA, Salonen JT: Cardiorespiratory fitness and the progression of carotid atherosclerosis in middle-aged men. *Ann Intern Med* 134: 12–20, 2001

31. van der Meer IM, Iglesias del Sol A, Hak AE, Bots ML, Hofman A, Witteman JC: Risk factors for progression of atherosclerosis measured at multiple sites in the arterial tree: the Rotterdam Study. *Stroke* 34:2374–2379, 2003

32. Chambless LE, Folsom AR, Davis V, Sharrett R, Heiss G, Sorlie P, Szklo M, Howard G, Evans GW: Risk factors for progression of common carotid atherosclerosis: the Atherosclerosis Risk in Communities Study, 1987–1998. *Am J Epidemiol* 155: 38–47, 2002

33. Wagenknecht LE, Zaccaro D, Espeland MA, Karter AJ, O'Leary DH, Haffner SM: Diabetes and progression of carotid atherosclerosis: the Insulin Resistance Atherosclerosis Study. *Arterioscler Thromb Vasc Biol* 23:1035–1041, 2003

34. Bots ML, Evans GW, Riley WA, Grobbee DE: Carotid intima-media thickness measurements in intervention studies: design options, progression rates, and sample size considerations: a point of view. *Stroke* 34:2985–2994, 2003

35. Colhoun HM, Betteridge DJ, Durrington PN, Hitman GA, Neil HA, Livingstone SJ, Thomason MJ, Mackness MI, Charlton-Menys V, Fuller JH: Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomized placebo-controlled trial. *Lancet* 364:685–696, 2004

36. Vijan S, Hayward RA: Pharmacologic lipid-lowering therapy in type 2 diabetes mellitus: background paper for the American College of Physicians. *Ann Intern Med* 140:650–658, 2004

37. Schmidt AM, Yan SD, Wautier JL, Stern D: Activation of receptor for advanced glycation end products: a mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. *Circ Res* 84:489–497, 1999

38. Sakata N, Takeuchi K, Noda K, Saku K, Tachikawa Y, Tashiro T, Nagai R, Horiuchi S: Calcification of the medial layer of the internal thoracic artery in diabetic patients: relevance of glycoxidation. *J Vasc Res* 40:567–574, 2003

Chapter 4

The effect of statin therapy on endothelial function in type 2 diabetes mellitus without manifest cardiovascular disease

ED Beishuizen[1], JT Tamsma[1], JW Jukema [2],
MA van de Ree [3], JCM van der Vijver[4],
AE Meinders [1], MV Huisman[1]

[1] Department of General Internal Medicine and Endocrinology , Leiden University Medical Center

[2] Department of Cardiology, Leiden University Medical Center

[3] Department of Internal Medicine, Diakonessenhuis, Utrecht

[4] Department of Internal Medicine, Leyenburg Hospital, The Hague

The Netherlands

Diabetes Care 2005; 28: 1668-1674

ABSTRACT

Objective

Cardiovascular disease is the most important cause of mortality in patients with type 2 diabetes mellitus (DM2) and is preceded by endothelial dysfunction. Flow Mediated Dilation (FMD) is a non-invasive technique for measuring endothelial dysfunction. We aimed to determine the effect of long-term statin therapy versus placebo on FMD in patients with DM2 without manifest cardiovascular disease.

Research Design and Methods

A randomized, placebo-controlled, double-blind trial was performed in 250 patients with DM2. Patients were given 0.4 mg cerivastatin or placebo daily. In August 2001, when cerivastatin was withdrawn from the market, 0.4 mg cerivastatin was replaced by 20 mg simvastatin, without deblinding the study. The primary endpoint was the change in FMD, measured by B-mode ultrasound, after 2 years.

Results

Determinants of baseline FMD were diabetes duration, common carotid intima-media thickness (IMT) and brachial artery diameter. FMD at baseline was 1.51 % in the placebo group and 1.66 % in the statin group and did not change significantly after two years.

Conclusions

There was no effect of 2 years' statin therapy on FMD in DM2. Statin-induced improvement of cardiovascular risk in patients with DM2 may be mediated through mechanisms other than increased nitric oxide availability.

INTRODUCTION

Cardiovascular disease (CVD) is the most important cause of mortality in patients with type 2 diabetes mellitus (DM2) [1]. Endothelial dysfunction precedes the development of atherosclerotic plaques and is believed to be reversible[2]. Nitric oxide (NO) is a key molecule in this process: it modulates blood flow and vascular permeability, it limits inflammation and coagulation and diminishes vascular smooth muscle cell proliferation and migration. DM2 is associated with endothelial dysfunction, the underlying mechanisms are complex and related to hyperglycemia (sorbitol, hexosamin, Protein Kinase C, and Advanced Glycemic Endproducts pathways) and insulin resistance, resulting in mitochondrial superoxide overproduction and thus decreased NO availability[3]. Regarding insulin, its vasodilatory capacity is at least in part NO dependent[4;5], thus explaining how insulin resistance might be related to endothelial dysfunction.

Flow Mediated Dilation (FMD) of the brachial artery is a non-invasive technique for measuring endothelial function. FMD of the brachial artery has been shown to be the result of endothelium-derived NO release [6] and is related to coronary vasoreactivity[7]. FMD has proven to be predictive for the presence of coronary artery disease [8;9], for future cardiovascular events[10-12] and for postoperative cardiovascular events [13] in high-risk populations. Improvement in FMD predicts a favourable cardiovascular outcome in postmenopausal hypertensive women[2]. However, in patients at lower risk, FMD was not independently associated with outcome[14]. FMD is impaired in patients with DM2 with FMD values reported from 4.47-12.3 % in controls versus 2.96-6.1% in DM2 patients in cross-sectional studies[15-22].

HMG-coenzyme A reductase inhibitors (statins) have been shown to reverse endothelial dysfunction in hypercholesterolemic non-diabetic patients, possibly through upregulation of endothelial Nitric Oxide Synthase expression [23-25], resulting in increased NO production. Statins also inhibit superoxide production [25], thereby reducing NO breakdown. The net effect is an increase in NO availability, theoretically within days after starting statin therapy. This may explain the rapid improvement in endothelial dysfunction observed in several studies in non-diabetics[26]. In patients with type 2 diabetes, the results of studies with short-term statin therapy are, however, contradictory with respect to FMD. We therefore conducted a randomized, placebo- controlled trial to evaluate the effect of 2 years' statin therapy on endothelial function in patients with DM2 without CVD.

RESEARCH DESIGN AND METHODS

Subjects and design

The study design and baseline characteristics of the original patient population have been described elsewhere [27]. Briefly, 250 patients with DM2 for at least one year, aged 30-80 years,

without CVD were included in this randomized, double-blind, clinical trial. Patients were given 0.4 mg cerivastatin (Bayer B.V., Mijdrecht , The Netherlands) or placebo daily for 2 years. After the withdrawal of cerivastatin from the market, 0.4 mg cerivastatin was replaced by 20 mg simvastatin (Merck Sharp & Dome, Haarlem, the Netherlands), without deblinding the study. Only patients who completed the study were included in the present analysis. There were no significant differences in demographic or lipid parameters between the full cohort (n=250) and the patients in this study (n=182), except for race, as more Caucasians than non-Caucasians completed the study (data not shown). Eligible patients gave their written informed consent. The study was performed at the Leyenburg Hospital, The Hague. The study was approved by the hospital's Medical Ethics Committee.

Study Objectives

The primary endpoint of the study was the change in FMD between 24 months and baseline. Secondary endpoints were the change in absolute diameter (D_{max}-D), the time to peak (T_{max}), the change in Nitroglycerin Mediated Dilation (NMD) and the FMD-to-NMD ratio(FMD/NMD). Comparisons between standard measurements for FMD at 1 minute after cuff deflation and for NMD at 3,4 or 5 minutes after Nitroglycerin administration and real maximum values obtained by beat-to-beat analysis were analyzed as an exploratory endpoint.

Follow-up

Patients returned to the study site after a 12 hours fast at 3, 6, 12, 18 and 24 months when blinded lipid and safety measurements (creatinin kinase, ALT) were performed. Ultrasound measurements were performed at baseline and 24 months. Two years follow-up for clinical events was performed for all 250 patients.

Ultrasound measurements

Ultrasound imaging was performed with an Acuson Aspen scanner with a linear array 7.5 MHz probe. All images were recorded digitally for off-line, blinded, analysis by an independent core laboratory, Heart Core, Leiden, the Netherlands. During the study, all measurements were performed by the same two, certified, ultrasonographers.

Fasting subjects were examined in the supine position. Heart rate was continuously monitored by three-lead ECG. Mean common carotid artery Intima-Media Thickness (CCA IMT) was measured as reported earlier [27]. Briefly, the left and right distal 1.0 cm of the common carotid arteries, near and far walls, were examined longitudinally in the angle resulting in an optimal and maximal IMT (while avoiding plaques). For each segment, three R-wave triggered images were stored. Mean IMT was measured, when possible, over the entire 1 cm of the vessel segment. CCA IMT was obtained by averaging the mean IMT's of far and near wall, left and right.

For FMD the right arm was placed in extension in the elbow, hand in supination, wrist and elbow supported by foam cushions. An optimal longitudinal image of the brachial artery at, or just above the elbow, was established and kept stable using a specially designed fixative. To obtain clearer images, a water bag was placed between the transducer and the skin. At baseline, 15 consecutive R-wave triggered beats were stored. A cuff placed just distally from the elbow was inflated to 50 mm Hg above systolic blood pressure (up to a maximum of 230 mm Hg) for four minutes. After deflation, R-wave frozen images were recorded for every beat, during 5 minutes. After 10 minutes rest again 15 R-wave triggered beats were stored. Subsequently two puffs of nitroglycerin (0.8 mg) spray were given sublingually, upon which again R-wave frozen images were recorded for every beat during 5 minutes.

Lumen diameter (D) was defined as the distance between the media-adventitia interfaces of far and near wall. Using an automated contour detection system, D was measured semi-automatically by placing a cursor on the media-adventitia interfaces. FMD was defined as the percentage increase in brachial artery diameter within 30 to 120 seconds after ischemia ((D_{max}-D)/ D). NMD is defined as the percentage increase within five minutes after nitroglycerin.

Earlier studies in our institute reported reliability coefficients of 99%, 99% and 67% for baseline diameter, peak diameter and FMD respectively [28]. In a recent report on variability of FMD (using a continuous method like we did) in DM2, CVs for baseline diameter, peak diameter and FMD were 2.7, 2.5 and 29.7%, respectively[29].

Laboratory investigations

All laboratory measurements were performed at the Department of Clinical Chemistry and Hematology of the Leyenburg Hospital, according to ISO 15189 standard procedures. Blood samples were collected from the subjects after a 12 hour fast. EDTA tubes were used for the determination of HbA1c. Liver enzymes and lipids were measured in serum. A urine sample was collected for the determination of the albumin-to-creatinin ratio. Serum or plasma was isolated by centrifugation at 1700 g (2900 rpm) for 5 minutes.

Serum levels of total cholesterol and triglycerides were measured by enzymatic methods on a Synchron LX20-analyzer (Beckman Coulter, Brea, USA). LDL cholesterol was calculated according to the Friedewald formula. If triglycerides were > 4.5 mmol/l, LDL cholesterol was measured directly with the use of a reagent kit (Genzyme Diagnostics). HDL cholesterol levels were determined after dextran sulfate-magnesium precipitation of apolipoprotein B-containing lipoproteins. Creatinin kinase and ALT were measured by an enzymatic rate method on a Synchron LX20 multichannel chemistry analyzer, according to IFCC-methods. HbA1c was measured by HPLC on a Variant II (BioRad, USA). For the urine sample, a Jaffé rate method was used for the measurement of creatinine on a Synchron LX20-analyzer, while albumin was measured by rate nephelometry.

Statistical analysis

The number of patients needed to detect a difference in FMD of 2% after 2 years (expected SD 4%) with a power of 80 % (α = 0.05) was 63 patients in each group. The primary treatment comparison was between placebo and statin therapy in patients completing the study, as on-treatment analysis. Changes from baseline within each treatment group were analyzed using Student's paired t-test. Comparisons of the effects between the treatment groups were performed using Student's independent samples t-test. Stepwise regression techniques were used to investigate the effect on baseline FMD and on changes in FMD of baseline characteristics, carotid IMT and duration of cerivastatin versus simvastatin use. To test the equivalence of cerivastatin 0.4 mg and simvastatin 20 mg, LDL levels before and after the switch to simvastatin were compared using Student's paired t-test. Correlation between changes in FMD and changes in lipid levels were evaluated by calculating Pearson's correlation coefficients. Comparison between beat-to-beat analysis and standard methods was performed using the Student's paired t-test and Bland Altman analysis[30].

Analyses were performed using SPSS 11.0 for Windows software. All analyses were 2-sided, with a level of significance of α = 0.05.

RESULTS

The characteristics of the study population are given in Table 1. No statistical differences between the groups were observed.

Table 1. Baseline Characteristics of 182 patients

	Placebo (n=79)	Statin (n=103)
Male sex	38 (48)	52 (51)
Age (years)	59 ± 10	59 ± 11
Ethnicity		
Caucasian	60 (76)	72 (70)
Asian-Indian	10 (13)	21 (20)
Other	9 (11)	10 (10)
BMI (kg/m^2)	31.2 ± 6.0	30.5 ± 5.4
Waist-to-hip ratio	1.00 ± 0.09	0.98 ± 0.08
Current smoker	19 (24)	27 (26)
Hypertension	46 (58)	49 (48)
Diabetes duration (years)	9 ± 8	8 ± 7
Insulin use	45 (57)	51 (50)
HbA1c (%)	7.68 ± 1.31	7.50 ± 0.98
Microalbuminuria *	12 (15)	21 (20)
CCA IMT (mm)	0.780 ± 0.129	0.763 ± 0.124

Data are means ± SD or n (%).

* Men, > 2.5 g/mol creatinine;women > 3.5 g/mol creatinine

CCA IMT: Intima-media Thickness of the common carotid artery.

Lipids

LDL cholesterol was 3.44 ± 0.71 mmol/l at baseline and 2.58 ± 0.95 mmol/l at 2 years (-25 %, p < 0.001) in the statin group and 3.55 ± 0.71 mmol/l at baseline and 3.78 ± 0.81 mmol/l at 2 years (+8 %, p=0.003) in the placebo group (p < 0.001). HDL cholesterol was 1.23 ± 0.39 mmol/l at baseline and 1.20 ± 0.36 mmol/l at 2 years in the statin group and 1.21 ± 0.37 mmol/l at baseline and 1.22 ± 0.38 mmol/l at 2 years in the placebo group. Triglycerides were 1.88 ± 0.79 mmol/l at baseline and 1.72 ± 1.22 mmol/l at 2 years in the statin group and 1.82 ± 0.97 mmol/l at baseline and 1.60 ± 1.38 mmol/l at 2 years in the placebo group. Changes in HDL cholesterol and triglycerides were not significantly different compared with baseline or the placebo group. Average LDL cholesterol levels were higher after the switch to simvastatin (2.34 before versus 2.56 mmol/l after the switch, p < 0.001).

FMD

Baseline FMD was not significantly different between the groups. Baseline FMD as in the group of 182 patients who completed the study was not significantly different from baseline FMD in the drop-outs (data not shown). For the 182 patients who completed the study, FMD in the placebo group was 1.51 % at baseline and 1.59 % at 2 years (p =0.78), in the statin group it was 1.66 % at baseline and 2.10 % at 2 years (p=0.10)(Table 2). There was no significant difference between the change in FMD in the statin group and the placebo group

Table 2. Parameters for endothelial function of 182 patients

	Baseline	2 years	Mean change [95% CI]	p
Placebo (n= 79)				
Primary endpoint				
FMD (%)	1.51 ± 1.73	1.59 ± 1.84	0.08 [-0.50 to 0.66]	0.78
Secondary endpoints				
D (mm)	4.77 ± 0.55	4.82 ± 0.58	0.05 [-0.03 to 0.12]	0.22
D_{max}-D (mm)	0.07 ± 0.08	0.08 ± 0.09	0.01 [-0.02 to 0.03]	0.61
T_{max} (sec)	65 ± 30	64 ± 29	-1 [-11 to 8]	0.78
NMD (%)	10.24 ± 4.40	10.28 ± 4.32	0.04 [-0.87 to 0.94]	0.94
FMD/NMD	0.14 ± 0.19	0.18 ± 0.22	0.04 [-0.03 to 0.10]	0.31
Statin (n= 103)				
Primary endpoint				
FMD (%)	1.66 ± 1.75	2.10 ± 2.20	0.44 [-0.08 to 0.96]	0.10
Secondary endpoints				
D(mm)	4.67 ± 0.70	4.67 ± 0.69	0.00 [-0.08 to 0.08]	0.97
D_{max}-D(mm)	0.08 ± 0.08	0.09 ± 0.10	0.02 [0.00 to 0.04]	0.10
T_{max} (sec)	64 ± 28	61 ± 26	-3 [-10 to 4]	0.37
NMD (%)	10.98 ± 5.73	10.27 ± 4.56	-0.71[-1.64 to 0.22]	0.13
FMD/NMD	0.19 ± 0.30	0.23 ± 0.26	0.04 [-0.04 to 0.11]	0.36

Data are means ± SD or means [95 % CI]

Mean change = mean change from baseline to 2 years

D: brachial artery diameter; D_{max}: maximal brachial artery diameter after ischemia;

T_{max}: time to reach maximal brachial artery diameter

(mean difference 0.36 % [95% CI –0.42 to 1.13 %] p=0.37). We performed an intention-to-treat analysis for the whole group of 250 patients by using the method of 'last observation carried forward' for missing values: FMD in the placebo group was 1.69 % at baseline and 1.75 % at 2 years (p =0.78), in the statin group it was 1.65 % at baseline and 2.02 % at 2 years (p=0.10). There was no significant difference between the change in FMD in the statin group and the placebo group (mean difference 0.32 % [95% CI –0.89 to 0.26 %] p=0.28). There was also no significant difference between the changes in absolute increase in diameter after ischemia, T_{max}, NMD and the FMD-to-NMD ratio.

Determinants for ***baseline FMD*** were age (r = -0.145; p = 0.055), systolic blood pressure (r = -0.192; p =0.011), diabetes duration (r = -0.160; p = 0.034) and baseline brachial artery diameter (r = -0.582; p < 0.001). Baseline CCA IMT as a continuous variable was not a determinant of baseline FMD. However, when split into quartiles, FMD at baseline was significantly lower in the highest CCA IMT quartile compared with the three lower CCA IMT quartiles (0.94 % versus 1.77 %, p=0.006). When included into a regression model, only highest quartile CCA IMT, diabetes duration and baseline brachial artery diameter remained significant determinants and together explained 11% of the variance in baseline FMD.

Baseline FMD and changes in FMD were not correlated with LDL cholesterol or any other lipid parameter. Baseline FMD and changes in FMD were also not related to sex, race, insulin use, anti-hypertensive medication, HbA1c, anthropometric parameters and smoking habits. ***Changes in FMD*** were not related to baseline CCA IMT. Changes in FMD were negatively correlated to changes in CCA IMT in the placebo group (r = -0.259; p = 0.029). Thus, an increase in CCA IMT in the placebo group during follow-up was associated with a decrease in FMD. This could not be observed in the statin group.

The effect of the two statins used was analyzed by correcting the change in FMD for duration of cerivastatin treatment (range 6 to 23 months). This did not change the results.

The D_{max}, FMD and NMD as determined by beat-to-beat analysis were significantly higher compared with values obtained at fixed times. The extent of these differences was not related to absolute values. However, standard deviations of the baseline values and confidence intervals of the changes after two years were not lower in the beat-to-beat analysis (data not shown). When repeating the analysis with fixed times values as an outcome measure, results did not change.

CONCLUSIONS

Patients with DM2 have a high-risk of cardiovascular events and endothelial dysfunction can be viewed as an early sign of atherosclerosis. No long-term, blinded, placebo-controlled trials on the effect of statin therapy on endothelial function in DM2 have been reported. The

present study shows that in our patient group endothelial dysfunction is not reversible with medium-dose statin therapy.

Several earlier studies have been performed to evaluate the effect of statin therapy on FMD in patients with DM2. In Table 3 these studies are summarized. In a randomized study, van Venrooy et al. [28] did not find an effect of 30 weeks atorvastatin (10 or 80 mg) versus placebo on FMD. Ceriello et al. [16] reported an improvement in FMD after simvastatin 40 mg given for only three to six days. Recently, Economides et al. reported a non-significant improvement in FMD after 12 weeks atorvastatin 20 mg [31]. The other studies are not randomized trials or open label trials [21 32 33]. There are several explanations for the discrepancy in the results of these studies. All studies have included patients without CVD and age, diabetes duration and HbA1c seem quite comparable. However, FMD methodology was not always clearly defined. First, the way D_{max} is determined is critical. Simply measuring once, one minute after cuff deflation, or measuring every 15 seconds, can result in underestimation, but in case of outliers, also in overestimation of FMD. Beat-to-beat analysis results in a more precise estimate of D_{max}, but did not lead to lower confidence intervals in the present study. Second, several authors do not mention their baseline lumen diameters, which is an established determinant of FMD [34]. If lumen diameter is defined as the distance between the intima-lumen interfaces instead of media-adventitia interfaces of the vessel wall, lumen diameter decreases and FMD increases. Third, some authors do not mention whether the cuff is placed around the forearm or upper arm. This is a critical issue because the latter location results in a higher FMD.

Baseline FMD in our patients was low in comparison to the diabetic populations in the intervention studies mentioned, but comparable to another Dutch study, the Hoorn study (FMD 2.96 %) [22] and to a cross-sectional study (FMD 1.9 %) in diabetic patients with microalbuminuria[35]. In our study with long-term statin therapy, more patients per treatment arm were included than in any other study and we used the beat-to-beat analysis for optimal precision. Moreover, given the confidence interval of the mean difference in FMD change between placebo and statin, there is a 95 % certainty that there is no treatment effect greater than an absolute difference in FMD of 1.13 %.

There is much debate whether statin induced improvement of endothelial function is mediated through a change in lipid profile, through so-called pleiotropic effects or both. In the present study we found no relation between (changes in) lipid profile and (changes in) FMD. There is also much discussion about possible differing pleiotropic effects between the different statins [36]. In our study, because of unforeseen circumstances, two different statins have been used and we found no difference in effect on FMD between the statins.

Until recently, the value of statin therapy in diabetic patients was not clear in the setting of primary prevention. However, a recent meta-analysis[37] and the CARDS trial, in which diabetic patients with at least one additional cardiovascular risk factor were included[38], reported marked cardiovascular risk reduction. We also found a reduced cardiovascular event rate in the statin-treated group in the present study population as reported before [27]. Event

Table 3. Intervention studies on the effect of statins on FMD in patients with DM2 without CVD

Author	N	Design	Dmax *method*	Inflation *mmHg*	Cuff	Statin dose *mg*	LDL↓ %	F-up *weeks*	D *mm*	FMD_{bl} %	$FMD_{f\text{-}up}$ %	p	NMD_{bl} %	$NMD_{f\text{-}up}$ %
Sheu[21]	21	non-rand	NA	200	NA	simva 10	36	24	4.71	6.1	7.7	NS	14.5	13.3
Sheu[32]	6	non-rand	NA	200	NA	simva 20-40	>2.1 mmol/L	12	NA	4.4	8.2	0.173†	NA	NA
	6						<2.1 mmol/L	12	NA	5.6	13.6	<0.028†	NA	NA
Tsunekawa[33]	14	RCT open	at 60 s.	250	forearm	ceriva 0.15	2	0.5	NA	4.0*	8.5*	<0.05	7.0*	7.4*
	8						21	14	NA	4.0*	8.5*	<0.05	6.5*	7.5*
Venrooij[28]	46	RCT db	per 15 s.	20>BP_{sys}	forearm	atorva 10	46	30	4.89	3.41	3.20	>0.8	6.80	6.87
	43					atorva 80	51	30	4.77	3.18	3.10	>0.8	6.01	6.59
Ceriello[16]	30	cross-over RCT db	45-90 s. b-t-b	300	forearm	simva 40	3	0.5	NA	4.8	7.3	<0.001	NA	NA
	30						28	14	NA	4.9	9.2	<0.05	NA	NA
Economides[31]	19	RCT db	NA	50>BP_{sys}	forearm	atorva20	41	14	3.7	4.2	5.6	0.07†	12.5	11.9
present study	103	RCT db	30-120 s. b-t-b	50>BP_{sys}	forearm	ceriva 0.4 / simva 20	25	104	4.67	1.66	2.10	0.37	10.98	10.27

N = number of patients in the statin treated group; NA = data not available

non-rand = non-randomized trial; RCT = randomized controlled trial; db = double blind; b-t-b = beat-to-beat analysis

BP_{sys} = systolic blood pressure; LDL↓ % = percentage decrease in LDL cholesterol; F-up = Follow-up

D = lumendiameter; FMD_{bl} = FMD at baseline; $FMD_{f\text{-}up}$ = FMD at follow-up; NMD_{bl} = NMD at baseline; $NMD_{f\text{-}up}$ = NMD at follow-up

p = p-value for comparison of changes in FMD between statin and placebo; † p-value for comparison of FMD at follow-up with FMD at baseline

* = estimated from figure

reduction on the one hand and no difference in FMD on the other, imply that statin-induced risk reduction in DM2 is either not mediated through restoration of endothelial dependent dilation or that FMD is not a proper test to detect changes in endothelial dysfunction in DM2 patients. The latter possibility is less likely, because forearm blood flow measured by venous occlusion plethysmography, another parameter for endothelial function, also showed no improvement after statin therapy in diabetic patients[39;40]. Other interventions in patients with recent-onset DM2 have resulted in an improvement in FMD[41], indicating that FMD is not simply irreversibly impaired in DM2. Moreover, diabetes duration, carotid IMT and vessel diameter together only explain 11% of the variance in FMD, indicating that irreversible diabetic vessel wall changes may not have an important impact on FMD in this population. Therefore, we conclude that statin induced cardiovascular risk reduction in DM2 is probably not mediated through improved NO availability. Other mechanisms, such as suppression of inflammatory response, improvement of plaque stability and reduced thrombogenic potential of the endothelial cell [42], are possible alternative explanations for the beneficial effect of statin therapy in diabetic subjects. Our results imply that in patients with DM2, FMD is not a proper intermediate endpoint for statin studies. Until now, data on the prognostic value of FMD for future cardiovascular events in patients with DM2 are lacking.

We feel that the present study adds strongly to the evidence that medium-dose statin therapy has no effect on FMD in DM2 subjects without manifest CVD. FMD is impaired in diabetes of longer duration and with higher carotid IMT. Beat-to-beat analysis gives a more precise estimate of D_{max}, but did not lead to lower confidence intervals in the present study. In patients with DM2, statin-induced improvement of cardiovascular risk may be mediated through mechanisms other than increased NO availability.

REFERENCES

1. Stamler J, Vaccaro O, Neaton JD, Wentworth D: Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care* 16:434-444, 1993
2. Modena MG, Bonetti L, Coppi F, Bursi F, Rossi R: Prognostic role of reversible endothelial dysfunction in hypertensive postmenopausal women. *J.Am.Coll.Cardiol.* 40:505-510, 2002
3. Creager MA, Luscher TF, Cosentino F, Beckman JA: Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: Part I. *Circulation* 108:1527-1532, 2003
4. Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron AD: Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. *J.Clin.Invest* 94:1172-1179, 1994
5. Baron AD: Vascular reactivity. *Am.J.Cardiol.* 84:25J-27J, 1999
6. Joannides R, Haefeli WE, Linder L, Richard V, Bakkali EH, Thuillez C, Luscher TF: Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation* 91:1314-1319, 1995
7. Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, Lieberman EH, Ganz P, Creager MA, Yeung AC, .: Close relation of endothelial function in the human coronary and peripheral circulations. *J.Am.Coll.Cardiol.* 26:1235-1241, 1995
8. Schroeder S, Enderle MD, Ossen R, Meisner C, Baumbach A, Pfohl M, Herdeg C, Oberhoff M, Haering HU, Karsch KR: Non-invasive determination of endothelium-mediated vasodilation as a screening test for coronary artery disease: pilot study to assess the predictive value in comparison with angina pectoris, exercise electrocardiography, and myocardial perfusion imaging. *Am.Heart J.* 138:731-739, 1999
9. Teragawa H, Kato M, Kurokawa J, Yamagata T, Matsuura H, Chayama K: Usefulness of flow mediated dilation of the brachial artery and/or the intima-media thickness of the carotid artery in predicting coronary narrowing in patients suspected of having coronary artery disease. *Am.J.Cardiol.* 88:1147-1151, 2001
10. Neunteufl T, Heher S, Katzenschlager R, Wolfl G, Kostner K, Maurer G, Weidinger F: Late prognostic value of flow mediated dilation in the brachial artery of patients with chest pain. *Am.J.Cardiol.* 86:207-210, 2000
11. Chan SY, Mancini GB, Kuramoto L, Schulzer M, Frohlich J, Ignaszewski A: The prognostic importance of endothelial dysfunction and carotid atheroma burden in patients with coronary artery disease. *J.Am.Coll.Cardiol.* 42:1037-1043, 2003
12. Gokce N, Keaney JF, Jr., Hunter LM, Watkins MT, Nedeljkovic ZS, Menzoian JO, Vita JA: Predictive value of non-invasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. *J.Am.Coll.Cardiol.* 41:1769-1775, 2003
13. Gokce N, Keaney JF, Jr., Hunter LM, Watkins MT, Menzoian JO, Vita JA: Risk stratification for postoperative cardiovascular events via non-invasive assessment of endothelial function: a prospective study. *Circulation* 105:1567-1572, 2002
14. Fathi R, Haluska B, Isbel N, Short L, Marwick TH: The relative importance of vascular structure and function in predicting cardiovascular events. *J.Am.Coll.Cardiol.* 43:616-623, 2004
15. Tan KC, Chow WS, Ai VH, Metz C, Bucala R, Lam KS: Advanced glycation end products and endothelial dysfunction in type 2 diabetes. *Diabetes Care* 25:1055-1059, 2002
16. Ceriello A, Taboga C, Tonutti L, Quagliaro L, Piconi L, Bais B, Da Ros R, Motz E: Evidence for an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial dysfunction and oxidative stress generation: effects of short- and long-term simvastatin treatment. *Circulation* 106:1211-1218, 2002

17. Kawano H, Motoyama T, Hirashima O, Hirai N, Miyao Y, Sakamoto T, Kugiyama K, Ogawa H, Yasue H: Hyperglycemia rapidly suppresses flow mediated endothelium-dependent vasodilation of brachial artery. *J.Am.Coll.Cardiol.* 34:146-154, 1999

18. Goodfellow J, Ramsey MW, Luddington LA, Jones CJ, Coates PA, Dunstan F, Lewis MJ, Owens DR, Henderson AH: Endothelium and inelastic arteries: an early marker of vascular dysfunction in non-insulin dependent diabetes. *BMJ* 312:744-745, 1996

19. Balletshofer BM, Rittig K, Enderle MD, Volk A, Maerker E, Jacob S, Matthaei S, Rett K, Haring HU: Endothelial dysfunction is detectable in young normotensive first-degree relatives of subjects with type 2 diabetes in association with insulin resistance. *Circulation* 101:1780-1784, 2000

20. Tan KC, Ai VH, Chow WS, Chau MT, Leong L, Lam KS: Influence of low density lipoprotein (LDL) subfraction profile and LDL oxidation on endothelium-dependent and independent vasodilation in patients with type 2 diabetes. *J.Clin.Endocrinol.Metab* 84:3212-3216, 1999

21. Sheu WH, Juang BL, Chen YT, Lee WJ: Endothelial dysfunction is not reversed by simvastatin treatment in type 2 diabetic patients with hypercholesterolemia. *Diabetes Care* 22:1224-1225, 1999

22. Henry RM, Ferreira I, Kostense PJ, Dekker JM, Nijpels G, Heine RJ, Kamp O, Bouter LM, Stehouwer CD: Type 2 diabetes is associated with impaired endothelium-dependent, flow mediated dilation, but impaired glucose metabolism is not; The Hoorn Study. *Atherosclerosis* 174:49-56, 2004

23. Kaesemeyer WH, Caldwell RB, Huang J, Caldwell RW: Pravastatin sodium activates endothelial nitric oxide synthase independent of its cholesterol-lowering actions. *J.Am.Coll.Cardiol.* 33:234-241, 1999

24. Laufs U, La F, V, Plutzky J, Liao JK: Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. *Circulation* 97:1129-1135, 1998

25. Wagner AH, Kohler T, Ruckschloss U, Just I, Hecker M: Improvement of nitric oxide-dependent vasodilatation by HMG-CoA reductase inhibitors through attenuation of endothelial superoxide anion formation. *Arterioscler.Thromb.Vasc.Biol.* 20:61-69, 2000

26. Vogel RA, Corretti MC, Plotnick GD: Changes in flow mediated brachial artery vasoactivity with lowering of desirable cholesterol levels in healthy middle-aged men. *Am.J.Cardiol.* 77:37-40, 1996

27. Beishuizen ED, van de Ree MA, Jukema JW, Tamsma JT, van der Vijver JC, Meinders AE, Putter H, Huisman MV: Two-year statin therapy does not alter the progression of intima-media thickness in patients with type 2 diabetes without manifest cardiovascular disease. *Diabetes Care* 27 :2887-2892, 2004.

28. van Venrooij FV, van de Ree MA, Bots ML, Stolk RP, Huisman MV, Banga JD: Aggressive lipid lowering does not improve endothelial function in type 2 diabetes: the Diabetes Atorvastatin Lipid Intervention (DALI) Study: a randomized, double-blind, placebo-controlled trial. *Diabetes Care* 25:1211-1216, 2002

29. West SG, Wagner P, Schoemer SL, Hecker KD, Hurston KL, Likos KA, Boseska L, Ulbrecht J, Hinderliter AL: Biological correlates of day-to-day variation in flow mediated dilation in individuals with Type 2 diabetes: a study of test-retest reliability. *Diabetologia* 47:1625-1631, 2004

30. Bland JM, Altman DG: Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 327:307-310, 1986

31. Economides PA, Caselli A, Tiani E, Khaodhiar L, Horton ES, Veves A: The effects of atorvastatin on endothelial function in diabetic patients and subjects at risk for type 2 diabetes. *J.Clin.Endocrinol. Metab* 89:740-747, 2004

32. Sheu WH, Chen YT, Lee WJ: Improvement in endothelial dysfunction with LDL cholesterol level < 80 mg/dl in type 2 diabetic patients. *Diabetes Care* 24:1499-1501, 2001

33. Tsunekawa T, Hayashi T, Kano H, Sumi D, Matsui-Hirai H, Thakur NK, Egashira K, Iguchi A: Cerivastatin, a hydroxymethylglutaryl coenzyme a reductase inhibitor, improves endothelial function in elderly diabetic patients within 3 days. *Circulation* 104:376-379, 2001

34. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R: Guidelines for the ultrasound assessment of endothelial-dependent flow mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J.Am.Coll.Cardiol.* 39:257-265, 2002

35. Papaioannou GI, Seip RL, Grey NJ, Katten D, Taylor A, Inzucchi SE, Young LH, Chyun DA, Davey JA, Wackers FJ, Iskandrian AE, Ratner RE, Robinson EC, Carolan S, Engel S, Heller GV: Brachial artery reactivity in asymptomatic patients with type 2 diabetes mellitus and microalbuminuria (from the Detection of Ischemia in Asymptomatic Diabetics-brachial artery reactivity study). *Am.J.Cardiol.* 94:294-299, 2004

36. Sakabe K, Fukuda N, Wakayama K, Nada T, Shinohara H, Tamura Y: Time course differences for statin-induced pleiotropic effects in hypercholesterolemic patients. *Int.J.Cardiol.* 94:111-117, 2004

37. Vijan S, Hayward RA: Pharmacologic lipid-lowering therapy in type 2 diabetes mellitus: background paper for the American College of Physicians. *Ann.Intern.Med.* 140:650-658, 2004

38. Colhoun HM, Betteridge DJ, Durrington PN, Hitman GA, Neil HA, Livingstone SJ, Thomason MJ, Mackness MI, Charlton-Menys V, Fuller JH: Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomized placebo-controlled trial. *Lancet* 364:685-696, 2004

39. van de Ree MA, Huisman MV, de Man FH, van der Vijver JC, Meinders AE, Blauw GJ: Impaired endothelium-dependent vasodilation in type 2 diabetes mellitus and the lack of effect of simvastatin. *Cardiovasc.Res.* 52:299-305, 2001

40. van Etten RW, de Koning EJ, Honing ML, Stroes ES, Gaillard CA, Rabelink TJ: Intensive lipid lowering by statin therapy does not improve vasoreactivity in patients with type 2 diabetes. *Arterioscler. Thromb.Vasc.Biol.* 22:799-804, 2002

41. Caballero AE, Saouaf R, Lim SC, Hamdy O, Abou-Elenin K, O'Connor C, Logerfo FW, Horton ES, Veves A: The effects of troglitazone, an insulin-sensitizing agent, on the endothelial function in early and late type 2 diabetes: a placebo-controlled randomized clinical trial. *Metabolism* 52:173-180, 2003

42. Wolfrum S, Jensen KS, Liao JK: Endothelium-dependent effects of statins. *Arterioscler.Thromb. Vasc.Biol.* 23:729-736, 2003

Chapter 5

Differential effects of statin therapy on CRP in patients with type 2 diabetes with and without the metabolic syndrome

ED Beishuizen[1], JT. Tamsma[1], JW Jukema [2], MA van de Ree [3], JCM van der Vijver[4], H van Pelt[5], MV Huisman[1]

1 Department of General Internal Medicine and Endocrinology, Leiden University Medical Center, Leiden
2 Department of Cardiology, Leiden University Medical Center, Leiden
3 Department of Internal Medicine, Diakonessenhuis, Utrecht
4 Department of Internal Medicine, HAGA Hospital, The Hague
5 Department of Clinical Chemistry, Leiden University Medical Center, Leiden

The Netherlands

submitted

ABSTRACT

Objective

C-reactive protein (CRP) is a marker for the inflammatory process of atherosclerosis. We evaluated the effect of statin therapy on CRP in patients with type 2 diabetes mellitus (DM2) without manifest cardiovascular disease.

Research Design and Methods

A randomized, placebo-controlled double-blind trial was performed in 250 patients with DM2 without manifest cardiovascular disease. Patients were given 0.4 mg cerivastatin or placebo daily. The primary endpoint was the change in high sensitivity CRP after 2 years.

Results

CRP in the statin group was 1.58 mg/L at baseline and 1.69 mg/L at 2 years (p= 0.413), in the placebo group it increased from 2.03 mg/L at baseline to 2.54 mg/L at 2 years (p = 0.058) (p= 0.269 for comparison between the groups). In a high-risk subgroup with the metabolic syndrome and LDL levels > 2.6 mmol/L (40 % of the cohort) CRP levels increased significantly in the placebo group (from 2.97 mg/L at baseline to 3.99 mg/L at 2 years, p=0.036) in comparison to the statin group (from 2.13 mg/L at baseline to 2.10 mg/L at 2 years, p=0.885) (p=0.042 for comparison between the groups)

Conclusions

There was no effect of two year statin therapy on CRP in patients with DM2 without manifest cardiovascular disease, except in a subgroup with the metabolic syndrome and LDL > 2.6 mmol/L. Studies supporting risk stratified therapy in primary prevention in DM2 are needed.

INTRODUCTION

Cardiovascular disease (CVD) is the most important cause of mortality in patients with type 2 diabetes mellitus (DM2)[1]. C-reactive protein (CRP) is a marker for the chronic inflammatory process in atherosclerotic plaques, and probably has pro-atherogenic properties of its own[2-4]. When measured with high sensitivity assays, CRP levels are highly reproducible, unaffected by food intake and with no circadian variation. CRP is a strong predictor of future cardiovascular events, independent of traditional risk factors such as LDL cholesterol[5-7]. CRP levels are associated with components of the metabolic syndrome (MS) such as triglycerides, obesity and insulin sensitivity[8,9]. Finally, CRP might be predictive of incident DM2[10-12].

In DM2 without coronary artery disease, levels of CRP are higher than in non-diabetic controls[13]. CRP levels independently predict future cardiovascular events in DM2 in some studies[14,15]. Importantly, in the Hoorn study[16], the association of CRP with future CVD events in DM2 was not independent of classical risk factors.

A meta-analysis of intervention studies with statins in the setting of secondary prevention after a cardiovascular event has shown a correlation between reduced cardiovascular events and reduction in CRP, independent of LDL cholesterol lowering[17]. Results from intervention studies on the effects of statin therapy on CRP in DM2 have shown contradictory results[18-23]. The present study is an analysis of CRP within a randomized, placebo-controlled trial that has evaluated the effect of 2 years' statin therapy on CRP as a pre-specified secondary endpoint in patients with DM2 without CVD.

RESEARCH DESIGN AND METHODS

Subjects and design

The study design and baseline characteristics of the original patient population have been described elsewhere[24]. Briefly, 250 patients with DM2 for at least one year, aged 30-80 years, without CVD were included between August 1999 and February 2001 in this randomized, double-blind, clinical trial. Patients were given 0.4 mg cerivastatin (Bayer B.V., Mijdrecht , The Netherlands) or placebo daily for 2 years. After the withdrawal of cerivastatin from the market, 0.4 mg cerivastatin was replaced by 20 mg simvastatin (Merck Sharp & Dome, Haarlem, the Netherlands), without unblinding the study. At that moment, all the patients had been randomized with a mean follow-up of 15 months (range 6-23 months).

Eligible patients gave their written informed consent. The study was performed at the HAGA Hospital, The Hague. The study was approved by the hospital's Medical Ethics Committee.

Study Objectives

The primary endpoint of this sub-study was the change in CRP between 24 months and baseline. The relationship between CRP and MS score was a secondary endpoint.

Follow-up

Patients returned to the study site after 12 hours fast at 3, 6, 12, 18 and 24 months when blinded lipid and safety measurements (creatinin kinase, ALT) were performed. CRP was measured at baseline and at 24 months.

Laboratory investigations

Lipid and safety measurements were performed at the Department of Clinical Chemistry and Hematology of the HAGA Hospital, according to ISO 15189 standard procedures. Blood samples were collected from the subjects after a 12 hour fast. EDTA tubes were used for the determination of HbA1c. Liver enzymes and lipids were measured in serum. A urine sample was collected for the determination of the albumin-to-creatinin ratio.

The high sensitive CRP assay was performed in the Leiden University Medical Center with the Tina Quant C-reactive protein (latex) high sensitive assay from Roche using particle enhanced immunoturbidimetry on a Roche Module P(Basel, Switzerland). The lower detection limit (analytical sensitivity) is 0.03 mg/L and the functional sensitivity 0.11 mg/L. The intra-assay CV is 1.34% at 0.55 mg/L and the inter-assay CV is 5.70% at 0.52 mg/L. All CRP assays were performed after completion of the study.

Statistical analysis

The primary treatment comparison was between placebo and statin therapy in patients completing the study, as on-treatment analysis. CRP values more than 15 mg/L were excluded. As CRP values were not normally distributed, logarithmic transformations were used. Changes within each treatment group were analyzed using Student's paired t-test. Comparisons of the effects between the treatment groups were performed using Student's independent samples t-test. Analysis of the baseline data was performed in all randomized patients. Stepwise regression techniques were used to investigate the effect of baseline characteristics on baseline CRP and on changes in CRP. ANOVA was used to investigate the relation between the MS score (1 point for every criterion (waist, triglycerides, HDL cholesterol and blood pressure) according to the NCEP/ATPIII criteria[25]) and baseline CRP. In addition, the effect of statin treatment on CRP was analyzed in a high-risk patient group with 3 or 4 additional MS criteria on top of their diabetes and LDL cholesterol levels > 2.6 mmol/L[26]. To test the equivalence of cerivastatin 0.4 mg and simvastatin 20 mg, LDL levels before and after the switch to simvastatin were compared using Student's paired t-test. Correlation between changes in CRP and changes in other parameters were evaluated with Pearson's correlation coefficients.

Analyses were performed using SPSS 11.0 for Windows software. All analyses were 2-sided, with a level of significance of $\alpha = 0.05$.

RESULTS

The characteristics of the study population are given in Table 1. No statistical differences between the groups were observed. 68 patients did not complete the study. This relatively high drop-out rate was mainly caused by the withdrawal of cerivastatin from the market[24]. There were no significant differences in demographic or lipid parameters between the full cohort (n=250) and the patients completing the study (n=182), except for race as more Caucasians than non-Caucasians completed the study (data not shown).

Table 1 Baseline Characteristics of 250 Randomized Patients

	Placebo (n=125)	Statin (n=125)
Male sex	57 (46)	61 (49)
Age (years)	58.2 ± 11.4	58.8 ±11.3
Ethnicity:		
Caucasian	86 (69)	83 (66)
Asian-Indians	20 (16)	28 (22)
other	19 (15)	14 (11)
Current smoker	33 (26)	28 (22)
Hypertension	66 (53)	60 (48)
Diabetes duration (years)*	7 ± 8	6 ± 7
Insulin use	69 (55)	62 (50)

Data are means ± SD or numbers of patients (%). *Median ± SD.

CRP measurements (Table 2)

Baseline CRP was not significantly different between the groups. Baseline CRP in the drop-outs did not differ from values in patients completing the study. A total of 149 patients had analyzable CRP data (i.e. < 15 mg/L) at baseline and 24 months.

There was no significant difference between the change in CRP in 2 years in the statin group and the placebo group (mean difference 0.53 mg/L [95% CI –0.42 to 1.48 mg/L] p=0.269). CRP in the placebo group increased from 2.03 mg/L at baseline to 2.54 mg/L at 2 years (p = 0.058), in the statin group it was 1.58 mg/L at baseline and 1.69 mg/L at 2 years (p= 0.413).

Determinants for baseline CRP in univariate analysis were waist, Body Mass Index (BMI), HbA1c, age, gender (higher in women), ethnicity (higher in caucasians and Asian-Indians), smoking, triglycerides, apoB/LDL cholesterol, diabetes medication and MS score. When included into a regression model, age (beta = -0.005, p= 0.031), BMI (beta = 0.019, p<0.001), HbA1c(beta = 0.049, p=0.016), gender(beta = 0.171, p=0.003), ethnicity (beta = -0.196,

Table 2 CRP and metabolic changes

	Parameter	Baseline	24 months	p *0-24 months*
Placebo	CRP(mg/L)	2.03	2.54	0.058
n=64	ALT (IU/L)	33	35	0.783
	HbA1c (%)	7.62	7.57	0.764
	BPsyst (mmHg)	137	134	0.071
	BPdiast (mmHg)	77	74	<0.001
	BMI (kg/m^2)	30.2	30.3	0.696
	Waist(m)	1.03	1.02	0.176
	MS score	2.48	2.22	0.031
	Microalbuminuria (g/mol creat)	3.13	9.74	0.019
Statin	CRP(mg/L)	1.58	1.69	0.413
n=85	ALT (IU/L)	25	26	0.410
	HbA1c (%)	7.49	7.63	0.235
	BPsyst (mmHg)	137	132	0.007
	BPdiast (mmHg)	77	74	<0.001
	BMI (kg/m^2)	30.1	30.4	0.145
	Waist (m)	1.02	1.01	0.369
	MS score	2.18	2.04	0.141
	Microalbuminuria (g/mol creat)	2.48	5.81	0.053

Data are geometric means for CRP and means for other parameters

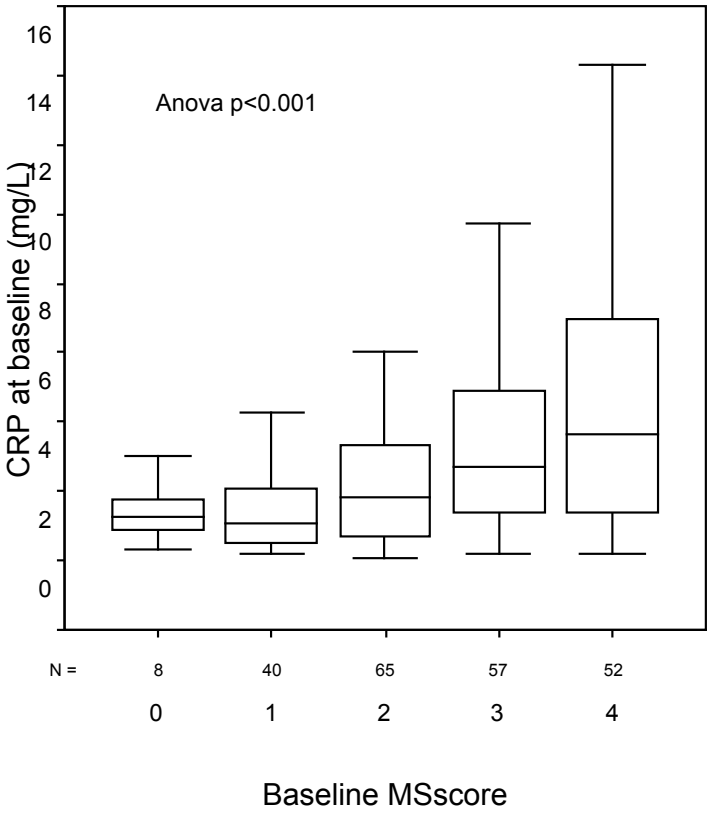
p=0.011) and MS score (beta = 0.071, p=0.010) remained significant determinants and together explained 29% of the variance in baseline CRP. The relation between MS score and CRP was linear at baseline(ANOVA p for linearity <0.001)(Figure 1). After 2 years, this relation remained statistically significant only in the placebo group. Further analysis on this issue revealed that in a high-risk subgroup with 3 or more additional MS criteria (on top of their diabetes) and LDL levels > 2.6 mmol/L, which comprised 40% of the cohort (29 patients in the placebo group and 30 patients in the statin group), two years' CRP levels increased significantly in the placebo group (from 2.97 at baseline to 3.99 at 2 years, p=0.036) in comparison to the statin group (from 2.13 mg/L at baseline to 2.10mg/L at 2 years, p=0.885) (Figure 2, p=0.042 for comparison between placebo and statin)

Changes in CRP were not related to baseline characteristics, changes in lipid levels, body weight or Hba1c. The effect of the two statins used was analyzed by correcting the change in CRP for duration of simvastatin treatment (range 1 to 18 months). This did not change the results.

Lipids

LDL cholesterol was 3.44 ± 0.71 mmol/L at baseline and 2.58 ± 0.95 mmol/L at 2 years (-25 %, p < 0.001) in the statin group and 3.55 ± 0.71 mmol/L at baseline and 3.78 ± 0.81 mmol/L at 2 years (+8 %, p=0.003) in the placebo group (p < 0.001). HDL cholesterol was 1.23 ± 0.39

Figure 1. Relation between MS score and CRP at baseline

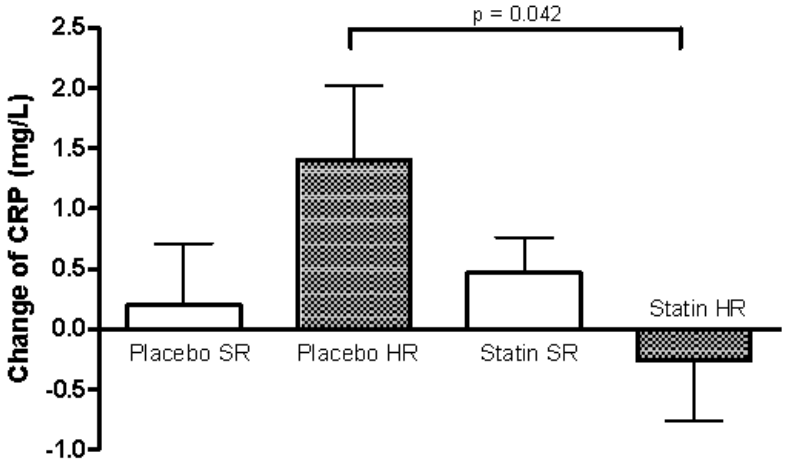


mmol/L at baseline and 1.20 ± 0.36 mmol/L at 2 years in the statin group and 1.21 ± 0.37 mmol/L at baseline and 1.22 ± 0.38 mmol/L at 2 years in the placebo group. Triglycerides were 1.82 ± 0.97 mmol/L at baseline and 1.60 ± 1.38 mmol/L at 2 years in the statin group and 1.88 ± 0.79 mmol/L at baseline and 1.72 ± 1.22 mmol/L at 2 years in the placebo group. Changes in HDL cholesterol and triglycerides were not significantly different compared to baseline or compared to the placebo group , except for the reduction in triglycerides in the statin group after 2 years (p=0.043). Average LDL cholesterol levels were higher after the switch to simvastatin (2.34 before versus 2.56 mmol/L after the switch, $p < 0.001$).

CONCLUSIONS

Patients with DM2 have a high-risk of cardiovascular events. Many studies have been performed to evaluate new non-traditional risk factors for CVD. The number of studies in DM2

Figure 2. Effect of statin therapy on CRP, stratified by risk group



Changes in CRP (+ SE) after two years, stratified by high-risk (HR) (MS score 3 or 4 and LDL> 2,6 mmol/L) and standard risk (SR) (rest group).
p=0.042 for the difference in changes between placebo and statin in the high-risk group

however is sparse. This is the first randomized controlled trial on the effect of long-term statin therapy on CRP in DM2. We did not find an effect of 2 years' intermediate-dose statin therapy on CRP. Consistent with our results, Koh et al did not find an effect of 2 months of simvastatin 20 mg on CRP in a randomized, placebo-controlled crossover trial in DM2[22]. In patients with DM2 and low HDL levels, both 40 and 80 mg simvastatin significantly reduced CRP levels[19]. Other randomized placebo-controlled studies in DM2 show a dose dependent effect of atorvastatin on CRP[18,20]. Similarly, pravastatin 40 mg decreased CRP levels in an open, randomized, crossover study in DM2[21]. Balletshofer et al[23] found no effect of 12 weeks cerivastatin 0.2 mg and 0.8 mg on CRP in DM2; this study however included only 20 patients per group because the study was terminated after the withdrawal of cerivastatin.

Possible explanations for the inconsistent findings on the effect of statin therapy on CRP in DM2 are differences in patient inclusion criteria, differential effects of statins, dose-depending effects and duration of statin treatment.

In line with another study[9], CRP in our asymptomatic DM2 group was related to the MS and to other aspects of glucose metabolism. Interestingly, BMI and MS score were both independently associated with CRP. This is in concordance with the findings of Putz[27] and Mc Laughlin[28], implicating that the relationship between insulin resistance and CRP is only partly explained by obesity.

Data from NHANES III show that among people with DM2 and MS the prevalence of CVD was higher than among people with DM2 without MS[29]. We were able to identify a high-risk phenotype, present in 40 % of our cohort, with 3 or 4 additional MS criteria on top of their

diabetes and LDL levels > 2.6 mmol/L. These patients showed a significant effect of statin therapy on CRP in comparison to placebo. Intriguingly, this effect occurred only when the combination of MS and higher LDL levels was present. This important observation suggests that statins are most effective at reducing low grade inflammation in high-risk groups and supports risk stratification in the prescription of statin therapy in primary prevention in DM2. As advocated by the NCEP[26], statin therapy should be prescribed to DM2 individuals without CVD with LDL levels > 2.6 mmol/L and at least one additional cardiovascular risk factor.

We explored the effect of diabetes related factors as a possible cause of the (non-significant) rise in CRP in the placebo group. We found no changes in HbA1c after 24 months; blood pressure was significantly lower after 24 months although there was progression of microalbuminuria; One has to realize however, that in our regression model only 29% of the variance in CRP could be explained and that the diabetic state itself and genetic factors[30] might be major determinants of CRP.

Our study has limitations: the switch from cerivastatin to simvastatin was unplanned and we did not have the possibility to collect blood samples at the time of the switch. All our 24 month samples were collected after the switch, after 1-18 months of simvastatin treatment. In addition, we did not find an influence of the duration of simvastatin treatment on the 24 months results in regression analysis. The statin dose used was relatively low, but common in primary prevention during the time this study was started.

In conclusion, the present study showed no effect of two year statin therapy on CRP in patients with DM2 without manifest CVD. The beneficial effects of statin therapy on CRP in a high-risk subgroup with MS and LDL > 2.6 mmol/L supports the use of risk stratification in DM2. Prospective studies are needed to further substantiate these findings.

REFERENCES

1. Stamler J, Vaccaro O, Neaton JD, Wentworth D: Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care* 16:434-444, 1993
2. Verma S, Li SH, Badiwala MV, Weisel RD, Fedak PW, Li RK, Dhillon B, Mickle DA: Endothelin antagonism and interleukin-6 inhibition attenuate the proatherogenic effects of C-reactive protein. *Circulation* 105:1890-1896, 2002
3. Pasceri V, Willerson JT, Yeh ET: Direct proinflammatory effect of C-reactive protein on human endothelial cells. *Circulation* 102:2165-2168, 2000
4. Yeh ET: A new perspective on the biology of C-reactive protein. *Circ.Res.* 97:609-611, 2005
5. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR: Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N.Engl.J.Med.* 347:1557-1565, 2002
6. Ridker PM, Hennekens CH, Buring JE, Rifai N: C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N.Engl.J.Med.* 342:836-843, 2000
7. Ridker PM, Buring JE, Cook NR, Rifai N: C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. *Circulation* 107:391-397, 2003
8. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW: C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler.Thromb.Vasc.Biol.* 19:972-978, 1999
9. Kang ES, Kim HJ, Ahn CW, Park CW, Cha BS, Lim SK, Kim KR, Lee HC: Relationship of serum high sensitivity C-reactive protein to metabolic syndrome and microvascular complications in type 2 diabetes. *Diabetes Res.Clin.Pract.* 69:151-159, 2005
10. Snijder MB, Dekker JM, Visser M, Bouter LM, Stehouwer CD, Kostense PJ, Yudkin JS, Heine RJ, Nijpels G, Seidell JC: Associations of hip and thigh circumferences independent of waist circumference with the incidence of type 2 diabetes: the Hoorn Study. *Am.J.Clin.Nutr.* 77:1192-1197, 2003
11. Han TS, Sattar N, Williams K, Gonzalez-Villalpando C, Lean ME, Haffner SM: Prospective study of C-reactive protein in relation to the development of diabetes and metabolic syndrome in the Mexico City Diabetes Study. *Diabetes Care* 25:2016-2021, 2002
12. Freeman DJ, Norrie J, Caslake MJ, Gaw A, Ford I, Lowe GD, O'Reilly DS, Packard CJ, Sattar N: C-reactive protein is an independent predictor of risk for the development of diabetes in the West of Scotland Coronary Prevention Study. *Diabetes* 51:1596-1600, 2002
13. Wannamethee SG, Lowe GD, Shaper AG, Rumley A, Lennon L, Whincup PH: Insulin resistance, haemostatic and inflammatory markers and coronary heart disease risk factors in Type 2 diabetic men with and without coronary heart disease. *Diabetologia* 47:1557-1565, 2004
14. Schulze MB, Rimm EB, Li T, Rifai N, Stampfer MJ, Hu FB: C-reactive protein and incident cardiovascular events among men with diabetes. *Diabetes Care* 27:889-894, 2004
15. Matsumoto K, Sera Y, Abe Y, Ueki Y, Tominaga T, Miyake S: Inflammation and insulin resistance are independently related to all-cause of death and cardiovascular events in Japanese patients with type 2 diabetes mellitus. *Atherosclerosis* 169:317-321, 2003
16. 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 non-diabetic subjects: the Hoorn Study. *Arterioscler.Thromb.Vasc.Biol.* 19:3071-3078, 1999
17. Ridker PM, Cannon CP, Morrow D, Rifai N, Rose LM, McCabe CH, Pfeffer MA, Braunwald E: C-reactive protein levels and outcomes after statin therapy. *N.Engl.J.Med.* 352:20-28, 2005

18. van de Ree MA, Huisman MV, Princen HM, Meinders AE, Kluft C: Strong decrease of high sensitivity C-reactive protein with high-dose atorvastatin in patients with type 2 diabetes mellitus. *Atherosclerosis* 166:129-135, 2003

19. Miller M, Dobs A, Yuan Z, Battisti WP, Borisute H, Palmisano J: Effectiveness of simvastatin therapy in raising HDL-C in patients with type 2 diabetes and low HDL-C. *Curr.Med.Res.Opin.* 20:1087-1094, 2004

20. Tan KC, Chow WS, Tam SC, Ai VH, Lam CH, Lam KS: Atorvastatin lowers C-reactive protein and improves endothelium-dependent vasodilation in type 2 diabetes mellitus. *J.Clin.Endocrinol. Metab* 87:563-568, 2002

21. Sommeijer DW, MacGillavry MR, Meijers JC, Van Zanten AP, Reitsma PH, Ten Cate H: Anti-inflammatory and anticoagulant effects of pravastatin in patients with type 2 diabetes. *Diabetes Care* 27:468-473, 2004

22. Koh KK, Quon MJ, Han SH, Ahn JY, Jin DK, Kim HS, Kim DS, Shin EK: Vascular and metabolic effects of combined therapy with ramipril and simvastatin in patients with type 2 diabetes. *Hypertension* 45:1088-1093, 2005

23. Balletshofer BM, Goebbel S, Rittig K, Enderle M, Schmolzer I, Wascher TC, Ferenc PA, Westermeier T, Petzinna D, Matthaei S, Haring HU: Intense cholesterol lowering therapy with a HMG-CoA reductase inhibitor does not improve nitric oxide dependent endothelial function in type-2-diabetes--a multicenter, randomized, double-blind, three-arm placebo-controlled clinical trial. *Exp.Clin.Endocrinol.Diabetes* 113:324-330, 2005

24. Beishuizen ED, van de Ree MA, Jukema JW, Tamsma JT, van der Vijver JC, Meinders AE, Putter H, Huisman MV: Two-year statin therapy does not alter the progression of intima-media thickness in patients with type 2 diabetes without manifest cardiovascular disease. *Diabetes Care* 27:2887-2892, 2004

25. Grundy SM, Brewer HB, Jr., Cleeman JI, Smith SC, Jr., Lenfant C: Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation* 109:433-438, 2004

26. Grundy SM, Cleeman JI, Merz CN, Brewer HB, Jr., Clark LT, Hunninghake DB, Pasternak RC, Smith SC, Jr., Stone NJ: Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. *Circulation* 110:227-239, 2004

27. Putz DM, Goldner WS, Bar RS, Haynes WG, Sivitz WI: Adiponectin and C-reactive protein in obesity, type 2 diabetes, and monodrug therapy. *Metabolism* 53:1454-1461, 2004

28. McLaughlin T, Abbasi F, Lamendola C, Liang L, Reaven G, Schaaf P, Reaven P: Differentiation between obesity and insulin resistance in the association with C-reactive protein. *Circulation* 106:2908-2912, 2002

29. Alexander CM, Landsman PB, Teutsch SM, Haffner SM: NCEP-defined metabolic syndrome, diabetes, and prevalence of coronary heart disease among NHANES III participants age 50 years and older. *Diabetes* 52:1210-1214, 2003

30. Timpson NJ, Lawlor DA, Harbord RM, Gaunt TR, Day IN, Palmer LJ, Hattersley AT, Ebrahim S, Lowe GD, Rumley A, Davey SG: C-reactive protein and its role in metabolic syndrome: mendelian randomisation study. *Lancet* 366:1954-1959, 2005

Chapter 6

The impact of metabolic syndrome and CRP on vascular phenotype in type 2 diabetes mellitus

R Alizadeh Dehnavi[1], ED Beishuizen[1], MA van de Ree[2], S Le Cessie[3], MV Huisman[1], C Kluft[4], HMG Princen[4], JT Tamsma[1]

1 Department of General Internal Medicine and Endocrinology, Leiden University Medical Center

2 Department of Internal Medicine, Diakonessenhuis, Utrecht

3 Department of Medical Statistics and Bio-Informatics, Leiden University Medical Center

4 Gaubius Laboratory TNO-QoL, Leiden

The Netherlands

European Journal of Internal Medicine 2008; 19: 115-121

Members of the DALI Study Group: Erasmus Medical Centre Rotterdam, Department of Internal Medicine (I. Berk-Planken, N. Hoogerbrugge, H. Jansen); Erasmus University Rotterdam, Departments of Biochemistry and Clinical Chemistry (H. Jansen); Gaubius Laboratory TNO-PG, Leiden (H. M. G. Princen); Leiden University Medical Center (M. V. Huisman, M. A. van de Ree); University Medical Centre Utrecht, Julius Centre for General Practice and Patient Oriented Research (R. P. Stolk, F. V. van Venrooij); University Medical Centre Utrecht, Division of Internal Medicine (J. D. Banga, G. Dallinga-Thie, F.V. van Venrooij).

ABSTRACT

Objective

The burden of cardiovascular disease in diabetes mellitus type 2 (DM2) patients is variable. We hypothesize that metabolic syndrome (MS) and low-grade systemic inflammation modify the extent of atherosclerosis in DM2.

Research Design and Methods

Vascular phenotype was determined using the following endothelium-related, hemostatic, and sonographic endpoints in 62 DM2 patients with mild dyslipidemia: sVCAM, sE-selectin, von Willebrand factor (VWF), fibrinogen, s-thrombomodulin (sTM), tissue type Plasminogen Activator (tPA), Plasminogen Activator Inhibitor-1 (PAI-1), flow mediated dilation (FMD), and intima-media thickness (IMT). The impact of MS load (number of criteria present), MS components, and CRP on these parameters was assessed.

Results

Serum sVCAM, sTM, and tPA levels significantly increased with increasing MS load. IMT also significantly increased from 0.602 ± 0.034 (one MS criterion) to 0.843 ± 0.145 (four MS criteria, $p = 0.007$). LogCRP significantly correlated with fibrinogen, PAI-1, and IMT. In a multiple regression model with age and gender as covariates, MS load predicted sVCAM and sTM; CRP predicted PAI-1 and fibrinogen; MS load and CRP simultaneously predicted tPA and IMT. For each MS criterion present, IMT significantly increased by 0.04 mm. An increase in CRP from 1 to 3 mg/L resulted in a significant increase of 0.04 mm. Patients with four MS criteria and inflammation (CRP ≥ 3 mg/L) are predicted to have a 0.21 mm thicker IMT than those without. A second stepwise multiple regression analysis based on gender, traditional risk factors, diabetes-related parameters, renal function, individual MS criteria, and LogCRP as explanatory variables showed a significant effect of systolic and diastolic blood pressure, HDL, and LogCRP on IMT($r^2 = 0.36$, $p < 0.001$).

Conclusions

MS and low-grade chronic inflammation have an independent impact on vascular phenotype including IMT in DM2.

INTRODUCTION

Patients with diabetes mellitus type 2 (DM2) are known to suffer from increased rates of cardiovascular disease[1-4]. DM2 has even been regarded as a cardiovascular risk equivalent [5]. However, the burden of cardiovascular disease can vary considerably within this population. The metabolic syndrome (MS) is often present in DM2 patients and frequently coexists with elevated C-reactive protein (CRP) levels, a measure of chronic, low-grade inflammation [6]. Variability in MS load (a rough measure for the extent of metabolic dysregulation), defined as the number of MS criteria present, and the level of CRP has also been shown in DM2 patients. MS and CRP are both associated with increased cardiovascular morbidity and mortality but are not interchangeable and may even have additional predictive value [7]. CRP has been related to atherosclerotic vessel wall structural changes such as intima-media thickness (IMT) in several non-diabetic cohorts[8-12]. Moreover, the regulation of CRP has been argued to be independent of MS and is thought to be mostly due to inherited traits[13]. Thus, low-grade systemic inflammation could have a potential independent impact on vascular phenotype in DM2 patients. On the other hand, MS load has been directly related to coronary calcium scores, reflecting total body atherosclerotic burden in non-diabetic subjects[14]. MS also modified coronary heart disease prevalence in DM2 patients in an epidemiological study[4]. We hypothesize that MS and low-grade systemic inflammation, as measured by CRP, modify the extent of atherosclerotic disease burden in DM2 patients. We set out to study the impact of MS components, MS load, and CRP levels on endothelial, hemostatic, and ultrasonographically assessed vascular wall parameters in DM2.

RESEARCH DESIGN AND METHODS

Study design

This study was a single-center sub-study of the previously reported DALI study[15]. The study was carried out in accordance with the principles of the Declaration of Helsinki. The local medical ethics committee approved the study and all patients gave informed consent.

Patients

Male and female patients aged 45–75 years with DM2 (according to the American Diabetes Association classification) of at least 1-year duration were included in the study. Inclusion criteria were: fasting triglycerides between 1.5 and 6.0 mmol/L, total cholesterol between 4.0 and 8.0 mmol/L, and HbA1c < 10%. Patients were assessed after a washout period of lipid-lowering medication of at least 8 weeks. Patients with manifest or previous cardiovascular disease were excluded from the study. Pre-menopausal women and patients with acute liver disease, hepatic dysfunction, or impaired renal function (plasma creatinine > 150 µmol/L)

were excluded. Patients consuming more than four alcoholic drinks per day or on systemic steroids, androgens, cyclosporine, other immunosuppressive drugs, erythromycin, or mibefradil were also excluded.

Definition of MS criteria cut-off values and low-grade chronic systemic inflammation

The WHO cut-off values were used for the assessment of MS load[16]. The criteria were defined as follows: hypertension: systolic/diastolic ≥ 140/90 (mm Hg); triglyceride levels ≥ 1.7 mmol/L; and HDL cholesterol < 1.0 mmol/L (women) and < 0.9 mmol/L (men). Furthermore, waist circumference was used as a measure for obesity with a cut-off value of ≥ 94 cm in men and ≥ 80 cm in women. MS load was defined by the number of factors present exceeding the thresholds defined above, in addition to DM2. Five groups with respectively 0, 1, 2, 3, or 4 additional MS criteria could be defined. As for CRP, patients with levels over 15 mg/L were excluded from the evaluations on the suspicion of being a temporary outlier not representing the continuous habitual level[17].

Endpoints

The impact of MS and CRP on vascular phenotype in DM2 was assessed using sonographic vascular parameters, IMT, and flow mediated dilatation (FMD), as well as the following endothelium-related and hemostatic factors: fibrinogen, s-thrombomodulin (sTM), tissue type plasminogen-activator (tPA), plasminogen-activator inhibitor-1 (PAI-1), sVCAM, sE-selectin, and von Willebrand factor (VWF).

Laboratory investigations

Blood sampling and plasma lipid measurements, according to standard protocols, have been described previously[15]. CRP was measured in citrate, theophyline, adenosine, dipyridamol (CTAD) plasma with an enzyme immunoassay (EIA) using polyclonal antibodies (Dako, Copenhagen, Denmark) showing an intra- and inter-assay coefficient of variation around 2 mg/L of 2.7 and 4.3%, respectively[17]. Functional fibrinogen was measured in citrated plasma using a clotting rate method, essentially according to Clauss[18]. PAI-1 antigen was determined in CTAD plasma using an EIA (Innotest PAI-1, Innogenetics, Temse, Belgium)[19]. VWF antigen was measured in CTAD plasma with an in-house EIA using polyclonal rabbit antibodies to human VWF (DAKO, Copenhagen, Denmark)[20]. tPA antigen was determined in citrated plasma using an EIA (Immulyse™; Biopool, Umea, Sweden). An EIA was also used to measure sVCAM (Quantikine human sVCAM-1 from R&D systems, Abingdon, UK), sTM (Asserachrom s-Trombomodulin, from Diagnostica Stago, Asnières, France), and sE-selectin (Quantikine human sE-selectin from R&D systems, Abingdon, UK).

Flow mediated dilatation (FMD) and intima-media thickness (IMT)

These measurements were performed as described previously[21]. Patients were in a fasting state, did not use tobacco on the morning of the evaluation, and rested in the supine position for 10 min before the study. FMD of the brachial artery was obtained using three baseline images frozen on the R-wave of the electrocardiogram. After the induction of ischemia, R-wave-triggered brachial artery images were frozen and recorded every 15 s for 5 min (reactive hyperemic period). All images were analyzed off-line in a standardized and blinded manner.

For the measurement of IMT, patients were examined in the supine position with the neck slightly extended and rotated in the opposite direction. A 7.5-MHz linear array transducer was used (Aloka SD1400). A three-lead ECG was attached for R-wave triggering. Subsequently, the right and left distal 10 mm of the common carotid artery (CCA) were visualized under the angle with the clearest image of near and far walls. Three images were frozen on the R-wave of the ECG (end-diastole) and stored on S-VHS videotapes for off-line IMT analysis. Images from the videotape were displayed on a personal computer and the optimal images digitized. The mean carotid IMT of the distal 10 mm of the CCA was measured using the Artery Measurement System, which semi-automatically traced the trailing edges on the near wall and the leading edges of the far wall to provide for near and far wall IMT. The measurements of near walls and far walls, left and right, were averaged to provide an individual IMT.

Statistical analysis

Means between the groups were analyzed using ANOVA including an analysis for a linear trend. Percentages were compared using the chi-square test for trend. Since the distribution of CRP was skewed, a logarithmic transformation (log 10) was performed and used in correlation analysis and multiple regression analysis. Pearson's correlations were calculated for LogCRP and fibrinogen, sVCAM, sTM, tPA, PAI-1, sE-selectin, VWF, FMD, and IMT. A multiple regression analysis was performed with MS load and LogCRP as independent variables, age and gender as covariates, and the abovementioned parameters as dependent variables. In the case of sTM, renal clearance (calculated using the Cockcroft formula) was also added as a covariate as its plasma levels are known to be dependent on renal function[22-24]. The concept of MS load results in a binary response due to the fixed thresholds used for each parameter. Furthermore, the impact of traditional risk factors, diabetes-related factors, and renal function should be taken into account with regard to vascular phenotype. Therefore, a second stepwise multiple regression analysis was performed to examine the impact of the MS criteria used as continuous variables taking diabetes and cardiovascular risk factors into account. The following variables were used: traditional risk factors (age, gender, and cholesterol), waist circumference, systolic/diastolic blood pressure, HDL, triglycerides, fasting blood glucose, inflammation (LogCRP), DM2-related parameters (duration of DM2 and HbA1c) and, finally, renal function (Cockcroft clearance). Possible interactions were systematically assessed. As no relevant interactions were observed, none was taken into account in the final models. The

level of significance was set at $p < 0.05$. All analyses were performed using SPSS for Windows software, version 11.0.1.

RESULTS

Patient characteristics

Patient characteristics are given in Table 1. There were no patients without additional MS criteria. In addition to DM2, one other MS criterion was observed in 4 patients; two additional MS criteria were observed in 12 patients; three in 30 patients; and four in 16 patients. Hypertension, body mass index (BMI), waist circumference, weight, HDL cholesterol, and triglycerides significantly differed between the groups with different MS loads. A trend to differences was observed for fasting blood glucose. Renal function was similar for the groups.

Effect of MS load and CRP on vascular parameters (Table 2)

Mean sVCAM, fibrinogen, sTM, and tPA levels differed between the groups. The plasma levels of sVCAM, sTM, and tPA significantly increased with increasing MS load. LogCRP correlated with fibrinogen and PAI-1. Endothelial function as assessed by FMD was not significantly influenced by MS load or by inflammation. Imaging of the carotid artery yielded a mean IMT

Table 1 Patient characteristics and laboratory results.

	All patients (n=62)	DM + 1 MS criterion (n=4)	DM +2 MS criteria (n=12)	DM + 3 MS criteria (n=30)	DM + 4 MS criteria (n=16)	P value
Age (years)	59.6±6.98	55.5±3.70	59.8±6.86	59.7±6.66	60.4±8.34	0.66
Male/Female (n/n)	38/24	4/0	7/5	16/14	11/5	0.30
Current smoking (%)	21.0	0.0	41.7	23.3	6.3	0.72
Blood pressure (mmHg)						
Systolic	143.5±19.3	112.3±16.2	133.6±13.3	145.5±13.6	154.4±22.5	<0.001
Diastolic	84.2±9.1	76.0±9.8	77.1±10.5	85.8±7.1	87.9±7.9	0.002
Waist (cm)	105.4	86.0	104.3	105.2	111.4	0.004
BMI (kg/m^2)	30.8±5.2	22.86±1.8	31.1±6.4	30.9±4.0	32.5±5.4	0.007
Weight (kg)	90.9±16.5	68.8±8.0	91.2±19.9	89.6±12.3	98.8±17.5	0.008
HbA1c (%)	9.2±1.4	7.8±1.2	9.2±1.4	9.4±1.5	9.2±1.3	0.24
Glucose (mmol/L)	10.7±2.9	7.1±1.5	11.1±3.1	11.1±3.0	10.6±2.4	0.06
Cockcroft clearance (ml/min)	97.96±30	79.63±18	103.30±39	97.72±29	98.98±28	0.61
Total Cholesterol (mmol/L)	6.01±0.93	5.93±0.61	6.48±0.72	5.88±1.05	5.96±0.84	0.29
HDL cholesterol (mmol/L)	1.04±0.22	1.18±0.25	1.20±0.22	1.06±0.18	0.83±0.10	<0.001
Triglycerides (mmol/L)	2.50±0.88	2.22±0.68	1.98±0.56	2.32±0.65	3.31±0.99	<0.001

Data are means ± SD, except for gender and current smoking which are given as percentage.

Table 2 MS load and CRP vs vascular parameters

Factor	DM+1 MS criterion	DM+2 MS criteria	DM+3 MS criteria	DM+4 MS criteria	p-value	p-value	Correlation of factors with logCRP
					difference between groups	linearity	
sVCAM (µg/L)	427.9 (63.3)	389.5 (74.4)	453.8 (130.9)	510.5 (89.7)	0.04	0.01	NS
sE-selectin (µg/L)	54.1 (16.0)	59.9 (23.6)	59.8 (26.3)	66.7 (20.6)	NS	NS	NS
VWF-ag (%)	105.3 (38.3)	122.0 (52.3)	128.9 (53.4)	136.4 (47.7)	NS	NS	NS
fibrinogen (g/L)	2.91 (0.22)	3.90 (0.79)	3.72 (0.56)	3.91 (0.57)	0.03	NS(0.07)	r=0.62 p<0.001
sTM (µg/L)	27.85 (7.25)	28.31 (9.13)	25.98 (11.45)	38.70 (13.75)	0.008	0.03	NS
tPA (µg/L)	9.55 (1.92)	12.25 (2.34)	12.70 (2.80)	15.01 (4.37)	0.01	0.002	NS
PAI-1 (µg/L)	69.1 (28.8)	115.2 (81.9)	123.7 (73.1)	145.9 (83.3)	NS	NS(0.08)	r=0.37 p=0.003
Sonography							
FMD (%)	1.37 (1.88)	3.29 (1.31)	3.28 (5.29)	4.30 (3.43)	NS	NS	NS
IMT (mm)	0.602 (0.034)	0.794 (0.086)	0.800 (0.134)	0.843 (0.145)	0.01	0.007	r=0.29 p=0.02

Data are means (SD). NS = not significant

of 0.797 ± 0.135 mm. IMT was different between the groups and significantly increased with increasing MS load. Furthermore, IMT significantly correlated with LogCRP (Table 2).

Multiple regression analysis

1. Model including age, gender, MS load, and CRP (Table 3, model 1)

A multiple regression analysis was performed to assess the impact of MS load and LogCRP on the endpoints of the study using age and gender as covariates. The significant models are summarized in Table 3, model 1. MS load was a significant explanatory variable in the model for: sVCAM (β: 48.56[16.76, 80.37]) and sTM (β: 4.39[1.13, 7.64]). CRP significantly predicted plasma levels of fibrinogen (β: 0.86[0.50, 1.22]) and PAI-1 (β: 65.67[14.94, 116.40]). MS and CRP predicted tPA and IMT. For each MS criterion present, IMT significantly increased by 0.04 mm (SE 0.02, p = 0.03). In addition, an increase in CRP from 1 mg/L (log 1 = 0) to 3 mg/L (log 3 = 0.47) resulted in an increase of IMT by 0.04 mm (0.47 * 0.095). Thus, patients with four MS criteria and a CRP level of 3 mg/L are predicted to have a 0.21 mm thicker IMT than DM2 patients without MS and a CRP level of 1 mg/L. This implies a higher risk of reaching the upper limit of normal IMT (0.90 mm) in these patients[25].

2. Model based on gender, traditional risk factors, renal function, MS criteria, inflammation, and diabetes-related parameters (Table 3, model 2)

A stepwise, multiple regression approach was used including traditional risk factors, diabetes-related factors, and renal function as independent factors in addition to the individual MS criteria and LogCRP. Significant models were observed for all variables except FMD (p = 0.05). The predictive power was comparable to model 1 for sVCAM, fibrinogen, and tPA. The model improved for PAI-1 (explanatory variables: LogCRP, diabetes duration and HbA1c) and IMT. LogCRP (β: 0.09[0.007, 0.164]) and the following MS criteria were the significant determinants of IMT: systolic (β: 0.003[0.001, 0.005]) and diastolic (β: – 0.007[– 0.011, – 0.003]) blood pressure and HDL (β: – 0.27[– 0.44, – 0.11]); triglycerides contributed non-significantly: (β: – 0.03) [– 0.068, 0.013]). The model explained 36% (p < 0.001) of IMT. A decrease of 0.1 mmol/L HDL cholesterol increased IMT by 0.27 mm. The impact of CRP on IMT is very similar in model 1 and model 2 (β: 0.095 vs 0.09, respectively). Traditional risk factors and diabetes-related factors did not significantly contribute to the model explaining IMT in these patients.

Table 3. Multiple regression models

Dependent variable	Model 1 r^2 (p-value)	Model 2 r^2 (p-value)	Explanatory variables model 2
sVCAM (µg/L)	0.25 (0.002)	0.20 (0.03)	Gender[a], age, cholesterol, diastolic blood pressure, and LogCRP
sE-Selectin (µg/L)	NS	0.30 (0.003)	HbA1c [a], gender, DM2 duration, systolic blood pressure, Triglycerides, Cockroft clearance
VWF-ag (%)	NS	0.13 (0.02)	HbA1c [a], LogCRP
Fibrinogen (g/L)	0.41 (< 0.001)	0.46 (< 0.001)	Cholesterol[a], LogCRP[a], and systolic blood pressure
sTM (µg/L)	0.35[b] (< 0.001)	0.47 (< 0.001)	Gender[a], waist circumference[a], age, cholesterol, HbA1c, and Cockroft clearance
tPA (µg/L)	0.26 (0.001)	0.29 (0.001)	Gender[a], Triglycerides[a], LogCRP[a], age, and cholesterol
PAI-1 (µg/L)	0.16 (0.04)	0.30 (< 0.001)	LogCRP[a], DM2 duration[a], HbA1c [a], and HDL
FMD (%)	NS	0.10 (0.05)	Triglycerides[a], and gender
IMT (mm)	0.24 (0.003)	0.36 (< 0.001)	Systolic[a]/diastolic[a] blood pressure, HDL[a], LogCRP[a], and Triglycerides

Model 1: Age, gender, MS load, LogCRP.
Model 2: Stepwise model based on gender, traditional risk factors, renal function, MS criteria, inflammation, and diabetes-related parameters.
[a] : Significant explanatory variable; the other factors listed were found to have high betas, thus contributing to the model, but they did not reach the predefined level of significance.
[b] : Model using renal function as covariate, as described in text.

CONCLUSIONS

In this study, we observed that MS load, the presence of the individual defining criteria, and chronic low-grade inflammation independently contributed to vascular phenotype in this

DM2 population. The data support the hypothesis that, although partly interrelated, metabolic and inflammatory pathways both modify vascular phenotype.

MS is estimated to be present in 75–80% of DM2 patients and has been shown to influence cardiovascular risk [4,26] and fibrinolytic function[27,28]. In patients with MS but without diabetes, increased measures of atherosclerosis [14,29-31] and a higher incidence of cardiovascular events have been observed[27,32]. This has partly been attributed to impaired fibrinolytic function[27] due to altered regulation of PAI-1, tPA, and fibrinogen[28,33,34]. In patients with MS, it has been shown that most procoagulant features are associated with anthropometry[28], while fibrinogen is associated with inflammation[28]. High tPA and PAI-1 levels have previously been reported in DM2[35]. In line with these studies, we observed that tPA levels depended on both MS load and CRP, while fibrinogen and PAI-1 levels were solely explained by CRP. For tPA, model 2 (Table 3) points to triglycerides as the most relevant MS-related predicting factor. Diabetes-related factors were significant predictors for PAI-1 in addition to CRP, while none of the MS-defining criteria seemed to contribute to fibrinogen and PAI-1 levels. Thus, the main inhibitor of fibrinolysis was found to be severely influenced by the state of diabetes *per se* in alliance with low-grade systemic inflammation as measured by CRP.

An increased IMT was observed with increasing MS load, compatible with an increased burden of atherosclerosis (Table 3, model 1). Model 2 revealed that systolic and diastolic blood pressure and HDL were the MS criteria significantly defining IMT. HDL cholesterol levels had a strong inverse relationship with IMT, underlining the potential importance of HDL and reverse cholesterol transport pathways in mildly dyslipidemic DM2 patients. Previous studies reported on the positive relation between MS and IMT: in healthy children without any other risk factors[8]; in adult male dyslipidemic patients[9]; and in non-diabetic adults with cardiovascular disease or at high-risk of cardiovascular disease[10-12]. Our findings extend these observations to mildly dyslipidemic patients with DM2. Furthermore, our data support previously published reports showing that the presence of MS is strongly associated with the prevalence of cardiovascular disease in subjects with DM2[4].

MS has been found to be associated with low-grade systemic inflammation as assessed by CRP. CRP is thought to provide additional predictive information regarding cardiovascular risk in patients with MS[7]. The current study shows that, in the setting of DM2, CRP is directly related to vascular wall abnormalities and the abovementioned adverse changes in the fibrinolytic system. We observed that low-grade chronic systemic inflammation contributed to IMT in addition to the effects of MS and that, together, they could predict 36% of IMT ($p < 0.001$).

All parameters studied could have been influenced by the state of DM2 *per se*. This was indeed found to be the case for PAI-1 (Table 3, model 2). However, the state of diabetes *per se* is also likely to be relevant for FMD. We and others [15,36-38] have consistently observed low FMD in DM2. Endothelial dysfunction is most likely an early feature in the pathogenesis of atherosclerosis in patients with diabetes. If diabetes is present, the low FMD cannot deterio-

rate further by increasing MS load or low-grade inflammation. This hypothesis is, however, at variance with one other previously reported observation[39].

In summary, both MS and low-grade systemic inflammation modify vascular phenotype in patients with diabetes. Their impact is not mutually exclusive, nor is it synergistic for all parameters. Some hemostatic variables are especially influenced by inflammation (e.g., fibrinogen, PAI-1) while others are predicted by MS (e.g., sVCAM). Together, MS and inflammation significantly predict IMT, a direct measurement of the vascular wall and established surrogate marker for future cardiovascular events. Our study showed that, although MS load is a useful rough estimate for clinical practice, the power of the model to predict IMT improved when the MS criteria were used as individual, continuous variables. The use of individual MS factors, however, did not exclude systemic low-grade inflammation as an independent, contributing variable explaining vascular phenotype in DM2 patients.

REFERENCES

1. Kannel WB, McGee DL. Diabetes and cardiovascular disease. The Framingham study. JAMA 1979; 241(19):2035-2038.
2. Role of cardiovascular risk factors in prevention and treatment of macrovascular disease in diabetes. American Diabetes Association Diabetes Care 1989; 12(8):573-579.
3. Stamler J, Vaccaro O, Neaton JD, Wentworth D. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. Diabetes Care 1993; 16(2):434- 444.
4. Alexander CM, Landsman PB, Teutsch SM, Haffner SM. NCEP-defined metabolic syndrome, diabetes, and prevalence of coronary heart disease among NHANES III participants age 50 years and older. Diabetes 2003; 52(5):1210-1214.
5. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in non-diabetic subjects with and without prior myocardial infarction. N Engl J Med 1998; 339(4):229-234.
6. Ridker PM, Wilson PW, Grundy SM. Should C-reactive protein be added to metabolic syndrome and to assessment of global cardiovascular risk? Circulation 2004; 109(23):2818-2825.
7. Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. Circulation 2003;107(3):391-397.
8. Jarvisalo MJ, Harmoinen A, Hakanen M et al. Elevated serum C-reactive protein levels and early arterial changes in healthy children. Arterioscler Thromb Vasc Biol 2002; 22(8):1323-1328.
9. Blackburn R, Giral P, Bruckert E et al. Elevated C-reactive protein constitutes an independent predictor of advanced carotid plaques in dyslipidemic subjects. Arterioscler Thromb Vasc Bio l 2001; 21(12):1962-1968.
10. Heinrich J, Schulte H, Schonfeld R, Kohler E, Assmann G. Association of variables of coagulation, fibrinolysis and acute-phase with atherosclerosis in coronary and peripheral arteries and those arteries supplying the brain. Thromb Haemost 1995; 73(3):374-379.
11. Hak AE, Stehouwer CD, Bots ML et al. Associations of C-reactive protein with measures of obesity, insulin resistance, and subclinical atherosclerosis in healthy, middle-aged women. Arterioscler Thromb Vasc Biol 1999; 19(8):1986-1991.
12. Willeit J, Kiechl S, Oberhollenzer F et al. Distinct risk profiles of early and advanced atherosclerosis: prospective results from the Bruneck Study. Arterioscler Thromb Vasc Biol 2000; 20(2):529-537.
13. Timpson NJ, Lawlor DA, Harbord RM et al. C-reactive protein and its role in metabolic syndrome: mendelian randomisation study. Lancet 2005; 366(9501):1954-1959.
14. Arad Y, Newstein D, Cadet F, Roth M, Guerci AD. Association of multip le risk factors and insulin resistance with increased prevalence of asymptomatic coronary artery disease by an electron-beam computed tomographic study. Arterioscler Thromb Vasc Biol 2001; 21(12):2051-2058.
15. Diabetes Atorvastin Lipid Intervention (DALI) Study Group. The effect of aggressive versus standard lipid lowering by atorvastatin on diabetic dyslipidemia: the DALI study: a double-blind, randomized, placebo-controlled trial in patients with type 2 diabetes and diabetic dyslipidemia Diabetes Care 2001; 24(8):1335-1341.
16. World Health Organization, Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO Consultation, *Part 1: diagnosis and classification of diabetes mellitus*, World Health Organization, Geneva, Switzerland (1999) Available at: http://whqlibdoc.who.int/hq/1999/WHO_NCD_NCS_99.2.pdf. Accessed December 12, 2003.

17. de Maat MP, de Bart AC, Hennis BC et al. Interindividual and intraindividual variability in plasma fibrinogen, TPA antigen, PAI activity, and CRP in healthy, young volunteers and patients with angina pectoris. Arterioscler Thromb Vasc Biol 1996; 16(9):1156-1162.

18. Clauss A. [Rapid physiological coagulation method in determination of fibrinogen.]. Acta Haematol 1957; 17(4):237-246.

19. Meijer P, Pollet DE, Wauters J, Kluft C. Specificity of antigen assays of plasminogen activator inhibitor in plasma: Innotest PAI-1 immunoassay evaluated. Clin Chem 1994; 40(1):110-115.

20. Ingerslev J. A sensitive ELISA for von Willebrand factor (vWf:Ag). Scand J Clin Lab Invest 1987; 47(2):143-149.

21. van Venrooij FV, Van de Ree MA, Bots ML et al. Aggressive lipid lowering does not improve endothelial function in type 2 diabetes: the Diabetes Atorvastatin Lipid Intervention (DALI) Study: a randomized, double-blind, placebo-controlled trial. Diabetes Care 2002; 25(7):1211-1216.

22. Naumnik B, Borawski J, Pawlak K, Mysliwiec M. Renal function, proteinuria and ACE-inhib itor therapy as determinants of plasma levels of endothelial markers. Nephrol Dial Transplant 2002; 17(3):526-528.

23. Rustom R, Leggat H, Tomura HR, Hay CR, Bone JM. Plasma thrombomodulin in renal disease: effects of renal function and proteinuria. Clin Nephrol 1998; 50(6):337-341.

24. Aso Y, Inukai T, Takemura Y. Mechanisms of elevation of serum and urinary concentrations of soluble thrombomodulin in diabetic patients: possible application as a Metabolism 1998; 47(3):362-365.

25. van Dam MJ, de Groot E, Clee SM et al. Association between increased arterial-wall thickness and impairment in ABCA1-driven cholesterol efflux: an observational study. Lancet 2002; 359(9300):37-42.

26. Lempiainen P, Mykkanen L, Pyorala K, Laakso M, Kuusisto J. Insulin resistance syndrome predicts coronary heart disease events in elderly non-diabetic men. Circulation 1999; 100(2):123-128.

27. Anand SS, Yi Q, Gerstein H et al. Relationship of metabolic syndrome and fibrinolytic dysfunction to cardiovascular disease. Circulation 2003; 108(4):420-425.

28. Sakkinen PA, Wahl P, Cushman M, Lewis MR, Tracy RP. Clustering of procoagulation, inflammation, and fibrinolysis variables with metabolic factors in insulin resistance syndrome. Am J Epidemiol 2000; 152(10):897-907.

29. Bonora E, Kiechl S, Willeit J et al. Carotid atherosclerosis and coronary heart disease in the metabolic syndrome: prospective data from the Bruneck study. Diabetes Care 2003; 26(4):1251-1257.

30. Hulthe J, Bokemark L, Wikstrand J, Fagerberg B. The metabolic syndrome, LDL particle size, and atherosclerosis: the Atherosclerosis and Insulin Resistance (AIR) study. Arterioscler Thromb Vasc Biol 2000; 20(9):2140-2147.

31. Olijhoek JK, Van Der GY, Banga JD et al. The Metabolic Syndrome is associated with advanced vascular damage in patients with coronary heart disease, stroke, peripheral arterial disease or abdominal aortic aneurysm. Eur Heart J 2004; 25(4):342-348.

32. Hsia J, Bittner V, Tripputi M, Howard BV. Metabolic syndrome and coronary angiographic disease progression: The Women's Angiographic Vitamin & Estrogen trial. American Heart Journal 2003; 146(3):439-445.

33. Meigs JB, Mittleman MA, Nathan DM et al. Hyperinsulinemia, hyperglycemia, and impaired hemostasis: the Framingham Offspring Study. JAMA 2000; 283(2):221-228.

34. Juhan-Vague I, Alessi MC, Mavri A, Morange PE. Plasminogen activator inhibitor-1, inflammation, obesity, insulin resistance and vascular risk. J Thromb Haemost 2003; 1(7):1575-1579.

35. Sobel BE, Woodcock-Mitchell J, Schneider DJ et al. Increased plasminogen activator inhibitor type 1 in coronary artery atherectomy specimens from type 2 diabetic compared with non-diabetic

patients: a potential factor predisposing to thrombosis and its persistence. Circulation 1998; 97(22):2213-2221.

36. Beishuizen ED, Van de Ree MA, Jukema TJ et al. Two-year statin therapy does not alter the progression of intima-media thickness in patients with type 2 diabetes without manifest cardiovascular disease. Diabetes Care 2004; 27(12):2887-2892.

37. Evans M, Anderson RA, Graham J et al. Ciprofibrate therapy improves endothelial function and reduces postprandial lipemia and oxidative stress in type 2 diabetes mellitus. Circulation 2000; 101(15):1773-1779.

38. Beishuizen ED, Tamsma JT, Jukema J et al. The effect of statin therapy on endothelial function in type 2 diabetes without manifest cardiovascular disease. Diabetes Care 2005; 28(7):1668-1674.

39. Tan KC, Chow WS, Tam SC et al. Atorvastatin lowers C-reactive protein and improves endothelium dependent vasodilation in type 2 diabetes mellitus. J Clin Endocrino l Metab 2002; 87(2):563-568.

Chapter 7

Vascular phenotype and subclinical inflammation in diabetic Asian Indians without overt cardiovascular disease

A Ray [1], ED Beishuizen [1], A. Misra [2], MV Huisman [1], JT Tamsma [1]

[1] Department of General Internal Medicine and Endocrinology, Leiden University Medical Center, Leiden, The Netherlands

[2] Department of Medicine, All India Institute of Medical Sciences, New Delhi, India

Diabetes Research and Clinical Practice 2007; 76:390-396

ABSTRACT

Objective

Although Asian Indian (AI) patients with diabetes mellitus type 2 (DM2) are at high-risk for cardiovascular disease (CVD), not all patients develop CVD. The vascular phenotype of AI-DM2 without CVD has not been elucidated and may point to protective features.

Research Design and Methods

Using baseline data from a clinical trial we provide an initial description of vascular parameters in AI-DM2 compared to Europid Caucasian controls (ECs) matched for age and gender. Endpoints of the study were endothelial function, low-grade systemic inflammation (CRP) and carotid intima-media thickness (CIMT).

Results

AIs had longer duration of diabetes, worse glycemic control and more microangiopathy. Both groups demonstrated marked endothelial dysfunction. CRP levels were similar: 1.7 (4.9) mg/L in AIs and 2.8 (3.6) mg/L in ECs. CIMT values were significantly lower in AI-DM2 than EC-DM2 (0.655 mm (0.12) versus 0.711 mm (0.15), $p = 0.03$). Multiple regression analysis showed that variability in CRP was mainly determined by waist circumference, not by ethnicity. In contrast, ethnicity was a significantly explanatory variable for CIMT.

Conclusions

Vascular phenotype of AI-DM2 without CVD was characterized by endothelial dysfunction and relatively low levels of CRP, comparable to EC-DM2 controls. In contrast, lower CIMT values were observed in AI-DM2 despite longer duration of diabetes and worse metabolic control. We propose that mechanisms slowing its progression may have atheroprotective potential in AI-DM2.

INTRODUCTION

In the Netherlands a large community from the former Dutch colony of Surinam, originally of Asian Indian (AI) descent, has settled. Epidemiologic data suggest that the excess of type 2 diabetes mellitus (DM2) and cardiovascular disease (CVD) noted in AI populations across the world [1,2] is also present in AIs in the Netherlands[3,4]. An important pathogenic factor is the high prevalence of insulin resistance and DM2 in AIs. However, traditional risk factors do not fully explain the excess of CVD [5]. Several other risk factors such as low-grade systemic inflammation [6,7] and endothelial dysfunction [8,9] have been proposed to contribute to initiation and progression of atherosclerosis in AIs.

Despite the well-established high cardiovascular risk, not all AI-DM2 develop CVD. The vascular phenotype of AI-DM2 without CVD has not been elucidated and may point to protective features regarding the development of CVD. We aimed to provide a first evaluation of vascular parameters in AIs and matched EC controls with DM2 but without CVD.

RESEARCH DESIGN AND METHODS

Subjects

This study is a substudy of a previously reported randomized clinical trial. The study design and results of which have been described elsewhere [10–12]. Using this database, we were able to identify 48 subjects of AI descent and 48 EC subjects from the same cohort matched for age and gender. There were no differences in demographics between the two groups, both living in an urban area in the Netherlands. The predecessors of the AI population migrated from India to Surinam starting 1873. Most of our study subjects were first generation immigrants in this country and third or fourth generation out of India. Patients were eligible for the study if they had been diagnosed with DM2 for at least 1 year, aged 30–80 years and without CVD. CVD was defined as angina pectoris, clinically manifest coronary artery disease, ECG criteria for a past myocardial infarction, ischemic stroke, peripheral artery bypass surgery, percutaneous transluminal angioplasty or amputation because of atherosclerotic disease. Patients with marked dyslipidemia (fasting total cholesterol >6.9 mmol/L or triglycerides >6.0 mmol/L) were excluded from the original population, as prior statin therapy was an exclusion criterion in the clinical trial. Eligible patients gave their written informed consent. The study was approved by the hospital's Medical Ethics Committee.

Endpoints

The endpoints of this study were differences in inflammatory markers (serum C-reactive protein (CRP) and fibrinogen levels), endothelial function (as estimated using measurement of flow mediated dilation (FMD)) and carotid intima-media thickness (CIMT) as a

non-invasive measure of atherosclerosis. Furthermore, presence and risk of coronary atherosclerosis was assessed using measurement of silent myocardial ischemia (Ambulatory Electrocardiogram(AECG)) and UKPDS risk scores for CVD.

Clinical examination

Anthropometric measurements were performed by two observers using standardized methods. Waist circumference was measured midway between the iliac crest and the lowest costal margin at the end of normal expiration; hip circumference was measured at the maximal circumference at the level of the femoral trochanters. Blood pressure was measured using a standard sphygmomanometer after a 10 min resting period in supine position. Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥ 90 mmHg. The presence or absence of retinopathy was determined from the subject's medical files, wherein reports from ophthalmologists were retrieved.

Laboratory investigations

Lipid and safety measurements were performed at the Department of Clinical Chemistry and Hematology of the Leyenburg Hospital, according to ISO 15189 standard procedures. Blood samples were collected after an overnight fast. A urine sample was collected for the determination of the albumin over creatinine ratio. Serum or plasma was isolated by centrifugation at 2900 rpm for 5 min. Levels of total cholesterol and triglycerides were measured by enzymatic methods on a Synchron LX20-analyzer (Beckman Coulter, Brea, USA). LDL cholesterol was calculated according to the Friedewald formula [13]. If triglycerides were > 4.5 mmol/L, LDL cholesterol was measured directly with the use of a reagent kit (Genzyme Diagnostics). HDL cholesterol levels were determined after dextran sulfate-magnesium precipitation of apolipoprotein B-containing lipoproteins. Creatinine kinase and alaninaminotransferase were measured by an enzymatic rate method on a Synchron LX20 multichannel chemistry analyzer, according to IFCCmethods. HbA1c was measured by HPLC on a Variant II (BioRad, USA). For the urine sample, a Jaffe´ rate method was used for the measurement of creatinine on a Synchron LX20- analyzer, while albumin was measured by rate nephelometry. Presence of microalbuminuria was defined as >2.5 g albumin/ mol creatinine for men and >3.5 g albumin/mol creatinine for women.

The high-sensitivity CRP assay was performed in the Leiden University Medical Center with the Tinaquant CRP (latex) high-sensitive assay from Roche. This particle enhanced immunoturbidimetric assay was carried out on a Roche Module P using serum.

CVD risk scores, AECGs and metabolic syndrome criteria

Absolute 10-year risk scores for developing a cardiovascular event were calculated using the UKPDS risk engine Version 2.0 [14]. For patients using anti-hypertensive medication the systolic blood pressure was arbitrarily set at 160 mmHg. The AECG registration and analysis were

conducted as previously described [11]. Criteria for the presence of the metabolic syndrome (MS) were according to the European Group for the Study of Insulin Resistance modification of the WHO guidelines [15,16]: presence of DM2 (per definition in our population), and two or more of the following characteristics: waist circumference ≥ 94 cm in males and ≥ 80 cm in females; triglycerides >1.7 mmol/L; HDL cholesterol <0.9 mmol/L in males and <1.0 mmol/L in females; blood pressure ≥ 140/≥ 90 mmHg.

Ultrasound protocol

Ultrasound imaging was performed with an Acuson Aspen scanner with a linear array 7.5 MHz probe. For FMD, an optimal longitudinal image of the brachial artery at, or just above the elbow, was established and kept stable using a specially designed fixative. The exact FMD protocol was described earlier [12]. For CIMT, all images were recorded digitally for off-line, blinded, analysis by an independent core laboratory, Heartcore, Leiden, the Netherlands as described previously [10]. Briefly, the left and right distal 1.0 cm of the common carotid arteries, near and far walls, were examined longitudinally in the angle resulting in an optimal and maximal CIMT (while avoiding plaques). For each segment, three R-wave triggered images were stored. Mean CIMT was measured, when possible, over the entire 1 cm of the vessel segment. Mean common CIMT was obtained by averaging the mean IMTs of far and near wall, left and right.

Statistical analysis

All binary data were analyzed using the Pearson Chisquare test. All continuous outcome data were significantly skewed and therefore analyzed using the non-parametric Mann–Whitney test or log transformed (hsCRP, Lp(a)) before being analyzed using the Student's t-test. Values are reported as medians (IQR). p-Values <0.05 were considered statistically significant. Correlations were calculated with the Spearman's rank test. To test the impact of correlated parameters on the variability of the outcome variables a stepwise regression analysis was performed.

RESULTS

Patient characteristics are given in Table 1. Despite similar age distribution AIs had a significantly longer duration of diabetes (12.4 years versus 6.3 years; $p < 0.001$) and worse glycemic control as shown by higher median HbA1c levels (7.85% versus 7.20%; $p = 0.006$). Microangiopathy was observed more frequently in AIs, as shown by elevated prevalence of retinopathy (29% versus 6%; $p = 0.003$) and higher level of microalbuminuria (1.3 mg/L versus 0.6 mg/L; $p = 0.009$).

Table 1 Patient characteristics and laboratory findings

	Asian Indians (n=48)	European Caucasians (n=48)	p-values
Male gender	20 (42%)	20 (42%)	1.0
Age (years)*	50.7 (8.6)	50.9 (7.6)	0.89
Diabetes duration (years)*	12.4 (8.2)	6.3 (5.4)	<0.001
HbA1c (%)	7.58 (1.9)	7.20 (1.7)	0.006
Retinopathy	14 (29%)	3 (6 %)	0.003
Microalbuminuria	14 (29%)	8 (17%)	0.15
Microalbuminuria† (mg/L)	1.3 (7.7)	0.6 (1.1)	0.009
Family history of CVD	16 (33%)	13 (27%)	0.51
Hypertension	23 (48%)	19 (40%)	0.41
Smokers	20 (42%)	31 (65%)	0.024
UKPDS (%/10 years)	14.9 (14.1)	10.5 (13.7)	0.29
Creatinine (mcmol/L)	80.0 (30)	76.0 (19)	0.32
Clearance (mL/min)	81.2 (34.1)	101.9 (29.7)	<0.001
Total cholesterol (mmol/L)	5.2 (1.1)	5.5 (1.1)	0.26
HDL cholesterol (mmol/L)	1.1 (0.4)	1.2 (0.5)	0.26
LDL cholesterol (mmol/L)	3.3 (1.4)	3.5 (1.3)	0.83
Triglycerides (mmol/L)	1.6 (1.1)	1.7 (1.2)	0.51
Lipoprotein(a) † (mg/dL)	215.5 (410)	95.0 (316)	0.02
Fibrinogen (g/L)	3.6 (1.9)	3.2 (1.3)	0.88
CRP† (mg/L)	1.7 (4.9)	2.8 (3.6)	0.83
CRP ≥ 3.0 mg/L	14 (29%)	18 (38%)	0.39

All continuous data are expressed in medians (IQR) and compared using non-parametric test (Mann–Whitney) except * and †.

* Data were normally distributed and expressed in means (S.D.), compared using Student's t-test.

† Data were compared after log transformation using Student's t-test.

Cardiovascular risk and anthropometry

Smoking was less prevalent in AIs compared to ECs, both at present and ex-smokers. No significant differences were observed in hypertension (defined as systolic blood pressure ≥ 140 mmHg and /or diastolic blood pressure ≥ 90 mmHg or the use of anti-hypertensive medication) and family history of CVD in first degree relatives. Lipid parameters including plasma HDL cholesterol levels were comparable in both ethnic groups. Lp(a) was significantly different between the groups (215.5 mg/dL (410) in AIs versus 95.0 mg/dL (316) in ECs (p = 0.02)). The UKPDS risk scores for myocardial infarction were found to be 14.9%/10 years in AIs versus 10.5%/10 years in ECs (p = NS).

The anthropometric data are summarized in Table 2. AIs were significantly smaller and lighter. EC women had higher values for waist and hip circumference, as well as higher average BMI as compared to AI women. In men no differences were observed regarding these parameters. The MS score was fully comparable in the two groups, and did not change using ethnicity-specific cut-off values as recently proposed by the International Diabetes Federation (data not shown).

Table 2. Anthropometry and vascular parameters

	Asian Indians (n=48)	European Caucasians (n=48)	p-values
Height (cm)			
male	167.5 (12.0)	180.0 (11.0)	<0.001
female	156.5 (8.0)	164.5 (9.0)	<0.001
Weight (kg)			
male	77.0 (10.0)	87.0 (21.0)	0.003
female	76.0 (18.0)	96.0 (28.0)	0.001
Body mass index (kg/m^2)			
male	27.1 (5.2)	27.2 (4.9)	0.98
female	30.8 (7.0)	34.3 (8.9)	0.016
Waist circumference (cm)			
male	97.5 (13.0)	98.0 (15.0)	0.83
female	100.0 (19.0)	108.5 (20.0)	0.045
Hip circumference (cm)			
male	97.5 (8.0)	102.5 (8.0)	0.42
female	101.5 (11.0)	109.5 (19.0)	0.001
Waist/hip ratio			
male	1.00 (0.09)	0.99 (0.10)	0.33
female	0.98 (0.13)	0.99 (0.12)	0.87
Metabolic syndrome	33 (69%)	37 (77%)	0.36
MS score *	2.10 (1.1)	2.13 (0.87)	0.92
FMD (%)	1.56 (2.5)	1.88 (2.8)	0.44
CIMT (mm)	0.655 (0.12)	0.711 (0.15)	0.03
Abnormal AECG	9/47 (19.1%)	9/47 (19.1%)	1.0

All continuous data are expressed in medians (IQR) and compared using non-parametric test (Mann–Whitney) except *.

* Data were normally distributed and expressed in means (S.D.), compared using Student's t-test.

Inflammation, endothelial function and vascular parameters

No differences were observed between the groups for low-grade chronic inflammation as assessed by CRP. The median value was 1.7 mg/L (4.9) in AIs versus 2.8 mg/L (3.6) in ECs (p = 0.83). In addition the number of subjects with evidence of low-grade inflammation (defined as CRP levels ≥ 3 mg/L and <15 mg/L) did not significantly differ between the groups and was 14 (29%) in AIs and 18 (38%) in ECs (p = 0.39). Median serum levels of fibrinogen were comparable (3.6 g/L (1.9) in AIs and 3.2 g/L (1.3) in ECs; p = 0.88).

Endothelial dysfunction was observed in both groups with FMD levels under 2% (Table 2) but comparable between AIs and ECs. In both groups nine subjects (19.1%) had abnormal findings on their AECG suggesting silent ischemia. These 18 subjects had comparable values of IMT (0.730 mm (0.18) versus 0.680 mm (0.15) in subjects with normal AECGs, p = 0.472). Further analysis of these subjects revealed no ethnic difference for the number of ischemic episodes, the duration of ischemia or the ischemic burden (data not shown).

AIs were found to have significantly lower median CIMT values of 0.655 mm (0.12) compared to ECs (0.711 mm (0.15); p = 0.03). Luminal diameter was not a predetermined endpoint, but

was assessed in 66 cases (35 ECs and 31 AIs). In this subset AIs had smaller lumina (7.294 mm (1.12) versus 7.770 mm (1.05) in ECs; p = 0.02).

In a stepwise regression analysis log CRP, IMT, FMD and log Lp(a) were entered as dependent variables (Table 3). Log Lp(a) was included because it has consistently been found to be high in AI patients. Covariables that were taken into account were: age, race, duration of diabetes, HbA1c, smoking status, waist circumference, LDL- and HDL cholesterol, triglycerides and systolic blood pressure. FMD was impacted by age only. The strongest determinant of variance in Lp(a) levels was race. Waist circumference had the greatest impact on variance in CRP levels and race did not contribute significantly. Finally, age and race explained variance in CIMT.

Table 3 Multiregression analysis

	Age	Race	Waist circumference	Model p-value
CIMT (r^2=0.13)	ß = 0.004 (p=0.005)	ß = -0.47 (p=0.047)	-	0.003
FMD (r^2= 0.05)	ß = -0.01 (p=0.045)	-	-	0.045
Log CRP (r^2=0.16)	ß = -0.009 (p=0.136)	ß = 0.055 (p=0.589)	ß = 1.24 (p=0.001)	0.004
Log Lp(a) (r^2=0.11)	ß = 0.002 (p=0.799)	ß = 0.371 (p=0.003)	ß = 0.552 (p=0.137)	0.035

CONCLUSIONS

In this study we observed, for the first time, a low CIMT in AI-DM2 patients without CVD, compared to matched EC counterparts. Low CIMT was present despite longer duration of diabetes and worse glycemic control, the significance of the latter being illustrated by increased measures of microangiopathy in AI-DM2. Longer duration of diabetes and increased prevalence of microalbuminuria are in line with previous publications on AIs in the Netherlands, and elsewhere [17,18]. In addition, the high Lp(a) levels observed in AIs have been previously reported [18]. Thus, the low CIMT is a new and intriguing finding and it was found in a population with very similar characteristics to these earlier reports with one exception: absence of overt CVD, despite presence of DM2 in our study population.

Previous studies have suggested that endothelial function may be more vulnerable in AIs than in ECs and thus contributes to the development of atherosclerosis [19]. In our population of DM2 patients without CVD both ethnic groups exhibited endothelial dysfunction and we could not demonstrate ethnic differences.

CRP is a cardiovascular risk indicator with additional predictive power to the Framingham risk scores [20]. CRP levels were found to be higher in AI migrants compared to native populations in several [7,21] but not all [22] studies. We observed intermediate values of CRP in AI-DM2 and EC-DM2 with medians of, respectively, 1.7 and 2.8 mg/L. It could be hypothesized that an attenuated individual inflammatory response could be part of a protective phenotype, thus being in line with epidemiologic data relating CRP to CVD. The intermediate CRP levels

were observed in AI men and EC men with similar waist circumferences. Using the recent ethnicity specific cut-off values for waist circumference, AI-DM2 patients had a more outspoken abdominal obesity compared to EC-DM2. As several reports in literature link central and overall adiposity to CRP levels in AIs [7,21,23–26], we expected higher CRP levels in AI-DM2. These relatively low levels of CRP were therefore compatible with the hypothesis of an attenuated inflammatory response in these patients. Further studies should be performed to explore the possible abrogation of inflammation in high-risk subjects without overt CVD.

The most interesting observation was the relatively low CIMT values in AIs, despite longer duration of diabetes and worse glycemic control. This could be a race-related phenomenon, which is in line with the observation of smaller luminal diameters in AIs. To date, IMT studies on predictive power [27,28] have not taken diameter into account. In our subjects a significant variability of IMT for a given diameter was observed (data not shown), indicating the need for further explorative studies. There are no firm data on ethnicity-specific IMT values. We observed a median CIMT of 0.66 mm (± 0.12). Previous IMT studies in AIs have reported CIMT values of 0.59 mm (± 0.17) in non-diabetics and 0.63 mm (± 0.22) in DM2 patients living in South India [29]; and CIMT values of 0.93 ± 0.36 mm versus 0.85 ± 0.21 mm in DM2 with and without retinopathy, respectively, have been reported in the same population [30]. Based on our and other studies we calculated that future prospective comparative studies in different ethnic groups would require a sample size of at least 115 AIs versus 115 ECs to detect a 0.05 mm difference in CIMT with a power of 0.80 and a two-sided significance of 0.05.

The low CIMT observed may also have been due to pathophysiologic differences between AI-DM2 and EC-DM2 leading to slower progression of CIMT. Pathophysiologic changes directly related to diabetes seem unlikely as DM2 was milder in EC than in AI in this study. Thus, low CIMT is for instance not readily explained by decreased glycosylation of the extracellular matrix. Other candidate pathophysiologic mechanisms influencing CIMT progression in AI-DM2 could be endothelium-dependent, i.e. intrinsic or environmental acquired resistance against oxidative stress. In this regard the lower smoking rates in AI-DM2 may be of relevance. Mechanisms could also be endothelium-independent and more related to the pathophysiology of the intima. An attenuated intimal inflammatory response, in line with the intermediate levels of low-grade inflammation observed, would be such a mechanism.

In summary, the data presented provide a first description of vascular parameters in AI-DM2 from Surinam without CVD. In these patients, we observed ethnicity-defined, significantly lower CIMT than EC-DM2, despite presence of a number of robust cardiovascular risk factors. Following this interesting observation, reported for the first time, we propose that atheroprotective mechanisms are in play, slowing progression of CIMT and CVD. This and other similar AI cohorts should be intensively researched to unravel the protective factor(s).

REFERENCES

1. R. Balarajan, Ethnic differences in mortality from ischaemic heart disease and cerebrovascular disease in England andWales, BMJ 302 (6776) (1991) 560–564.
2. A. Misra, R.M. Pandey, J.R. Devi, R. Sharma, N.K. Vikram, N. Khanna, High prevalence of diabetes, obesity and dyslipidaemia in urban slum population in northern India, Int. J. Obes. 25 (11) (2001) 1722–1729.
3. I. Bongers, R.G. Westendorp, B. Stolk, H.A. Huysmans, J.P. Vandenbroucke, Early coronary heart disease together with type II diabetes mellitus in persons of Hindustani origin, Ned. Tijdschr. Geneeskd. 139 (1) (1995) 16–18.
4. B.J. Middelkoop, S.M. Kesarlal-Sadhoeram, G.N. Ramsaransing, H.W. Struben, Diabetes mellitus among South Asian inhabitants of The Hague: high prevalence and an age-specific socioeconomic gradient, Int. J. Epidemiol. 28 (6) (1999) 1119– 1123.
5. G.L. Beckles, G.J. Miller, B.R. Kirkwood, S.D. Alexis, D.C. Carson, N.T. Byam, High total and cardiovascular disease mortality in adults of Indian descent in Trinidad, unexplained by major coronary risk factors, Lancet 1 (8493) (1986) 1298–1301.
6. S.S. Anand, F. Razak, Q.L. Yi, B. Davis, R. Jacobs, V. Vuksan, et al., C-reactive protein as a screening test for cardiovascular risk in a multiethnic population, Arterioscler. Thromb. Vasc. Biol. 24 (8) (2004) 1509–1515.
7. J.C. Chambers, S. Eda, P. Bassett, Y. Karim, S.G. Thompson, J.R. Gallimore, et al., C reactive protein, insulin resistance, central obesity, and coronary heart disease risk in Indian Asians from the United Kingdom compared with European whites, Circulation 104 (2) (2001) 145–150.
8. A. Raji, M.D. Gerhard-Herman, M. Warren, S.G. Silverman, V. Raptopoulos, C.S. Mantzoros, et al., Insulin resistance and vascular dysfunction in non-diabetic Asian Indians, J. Clin. Endocrinol. Metab. 89 (8) (2004) 3965–3972.
9. K. Bhargava, G. Hansa, M. Bansal, S. Tandon, R.R. Kasliwal, Endothelium-dependent brachial artery flow mediated vasodilatation in patients with diabetes mellitus with and without coronary artery disease, J. Assoc. Physicians India 51 (2003) 355–358.
10. E.D. Beishuizen, M.A. van de Ree, J.W. Jukema, J.T. Tamsma, J.C. van derVijver, A.E. Meinders, et al., Two-year statin therapy does not alter the progression of intima-media thickness in patients with type 2 diabetes without manifest cardiovascular disease, Diabetes Care 27 (12) (2004) 2887–2892.
11. E.D. Beishuizen, J.W. Jukema, J.T. Tamsma, M.A. van de Ree, J.C. van der Vijver, H. Putter, et al., No effect of statin therapy on silent myocardial ischemia in patients with type 2 diabetes without manifest cardiovascular disease, Diabetes Care 28 (7) (2005) 1675–1679.
12. E.D. Beishuizen, J.T. Tamsma, J.W. Jukema, M.A. van de Ree, J.C. van der Vijver, A.E. Meinders, et al., The effect of statin therapy on endothelial function in type 2 diabetes without manifest cardiovascular disease, Diabetes Care 28 (7) (2005) 1668–1674.
13. W.T. Friedewald, R.I. Levy, D.S. Fredrickson, Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clin. Chem. 18 (6) (1972) 499–502.
14. R.J. Stevens,V.Kothari, A.I.Adler, I.M.Stratton, TheUKPDS risk engine: a model for the risk of coronary heart disease in type II diabetes (UKPDS 56), Clin. Sci. (Lond.) 101 (6) (2001) 671–679.
15. K.G. Alberti, P.Z. Zimmet, Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosisand classification of diabetes mellitus provisional report of a WHO consultation, Diabet. Med. 15 (7) (1998) 539–553.
16. B. Balkau, M.A. Charles, EuropeanGroup for the Study of InsulinResistance (EGIR), Comment on the provisional report from theWHO consultation, Diabet. Med. 16 (5) (1999) 442–443.

17. P.K. Chandie Shaw, L.A. van Es, L.C. Paul, F.R. Rosendaal, J.H. Souverijn, J.P. Vandenbroucke, Renal disease in relatives of Indo-Asian type 2 diabetic patients with end-stage diabetic nephropathy, Diabetologia 46 (5) (2003) 618–624.

18. S.S. Anand, E.A. Enas, J. Pogue, S. Haffner, T. Pearson, S. Yusuf, Elevated lipoprotein(a) levels in South Asians in North America, Metabolism 47 (2) (1998) 182–184.

19. J.C. Chambers, A. McGregor, J. Jean-Marie, J.S. Kooner, Abnormalities of vascular endothelial function may contribute to increased coronary heart disease risk in UK Indian Asians, Heart 81 (5) (1999) 501–504.

20. P.M. Ridker, N. Cook, Clinical usefulness of very high and very low levels of C-reactive protein across the full range of Framingham risk scores, Circulation 109 (16) (2004) 1955–1959.

21. N.G. Forouhi, N. Sattar, P.M. McKeigue, Relation of C-reactive protein to body fat distribution and features of the metabolic syndrome in Europeans and South Asians, Int. J. Obes. Relat. Metab. Disord. 25 (9) (2001) 1327–1331.

22. K. Chatha, N.R. Anderson, R. Gama, Ethnic variation in Creactive protein: UK resident Indo-Asians compared with Caucasians, J. Cardiovasc. Risk 9 (3) (2002) 139–141.

23. V. Mohan, R. Deepa, K. Velmurugan, G. Premalatha, Association of C-reactive protein with body fat, diabetes and coronary artery disease in Asian Indians: the Chennai Urban Rural Epidemiology Study (CURES-6), Diabet. Med. 22 (7) (2005) 863–870.

24. V. Dudeja, A. Misra, R.M. Pandey, G. Devina, G. Kumar, N.K. Vikram, BMI does not accurately predict overweight in Asian Indians in northern India, Br. J. Nutr. 86 (1) (2001) 105–112.

25. M.A. Banerji, N. Faridi, R.Atluri, R.L. Chaiken,H.E. Lebovitz, Body composition, visceral fat, leptin, and insulin resistance in Asian Indian men, J. Clin. Endocrinol. Metab. 84 (1) (1999) 137–144.

26. C. Snehalatha, V. Viswanathan, A. Ramachandran, Cutoff values for normal anthropometric variables in asian Indian adults, Diabetes Care 26 (5) (2003) 1380–1384.

27. M.L. Bots, A.W. Hoes, P.J. Koudstaal, A. Hofman, D.E. Grobbee, Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study, Circulation 96 (5) (1997) 1432–1437.

28. D.H. O'Leary, J.F. Polak, R.A. Kronmal, T.A. Manolio, G.L. Burke, S.K. Wolfson Jr., Cardiovascular Health Study Collaborative Research Group, Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults, N. Engl. J. Med. 340 (1) (1999) 14–22.

29. C. Snehalatha, V. Vijay, R.S. Mohan, K. Satyavani, S. Sivasankari, T. Megha, et al., Lack of association of insulin resistance and carotid intimal medial thickness in non-diabetic Asian Indian subjects, Diabetes Metab. Res. Rev. 17 (6) (2001) 444–447.

30. M. Rema, V. Mohan, R. Deepa, R. Ravikumar, Association of carotid intima-media thickness and arterial stiffness with diabetic retinopathy: The Chennai Urban Rural Epidemiology Study (CURES-2), Diabetes Care 27 (8) (2004) 1962–1967.

Chapter 8

No effect of statin therapy on silent myocardial ischemia in patients with type 2 diabetes without manifest cardiovascular disease

ED Beishuizen[1], JW Jukema [2], JT Tamsma [1],
MA van de Ree [3], JCM van der Vijver[4],
H Putter[5], AC Maan[2], AE Meinders[1], MV Huisman[1]

1 Department of General Internal Medicine and Endocrinology, Leiden University Medical Center
2 Department of Cardiology, Leiden University Medical Center
3 Department of Internal Medicine, Diakonessenhuis, Utrecht
4 Department of Internal Medicine, Leyenburg Hospital, The Hague
5 Department of Biostatistics, Leiden University Medical Center

The Netherlands

Diabetes Care 2005; 28: 1675-1679

ABSTRACT

Objective

Coronary artery disease is the most important cause of mortality in patients with type 2 diabetes mellitus (DM2). We aimed to determine the prevalence of silent myocardial ischemia (SMI) and the effect of statin therapy on SMI in DM2 patients without manifest cardiovascular disease.

Research Design and Methods

A randomized, placebo-controlled, double-blind trial was performed in 250 patients with DM2 without manifest cardiovascular disease. Patients were given either 0.4 mg cerivastatin or placebo daily. In August 2001, when cerivastatin was withdrawn from the market, cerivastatin 0.4 mg was replaced by 20 mg simvastatin without deblinding the study. The primary endpoint was the change in ischemic episodes, duration and burden as measured by 48 hours ambulatory electrocardiography (AECG) over 2 years.

Results

At baseline 47 out of 233 (20%) evaluable AEC G's showed evidence of ischemia. After 2 years, there was a trend towards more ischemia in both treatment groups, without significant differences between the changes in ischemic parameters (episodes:p=0.498; duration:p=0.697; burden:p=0.798) in the two treatment groups. Cardiovascular events occurred in 12 patients in the placebo group and in 2 patients in the statin group (p=0.006). There was no relationship between these cardiovascular events and the presence of SMI at baseline.

Conclusions

SMI occurred in 20 % of DM2 patients without manifest cardiovascular disease. There was no effect from two years of statin therapy on SMI. In contrast, we observed a significantly lower cardiovascular event rate on statin therapy. AECG may not be a proper tool for risk stratification in patients with DM2.

INTRODUCTION

Coronary artery disease is the most important cause of mortality in patients with type 2 diabetes mellitus (DM2)[1]. Individuals with diabetes not only have a higher risk for coronary events, but their outcome after such an event is worse [2] and more extensive atherosclerotic lesions are found at the first manifestation of coronary artery disease [3]. Periods of silent myocardial ischemia (SMI) might precede a first coronary event in DM2, especially if cardiac autonomic neuropathy is present[4]. Early detection of SMI is thus a potential tool for cardiovascular risk stratification in patients with DM2.

SMI can be detected with an exercise ECG, 24 or 48 hours ambulatory electrocardiography (AECG), or (stress) myocardial scintigraphy. Exercise testing requires a certain level of fitness of the patient. Myocardial scintigraphy is expensive, and both scintigraphy and exercise ECG are time consuming. In contrast, AECG can be applied in virtually every patient, is inexpensive, non-invasive and reflects daily life circumstances.

Treatment with HMG-Co-A reductase inhibitors (statins) [5,6] resulted in reduced SMI in non-diabetic patients with coronary artery disease. Data on the effect of statin therapy on SMI in DM2 are lacking. We conducted a randomized, placebo-controlled trial to determine the prevalence of SMI and to evaluate the effect from 2 years of statin therapy on SMI detected by AECG in patients with DM2 without cardiovascular disease.

RESEARCH DESIGN AND METHODS

Subjects and design

The study design and baseline characteristics of the original patient population have been described in detail elsewhere [7]. Briefly, 250 patients with DM2 for at least one year, aged 30-80 years, without cardiovascular disease (defined as angina pectoris, coronary artery disease, ECG criteria for a past myocardial infarction, ischemic stroke, peripheral artery bypass surgery, percutaneous transluminal angioplasty or amputation because of atherosclerotic disease) were included in this randomized, double-blind, clinical trial. Patients were given 0.4 mg cerivastatin (Bayer B.V., Mijdrecht, The Netherlands) or placebo daily for 2 years. After the withdrawal of cerivastatin from the market, 0.4 mg cerivastatin was replaced by 20 mg simvastatin (Merck Sharp & Dome, Haarlem, the Netherlands) without deblinding the study. At that moment, all the patients had been randomized with a mean follow-up of 15 months (range 6-23 months). Eligible patients gave their written informed consent. The study was performed at the Leyenburg Hospital, The Hague. The study was approved by the hospital's Medical Ethics Committee.

Study Objectives

The primary endpoint of the study was the change in ischemic episodes, ischemic duration and ischemic burden between 24 months and baseline. The following predefined cardiovascular events were evaluated during the study: cardiovascular death, nonfatal myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass graft surgery, nonfatal stroke, peripheral artery bypass graft, percutaneous transluminal angioplasty or amputation because of atherosclerotic disease.

Follow-up

Patients returned to the study site after a 12 hours fast at 3, 6, 12, 18 and 24 months for blinded plasma lipid and safety measurements. ECG and AECG measurements were performed at baseline and 24 months. A 2-year follow-up for clinical events was performed for all 250 patients. If there were signs of life threatening arrhythmia on the AECG, the patient was referred to a cardiologist.

ECG measurements

On a resting ECG the QT interval of lead V2 was measured from the beginning of the QRS complex to the end of the downslope of the T wave. The QT interval was corrected (QTc) for heart rate using Bazett's formula: $QTc = QT/\sqrt{RR}$. The Minnesota ECG criteria were used to detect a past Q-wave myocardial infarction [8].

AECG measurements

AECG's were recorded on a 3 channel Marquette 8500 tape recorder with electrodes positioned to obtain pseudo V5, V6 and aVF leads. The recordings were made over a continuous period of 48 hours, during which the patient completed a diary of physical activity and symptoms. The tapes were subsequently analyzed on a Marquette MARS 8000 Holter Analyzer by SEAL (Foundation for ECG Analysis Leiden, incorporated in the Leiden University Medical Center). The registrations were evaluated by blinded computer-assisted analysis by two certified technicians. The AECG results remained blinded for patients and their physicians.

Transient myocardial ischemia was defined as the presence of episodes showing > 0,1 mV (1 mm) horizontal or downsloping ST-segment depression, 80 ms after the J-point, lasting for > 60 seconds and separated by at least 60 seconds from the next ischemic episode. The total number of ischemic episodes, the total duration of ischemia, and total ischemic burden were assessed. For ischemic episodes and ischemic duration, any overlapping episodes in the different channels were not summed. Ischemic burden was defined as ischemic duration in minutes multiplied by ST-segment depression in millimetres, for each channel separately and then summed.

Not included in the AECG study were patients with non-ischemic ST-segment abnormalities, due to intraventricular conduction delay, bundle branch block, or atrial flutter or fibrillation.

AECG recordings of insufficient quality were rejected; only those in which at least 40 hours of ST-segment analysis could be performed in either lead V5 or V6 were included; if this criterion could not be met, at least 24 hours (50%) of data in both channels V5 and V6 were analyzed and compared with the matching hours of the corresponding recording (either baseline or at 24 months).

Laboratory investigations

All laboratory measurements were performed at the Department of Clinical Chemistry and Hematology of the Leyenburg Hospital, according to ISO 15189 standard procedures.

Statistical analysis

We assumed a 30 % prevalence of SMI in our DM2 patients[9-14]. From clinical studies in patients with coronary artery disease[5,6], we assumed an increase in prevalence to 35 % in the placebo group and a decrease in prevalence to 15 % in the statin group after two years. The number of patients needed to detect this difference with a power of 80 % ($\alpha = 0.05$) was 73 patients in each group.

The primary treatment comparison was between placebo and statin therapy in patients completing the study (on-treatment analysis). Differences between the groups were analyzed by Student's independent samples t-test, (Pearson's) Chi-squared test or Mann-Whitney test where appropriate. Changes from baseline within each treatment group were analyzed by Student's paired t-test, Mc Nemar Chi-squared test or Wilcoxon signed-rank test where appropriate. Comparisons of the effects between the treatment groups were performed using Mann-Whitney test.

Ischemic episodes, total ischemic duration and ischemic burden were categorized as 0 (no ischemia), 1, 2 or 3 according to tertiles at baseline in the patients with ischemia. With these ischemic scores, determinants of baseline ischemia were evaluated using ordinal regression techniques. The association between ischemia at baseline and cardiovascular events during follow-up was evaluated by Chi-squared test.

Analyses were performed using SPSS 11.0 for Windows software. All analyses were 2-sided, with a level of significance of $\alpha = 0.05$.

RESULTS

Of the 250 patients randomized, 233 had evaluable AECG at baseline. Of these 233 patients, 45 in the placebo group and 21 in the statin group dropped out during the study. There were 17 AECG recordings at baseline and 12 at follow-up that were not valid because of: intraventricular conduction delay (9), atrial fibrillation or flutter (5), background noise (2), technical problems with tape or recorder (8), or patient refusal or invalidation (5). This left 155 patients

with full valid AECG data both at baseline and at 24 months. Two patients were referred to a cardiologist because of arrhythmia at their 2-year AECG. One patient had frequent nocturnal sinus arrests; after cessation of labetolol treatment, a control AECG showed normalization. One patient had a 12-beat ventricular tachycardia; subsequent cardiological analysis revealed normal echocardiography results and no further action was taken.

The characteristics of the study population are given in Table 1. No statistical differences between the groups were observed. There were no differences in baseline characteristics between the 155 patients with valid AECG recordings and the other 78 patients.

Lipids

Mean LDL cholesterol in the 155 patients was 3.41 ± 0.72 mmol/l at baseline and 2.64 ± 0.96 mmol/l at 2 years (- 22 %, $p < 0.001$) in the statin group and 3.53 ± 0.72 mmol/l at baseline and 3.76 ± 0.83 mmol/l at 2 years (+ 8%, p=0.007) in the placebo group ($p < 0.001$ for difference between groups). Mean HDL cholesterol was 1.24 ± 0.41 mmol/l at baseline and 1.21 ± 0.38 mmol/l at 2 years (-1%, p=0.161) in the statin group and 1.21 ± 0.38 mmol/l at baseline and 1.21 ± 0.39 mmol/l at 2 years (+1%, p=0.866) in the placebo group (p=0.372 for difference between groups). Mean triglycerides were 1.80 ±0.95 mmol/l at baseline and 1.65 ± 1.49 mmol/l at 2 years (-11 %, p=0.218) in the statin group and 1.85 ± 0.80 mmol/l at baseline and

Table 1. Baseline Characteristics of 250 Randomized Patients

	Placebo (n=125)	Statin (n=125)
Male sex	57 (46)	61 (49)
Age (years)	58.2 ± 11.4	58.8 ±11.3
Ethnicity:		
Caucasian	86 (69)	83 (66)
Asian-Indian	20 (16)	28 (22)
other	19 (15)	14 (11)
BMI (kg/m^2)	31.0 ± 6.0	31.0 ± 6.3
Waist-to-hip ratio	0.99 ± 0.09	0.98 ± 0.08
Current smoker	33 (26)	28 (22)
Hypertension	66 (53)	60 (48)
Diabetes duration (years)*	7 ± 8	6 ± 7
Insulin use	69 (55)	62 (50)
HbA1c (%)	7.60 ± 1.48	7.53 ± 1.10
Vasoactive medication :	70 (56)	58 (46)
Beta blocking agents	16 (13)	14 (11)
Calcium channel blockers	16 (13)	15 (12)
renin-angiotensin system-inhibitors	46 (37)	47 (38)
Diuretics	29 (23)	34 (27)
Microalbuminuria†	19 (15)	24 (19)
Silent myocardial ischemia	26 (22)	21 (18)
QTc (seconds)	0.39 ± 0.03	0.40 ± 0.03

Data are means ± SD or numbers of patients (%) unless otherwise indicated.
*Median ± SD. † Men, > 2.5 g/mol creatinine; women, > 3.5 g/mol creatinine.

1.70 ± 1.26 mmol/l at 2 years (-4%, p=0.284) in the placebo group (p=0.436 for difference between groups). Average LDL cholesterol levels in the statin group were higher after the switch to simvastatin (2.37 before versus 2.63 mmol/l after the switch, $p < 0.001$).

AECG's

At baseline, 47 of 233 (20%) evaluable AECG's showed evidence of ischemia, equally distributed among the treatment groups. In the patient group completing the study with full AECG data (n=155), there was a nonsignificant difference between the placebo and statin group with a higher prevalence of ischemia in the placebo group at baseline (p=0.069, Pearson chi square with continuity correction). Patients in the group with complete AECG data had the same amount of baseline ischemic episodes as the noncompleting group. After two years, there was a nonsignificant trend toward more ischemic episodes, duration, and burden in the placebo group as well as in the statin group (Figure 1 and Table2). When the whole group was taken together (n=155), increases after 2 years in ischemic episodes and duration were significant (p = 0.019 and 0.018, respectively), with total prevalence of ischemia rising from 20.0 to 24.5 %. There were no significant differences between the changes in ischemic episodes (p=0.498), duration (p=0.697) or burden (p=0.798) in the two treatment groups. Correcting for baseline ischemia did not change these results.

Determinants for ***baseline*** ischemic episodes were QTc (p=0.011) and diastolic and systolic blood pressure (p=0.048 and 0.017, respectively). When included into an ordinal regression model, QTc and systolic blood pressure remained significant determinants and explained 6% of the variance (Nagelkerke pseudo r^2) in baseline ischemia.

The effect of the two statins used was analyzed by correcting the change in ischemia for duration of cerivastatin treatment (range 6 to 23 months). This did not change the results.

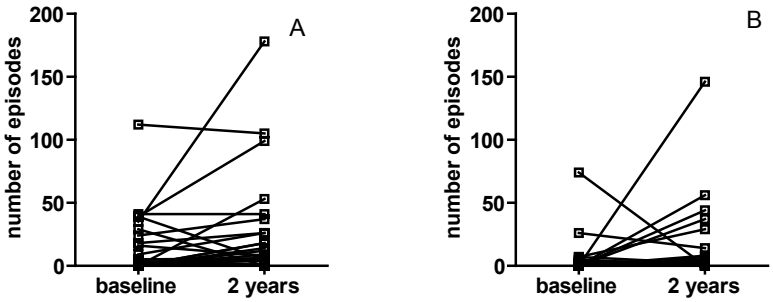


Figure 1. Changes in ischemic episodes in placebo group (A) (n=26) and statin group (B) (n=21) in patients with any ischemia at baseline or 2 years

Table 2. Parameters for ischemia in 155 patients with complete AECG data

		placebo(n=70)			statin(n=85)			
	score*	baseline	2 years	p**	baseline	2 years	p**	p†
episodes(nr)				0.056			0.191	0.498
0	0	51	48		73	69		
1-2	1	5	2		6	5		
3-15	2	5	10		4	6		
≥16	3	9	10		2	5		
duration(min)				0.151			0.050	0.697
0	0	51	48		73	69		
1-6.74	1	5	4		5	3		
6.75-72.74	2	6	8		5	8		
≥72.75	3	8	10		2	5		
burden(min*mm)				0.485			0.054	0.798
0	0	51	48		73	69		
≥0-6.33	1	5	3		5	2		
6.34-124.37	2	6	11		5	9		
≥124.38	3	8	8		2	5		

data are numbers of patients

* score: 0= no ischemia, 1-3= category according to tertiles of baseline ischemic parameters as described in text. ** Wilcoxon signed-rank test for change between baseline and 2 years. † Difference in change between placebo and statin groups.

Clinical events

As reported before [7], in the total population of 250 patients, cardiovascular events occurred in 12 patients in the placebo group and in two patients in the statin group (p=0.006). Ischemic episodes, duration, or burden at baseline were not related to the occurrence of cardiovascular events during the 2-year follow-up, neither in the placebo group nor in the statin group.

CONCLUSIONS

This is the first study on the effect of long-term statin therapy on SMI in DM2 patients. We did not find any effect of statin therapy on the occurrence of SMI, in spite of a significant reduction in cardiovascular events.

The reported prevalence of SMI in asymptomatic DM2 varies between 9 to 52% [4,9-20] and is strongly dependent on method of detection and on the population studied. In our DM2 population without prior cardiovascular disease with a broad range in age and diabetes duration we found a 20% incidence of SMI. In most cross-sectional studies the incidence of SMI is increased in DM2 compared with non-diabetic subjects [11,13]. It has been suggested that this is caused by diabetic autonomic neuropathy. However, recent clinical and epidemiological data suggest that this increase mainly reflects accelerated atherosclerosis [21]. This concept is confirmed by the modest relationship in our data with QTc, a parameter for diabetic cardiac

autonomic neuropathy[22]. Our findings are in concordance with the modest but consistent relationship that was found between other parameters of cardiac autonomic neuropathy, including Ewing tests [23], and SMI in a meta-analysis [24]. The relationship between QTc and SMI in patients with DM2 has not been studied before.

Other investigators found a relationship between SMI in DM2 and cholesterol [12,14], smoking[18], age[12,17], blood pressure [12,25] and microalbuminuria [4,12,17,18,25]. Our data do not confirm these data as we only found an association with blood pressure.

Results of studies on the predictive value of SMI in DM2 for cardiovascular events [4,10,16,17,26] show contradictive results and might be biased because treatment regimens were often influenced by the results of SMI testing [16,17,26].The studies are also difficult to compare because of inclusion of patients with type 1 diabetes [16,26] and because of various methodology to detect SMI : some studies use a combination of exercise ECG and myocardial scintigraphy [16,17,26], whereas others assess SMI with exercise ECG only [4,10]. To our knowledge, only one study on the predictive value of SMI in DM2 included AECG in their evaluation [16], showing that SMI as detected with a combination of exercise ECG, myocardial scintigraphy and AECG was a poor predictor of major cardiac events. In the present study, we did not find a relationship between the presence of 48 hour AECG detected SMI at baseline and the 2-year risk of cardiovascular events. However, our study was not designed nor powered for this purpose, so we cannot conclude from our data that SMI is not predictive for cardiovascular events in patients with DM2.

In non-diabetic patients with coronary artery disease, two earlier studies have reported a beneficial effect of statin therapy on ischemic episodes [5,6] and ischemic duration and burden [6] as detected with AECG. We did not find any effect of statin therapy on SMI in our DM2 population; from the present data, one can only speculate whether these contradictory findings are due to the fact that we studied patients with diabetes or to the fact that we studied asymptomatic patients. However, we did find a beneficial effect on cardiovascular event rates. This result implies that the beneficial effects of statin therapy may not directly be mediated through reduction of silent ischemic episodes, which is in line with the perception that most cardiovascular events do not evolve from progressive narrowing of the vessel lumen, but rather from thrombus formation on a ruptured nonobstructing instable plaque [27]. Searching for SMI might therefore not be a rational way of risk stratification in asymptomatic patients with DM2.

Our study has possible limitations. First, cerivastatin was withdrawn from the market, resulting in a change from cerivastatin to simvastatin. After correcting the change in ischemic parameters for duration of cerivastatin treatment, however, the results remained unchanged. Second, at baseline, patients with complete AECG data in the statin group tended to have less ischemia than in the placebo group. This might have lead to less power to detect a treatment effect. However, the clear trend toward more ischemia after 2 years, equal in both groups and reflecting the natural history of atherosclerosis in DM2, makes a type II error unlikely.

In conclusion, we found a 20% prevalence of SMI in asymptomatic patients with DM2. We did not find any effect from two years of statin therapy on SMI, despite a significant reduction in cardiovascular events. SMI as detected with 48 hour AECG may not be a proper tool for risk stratification in patients with DM2.

REFERENCES

1. Stamler J, Vaccaro O, Neaton JD, Wentworth D: Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care* 16:434-444, 1993
2. Miettinen H, Lehto S, Salomaa V, Mahonen M, Niemela M, Haffner SM, Pyorala K, Tuomilehto J: Impact of diabetes on mortality after the first myocardial infarction. The FINMONICA Myocardial Infarction Register Study Group. *Diabetes Care* 21:69-75, 1998
3. Influence of diabetes on 5-year mortality and morbidity in a randomized trial comparing CABG and PTCA in patients with multivessel disease: the Bypass Angioplasty Revascularization Investigation (BARI). *Circulation* 96:1761-1769, 1997
4. Rutter MK, Wahid ST, McComb JM, Marshall SM: Significance of silent ischemia and microalbuminuria in predicting coronary events in asymptomatic patients with type 2 diabetes. *J.Am.Coll. Cardiol.* 40:56-61, 2002
5. Andrews TC, Raby K, Barry J, Naimi CL, Allred E, Ganz P, Selwyn AP: Effect of cholesterol reduction on myocardial ischemia in patients with coronary disease. *Circulation* 95:324-328, 1997
6. van Boven AJ, Jukema JW, Zwinderman AH, Crijns HJ, Lie KI, Bruschke AV: Reduction of transient myocardial ischemia with pravastatin in addition to the conventional treatment in patients with angina pectoris. REGRESS Study Group. *Circulation* 94:1503-1505, 1996
7. Beishuizen ED, van de Ree MA, Jukema JW, Tamsma JT, van der Vijver JC, Meinders AE, Putter H, and Huisman MV: Two-year statin therapy does not alter the progression of intima-media thickness in patients with type 2 diabetes without manifest cardiovascular disease. *Diabetes Care* 27:2887-2892, 2004.
8. Blackburn H, Keys A, Simonson E, Rautaharju P, Punsar S: The electrocardiogram in population studies. A classification system. *Circulation* 21:1160-1175, 1960
9. Langer A, Freeman MR, Josse RG, Steiner G, Armstrong PW: Detection of silent myocardial ischemia in diabetes mellitus. *Am.J.Cardiol.* 67:1073-1078, 1991
10. Hume L, Oakley GD, Boulton AJ, Hardisty C, Ward JD: Asymptomatic myocardial ischemia in diabetes and its relationship to diabetic neuropathy: an exercise electrocardiography study in middle-aged diabetic men. *Diabetes Care* 9:384-388, 1986
11. Koistinen MJ: Prevalence of asymptomatic myocardial ischaemia in diabetic subjects. *BMJ* 301:92-95, 1990
12. Prevalence of unrecognized silent myocardial ischemia and its association with atherosclerotic risk factors in noninsulin-dependent diabetes mellitus. Milan Study on Atherosclerosis and Diabetes (MiSAD) Group. *Am.J.Cardiol.* 79:134-139, 1997
13. Chiariello M, Indolfi C, Cotecchia MR, Sifola C, Romano M, Condorelli M: Asymptomatic transient ST changes during ambulatory ECG monitoring in diabetic patients. *Am.Heart J.* 110:529-534, 1985
14. Naka M, Hiramatsu K, Aizawa T, Momose A, Yoshizawa K, Shigematsu S, Ishihara F, Niwa A, Yamada T: Silent myocardial ischemia in patients with non-insulin-dependent diabetes mellitus as judged by treadmill exercise testing and coronary angiography. *Am.Heart J.* 123:46-53, 1992
15. Janand-Delenne B, Savin B, Habib G, Bory M, Vague P, Lassmann-Vague V: Silent myocardial ischemia in patients with diabetes: who to screen. *Diabetes Care* 22:1396-1400, 1999
16. Valensi P, Sachs RN, Harfouche B, Lormeau B, Paries J, Cosson E, Paycha F, Leutenegger M, Attali JR: Predictive value of cardiac autonomic neuropathy in diabetic patients with or without silent myocardial ischemia. *Diabetes Care* 24:339-343, 2001

17. Inoguchi T, Yamashita T, Umeda F, Mihara H, Nakagaki O, Takada K, Kawano T, Murao H, Doi T, Nawata H: High incidence of silent myocardial ischemia in elderly patients with non insulin-dependent diabetes mellitus. *Diabetes Res.Clin.Pract.* 47:37-44, 2000

18. Gazzaruso C, Garzaniti A, Giordanetti S, Falcone C, De Amici E, Geroldi D, Fratino P: Assessment of asymptomatic coronary artery disease in apparently uncomplicated type 2 diabetic patients: a role for lipoprotein(a) and apolipoprotein(a) polymorphism. *Diabetes Care* 25:1418-1424, 2002

19. Bacci S, Villella M, Villella A, Langialonga T, Grilli M, Rauseo A, Mastroianno S, De Cosmo S, Fanelli R, Trischitta V: Screening for silent myocardial ischaemia in type 2 diabetic patients with additional atherogenic risk factors: applicability and accuracy of the exercise stress test. *Eur.J.Endocrinol.* 147:649-654, 2002

20. Wackers FJ, Young LH, Inzucchi SE, Chyun DA, Davey JA, Barrett EJ, Taillefer R, Wittlin SD, Heller GV, Filipchuk N, Engel S, Ratner RE, Iskandrian AE: Detection of silent myocardial ischemia in asymptomatic diabetic subjects: the DIAD study. *Diabetes Care* 27:1954-1961, 2004

21. Airaksinen KE: Silent coronary artery disease in diabetes--a feature of autonomic neuropathy or accelerated atherosclerosis? *Diabetologia* 44:259-266, 2001

22. Whitsel EA, Boyko EJ, Siscovick DS: Reassessing the role of QTc in the diagnosis of autonomic failure among patients with diabetes: a meta-analysis. *Diabetes Care* 23:241-247, 2000

23. Ewing DJ, Martyn CN, Young RJ, Clarke BF: The value of cardiovascular autonomic function tests: 10 years experience in diabetes. *Diabetes Care* 8:491-498, 1985

24. Vinik AI, Maser RE, Mitchell BD, Freeman R: Diabetic autonomic neuropathy. *Diabetes Care* 26:1553-1579, 2003

25. Fredenrich A, Castillo-Ros S, Hieronimus S, Baudouy M, Harter M, Canivet B: Screening for silent myocardial ischemia in diabetic patients. *Diabetes Care* 23:563-564, 2000

26. Cosson E, Paycha F, Paries J, Cattan S, Ramadan A, Meddah D, Attali JR, Valensi P: Detecting silent coronary stenoses and stratifying cardiac risk in patients with diabetes: ECG stress test or exercise myocardial scintigraphy? *Diabet.Med.* 21:342-348, 2004

27. Lee RT, Libby P: The unstable atheroma. *Arterioscler.Thromb.Vasc.Biol.* 17:1859-1867, 1997

Chapter 9

Summary and Conclusions

SUMMARY

Cardiovascular disease is the principal cause of mortality in patients with type 2 diabetes mellitus (DM2). Cardiovascular events are preceded by years of endothelial dysfunction, atherosclerotic plaque formation and finally the thrombotic occlusion after rupture of an unstable plaque. Hyperglycemia, insulin resistance and coexisting dyslipidemia, hypertension and obesity are responsible for the enhanced atherosclerotic process in DM2.

The typical dyslipidemia in DM2 consists of low levels of HDL cholesterol, high levels of triglycerides and small, dense, atherogenic LDL particles, where total LDL cholesterol levels are not higher than in the general population. LDL cholesterol has proven to be an important risk factor for coronary artery disease in DM2.

Since 1994, several trials have proven the beneficial effect of HMG-CoA reductase inhibitors (statins) on cardiovascular morbidity and mortality in the setting of secondary prevention and in primary prevention in high-risk patients. These early trials, however, did not include diabetes as a prespecified subgroup. In spite of the lack of randomized trials in DM2, guidelines advocated the use of statins in primary prevention based on the assumption that DM2 is a "coronary heart disease equivalent". In 2004, with the publication of the CARDS study, the beneficial effect of statin therapy in primary prevention, was proven.

The present thesis describes a study, designed at a timepoint when no primary prevention trial had yet been performed to investigate the effects of statin therapy on patients with DM2 without cardiovascular disease. We have performed a randomized, double-blind placebo-controlled trial in 250 DM2 patients without manifest cardiovascular disease. The aim of the trial was to study non-invasively the effect of two year statin therapy on the vessel wall.

In *Chapter 2* the techniques and current status in DM2 of these non-invasive vascular tools are desciribed. Non-invasive imaging techniques as parameters for atherosclerosis, may help in risk stratification and in implementation of tailored therapy for the individual patient. However, many of these vascular tools have not been validated in diabetic individuals.

Since the introduction in the early 1990's, intima-media thickness (IMT) of the vessel wall, especially of the carotid artery, is used more and more as a surrogate marker for atherosclerotic disease. IMT can be assessed non-invasively using B-mode ultrasound. Ultrasonographic IMT measurements of the *far* wall relate to histological IMT measurements. Carotid IMT is higher in DM2 versus controls, and IMT progression might be higher. In prospective studies, carotid IMT has proven to be a consistent and independent predictor for coronary events and stroke in the general population, but in DM2 only in combination with several other novel risk factors. Intervention studies in non-diabetic individuals with statins have shown that IMT progression can be stopped or even reversed. Intervention studies with lipid lowering regimens in diabetic subjects are sparse.

Flow Mediated Dilation (FMD) of the brachial artery, as first described by Celermajer, is a non-invasive technique for measuring endothelial function. FMD is measured with B-Mode ultrasound or a wall-track system. FMD is impaired in DM2, in some studies already in the prediabetic state. FMD has proven to be predictive for the presence of coronary artery disease and for future cardiovascular events in high-risk populations. There are however no studies on the predictive power of FMD in DM2. Intervention studies with statins in diabetic individuals have shown conflicting results regarding the effect on FMD.

Ambulatory ECG (AECG) can detect silent myocardial ischemia. The prevalence of silent myocardial ischemia in asymptomatic DM2 is reported from 9.1 to 52% and is strongly dependent on method of detection and on the population studied. The frequency of significant coronary artery stenoses in diabetic patients with silent myocardial ischemia varies between 22 and 94 %. Silent myocardial ischemia predicts future coronary events in asymptomatic diabetic subjects, as well as in diabetic patients with documented coronary artery disease. Treatment with beta-blockers or statins resulted in reduced silent myocardial ischemia in patients with coronary artery disease, but studies in DM2 were not performed.

Chapter 3 describes the primary endpoint of the study, the effect of two year statin therapy on IMT in patients with DM2 without manifest cardiovascular diesease. 250 patients were randomized to either 0.4 mg cerivastatin, or placebo daily. In August 2001, when cerivastatin was withdrawn from the market, 0.4 mg cerivastatin was replaced by 20 mg simvastatin, without deblinding the study. The primary endpoint was the change of mean common carotid IMT, as measured by B-mode ultrasound, over 2 years.

Common carotid IMT at baseline was 0.780 mm in the placebo group and 0.763 mm in the statin group and did not change significantly after two years. LDL cholesterol was reduced by 25 % in the statin group and increased by 8% in the placebo group ($p<0.001$). There was no significant difference in IMT change in any carotid segment between the groups. Cardiovascular events occurred in 12 patients in the placebo group and in 2 patients in the statin group ($p=0.006$). We discuss that prognostic tools other than IMT should be explored in this patient group.

Chapter 4 describes the effect of two year statin therapy on FMD in patients with DM2 without manifest cardiovascular disease. The primary endpoint was the change in FMD, measured by B-mode ultrasound, after 2 years.

Determinants of baseline FMD were diabetes duration, common carotid IMT and brachial artery diameter. FMD at baseline was 1.51 % in the placebo group and 1.66 % in the statin group and did not change significantly after two years.

In conclusion, there was no effect of 2 years' statin therapy on FMD in DM2. Statin induced improvement of cardiovascular risk in patients with DM2 may be mediated through other mechanisms than increased NO availability.

Chapter 5 describes the effect of two year statin therapy on C-reactive protein (CRP), a marker of the inflammatory process in atherosclerotic plaques. We evaluated the effect of statin therapy on CRP in 250 patients with DM2 without manifest cardiovascular disease. The primary endpoint was the change in high sensitivity CRP after 2 years. CRP in the statin group was 1.58 mg/L at baseline and 1.69 mg/L at 2 years (p= 0.413), in the placebo group it increased from 2.03 mg/L at baseline to 2.54 mg/L at 2 years (p = 0.058) (p= 0.269 for comparison between the groups). In a high-risk subgroup with the metabolic syndrome (MS) and LDL levels > 2.6 mmol/L (40 % of the cohort) CRP levels increased significantly in the placebo group (from 2.97 mg/L at baseline to 3.99 mg/L at 2 years, p=0.036) in comparison to the statin group (from 2.13 mg/L at baseline to 2.10 mg/L at 2 years, p=0.885) (p=0.042 for comparison between the groups).

In conclusion, there was no effect of two year statin therapy on CRP in patients with DM2 without manifest cardiovascular disease, except in a subgroup with the metabolic syndrome (MS) and LDL > 2.6 mmol/L. We discuss that studies supporting risk stratified therapy in primary prevention in DM2 are needed.

In *Chapter 6* the relationship between CRP and MS is further elaborated by analysing the baseline data of the DALI study. The DALI study was performed to evaluate the efficacy of atorvastatin 10 and 80 mg versus placebo in patients with DM2 and mild dyslipidemia without cardiovascular disease. Endpoints in the original study were lipid parameters and endothelial function as assessed by FMD. In the present substudy the baseline laboratory parameters for inflammation and hemostasis and the baseline sonographic parameters IMT and FMD were used to assess the impact of MS and low grade chronic inflammation as assessed by CRP on vascular phenotype in 62 DM2 patients.

Serum sVCAM, sTM, and tPA levels significantly increased with increasing MS load. IMT also significantly increased from 0.602 ± 0.034 (one MS criterion) to 0.843 ± 0.145 (four MS criteria, p = 0.007). LogCRP significantly correlated with fibrinogen, PAI-1, and IMT. In a multiple regression, model MS and low-grade chronic inflammation have an independent impact on vascular phenotype including IMT in DM2.

A total of 19 % of our original study population of 250 patients were Asian Indians from Surinam. Although Asian Indian patients with DM2 are at high-risk for cardiovascular disease, not all patients develop cardiovascular disease. The vascular phenotype of Asian Indians with DM2 without cardiovascular disease has not been elucidated and may point to protective features. In *Chapter 7* we analyzed the baseline data of our main study to provide an initial description of vascular parameters in Asian Indians compared with Europid Caucasian controls (all with DM2), matched for age and gender. Endpoints of the study were endothelial function as measured by FMD, low-grade systemic inflammation as assessed by CRP and carotid IMT. Asian Indians had longer duration of diabetes, worse glycemic control and more

microangiopathy. Both groups demonstrated marked endothelial dysfunction. CRP levels were similar: 1.7 (4.9) mg/L in Asian Indians and 2.8 (3.6) mg/L in Europid Caucasians. Carotid IMT values were significantly lower in Asian Indians than Europid Caucasians (0.655 mm (0.12) versus 0.711 mm (0.15), p = 0.03). Multiple regression analysis showed that variability in CRP was mainly determined by waist circumference, not by ethnicity. In contrast, ethnicity was a significantly explanatory variable for carotid IMT.
Vascular phenotype of Asian Indians with DM2 without CVD was characterized by endothelial dysfunction and relatively low levels of CRP, comparable to Europid Caucasian controls with DM2. In contrast, lower carotid IMT values were observed in Asian Indians despite longer duration of diabetes and worse metabolic control. We propose that mechanisms slowing its progression may have atheroprotective potential in Asian Indians with DM2.

In *Chapter 8* we aimed to determine the prevalence of silent myocardial ischemia (SMI) and the effect of statin therapy on SMI in DM2 patients without manifest cardiovascular disease.

The primary endpoint was the change in ischemic episodes, duration and burden as measured by 48 hours ambulatory electrocardiography (AECG) over 2 years. At baseline, 47 out of 233 (20%) evaluable AECG's showed evidence of ischemia. After 2 years, there was a trend towards more ischemia in both treatment groups, without significant differences between the changes in ischemic parameters (episodes:p=0.498; duration:p=0.697; burden:p=0.798) in the two treatment groups. Cardiovascular events occurred in 12 patients in the placebo group and in 2 patients in the statin group (p=0.006). There was no relation between these cardiovascular events and the presence of SMI at baseline. In conclusion, SMI occurred in 20 % of DM2 patients without manifest cardiovascular disease. There was no effect of two years' statin therapy on SMI. In contrast, we observed a significantly lower cardiovascular event rate on statin therapy. We discuss here that AECG may not be a proper tool for risk stratification in patients with DM2.

CONCLUSIONS

The present thesis describes the effect of statin therapy on the process of atherosclerosis in patients with DM2 without cardiovascular disease. We used several parameters to assess this process. We found no effect of two year statin therapy on carotid IMT as a reflection of the progress of atherosclerosis. We found no effect on endothelial function as assessed by FMD. The effect of statin therapy on CRP, as a marker for low grade inflammation, was only significant in a high-risk subgroup with the metabolic syndrome and a high LDL cholesterol. There was no effect of two-year statin therapy on the prevalence of silent myocardial ischemia. In spite of these findings, we observed a lower cardiovascular event rate in patients on

statin therapy, which is in line with other clinical trials. Several possible explanations for these findings should be discussed.

First, the vascular tools used were all validated in non-diabetic populations, but the prognostic value of most of them was not validated in patients with DM2. Vessel wall biology in DM 2 is distinct from that of a non-diabetic population and the question arises whether changes in the vessel wall induced by DM2 are reversible by statin therapy. The intimal and medial layers of the vessel wall in DM2 are most likely irreversibly changed by processes such as extracellular matrix glycosylation and media calcification. These changes may resist global regression based on interference with local intravascular cholesterol metabolism. It can be hypothesized that, although statins do not improve NO availability nor the irreversibly changed glycosylated extracellular matrix, it may well have an effect on outcome in DM2 patients by its beneficial influence on plaque vulnerability. This is in line with the perception that most cardiovascular events do not evolve from progressive narrowing of the vessel lumen (as assessed by IMT and silent myocardial ischemia), but from thrombus formation on a ruptured non-obstructing instable plaque. To date it remains unsolved however, whether these other, nonlipid ("pleiotropic") effects on the vascular wall play an important role in the risk reductions as seen in the clinical trials.

Second, cerivastatin was withdrawn from the market, at a time when inclusion was completed, resulting in a change from cerivastatin to simvastatin. The reduction in LDL cholesterol with simvastatin 20 mg was slightly lower than that achieved with cerivastatin 0.4 mg. After correcting the change in all described endpoints for duration of cerivastatin treatment however, the results remained unchanged, so we do not believe that this switch has had a major influence on our results.

Third, contrary to the postulated progression of mean IMT per 2 years, in the present study the placebo group did not show any progression in IMT. It could be argued that our patient population has been low-risk. However, we included diabetic patients with a broad range in age and diabetes duration, while their baseline CCA IMT was well comparable to those of patients in other studies. Moreover, the observed rate of first major vascular events in our placebo group is comparable with the diabetic subgroup without prior cardiovascular disease on placebo in the Heart Protection Study. Thus, it seems unlikely that our results have been influenced by any "healthy volunteer" effect.

Implications for clinical practice

The non-invasive vascular tools described in this thesis were not able to detect beneficial effects of statin therapy in DM2 patients. To date, IMT and CRP are frequently used as risk markers for atherosclerosis in the general population. Their prognostic clinical usefullness in patients with DM2 still remains to be established. Recent cholesterol guidelines do not include these risk markers in risk scores. ATP III (Adult Treatment Panel III) uses risk stratification by means of classical risk factors for their recommended LDL goals. The American Diabetes

Association recommends prescribing statins to all patients with DM2 in a primary prevention setting with LDL cholesterol levels > 3.5 mmol/L to achieve a 30 to 40 % reduction in LDL cholesterol. The Joint European Guidelines advocates an LDL goal of < 2.5 mmol/L for all patients with DM2.

Other non-invasive vascular tools, more aimed at detection of plaque characteristics, may have better prognostic value. The use of non-invasive vascular tools in daily practice for risk stratification and tailored therapy warrants further investigation.

Chapter 10

Samenvatting

SAMENVATTING

Hart- en vaatziekten, zoals een hartinfarct of een herseninfarct vormen de belangrijkste doodsoorzaak van patiënten met diabetes mellitus type 2 (DM2). Voordat een hartinfarct plaats vindt, zijn er al jaren voorafgegaan van slecht functioneren van de vaatwand (endotheeldysfunctie), de vorming van de atherosclerotische plaque ("dichtslibben") en tot slot de afsluiting van het vat door een stolsel nadat een instabiele plaque geruptureerd is. Hoge bloedsuikers, verminderde gevoeligheid voor insuline (insuline resistentie) en gelijktijdig optredende vetstofwisselingsstoornis (dyslipidemie), hoge bloeddruk (hypertensie) en overgewicht zijn verantwoordelijk voor het versnelde proces van atherosclerose in DM2. Het samen vóórkomen van deze risicofactoren wordt het metabool syndroom genoemd.

De typische diabetische dyslipidemie bestaat uit een verlaagd HDL cholesterol, verhoogde triglyceriden en kleine, dichte en daardoor atherogene LDL cholesterol partikels. Het totaal cholesterol is vaak niet hoger dan bij de normale bevolking. LDL cholesterol is wel een bewezen risicofactor voor hartziekten in patiënten met DM2.

Sinds 1994 zijn er verschillende onderzoeken gepubliceerd die het gunstig effect van de cholesterolverlagende statines bij patiënten met hart- en vaatziekten (secundaire preventie) hebben aangetoond. De eerste grote studies hebben echter niet speciaal naar diabetespatiënten gekeken. Omdat de sterfte aan hart- en vaatziekten bij diabetespatiënten die nooit een hartinfarct hebben doorgemaakt echter gelijk is aan de sterfte bij patiënten zonder diabetes maar met een doorgemaakt hartinfarct, worden diabetespatiënten in de richtlijnen beschouwd als hartpatiënten. Zonder dat het dus bewezen was, werd het advies om aan alle patiënten met DM2 een statine voor te schrijven. In 2004 verscheen de CARDS studie, wel speciaal gericht op patiënten met DM2 zonder doorgemaakt hartinfarct (primaire preventie): in deze studie werden minder hart- en vaatziekten gezien in de groep die behandeld werd met een statine.

In dit proefschrift wordt een onderzoek beschreven, dat ontworpen werd in de tijd dat dergelijke studies nog ontbraken. We hebben een gerandomiseerde, dubbelblinde, placebo gecontroleerde studie verricht in 250 patiënten met DM2 zonder manifeste hart- of vaatziekten. Het doel van het onderzoek was op een niet-invasieve manier de effecten van 2 jaar statinegebruik op de vaatwand te meten.

In Hoofdstuk 2 worden de technieken beschreven die gebruikt kunnen worden om de vaatwand te bestuderen. Dergelijke technieken kunnen een graadmeter zijn voor het proces van atherosclerose en hiermee helpen om een inschatting te maken van het risico op een hart- en vaatziekte. Veel van deze technieken zijn echter niet gevalideerd in patiënten met DM2.

De Intima-Media dikte (engelse afkorting: IMT) van de vaatwand van met name de halsslagaders, gemeten met behulp van ECHO, is al sinds de jaren negentig van de vorige eeuw in gebruik als parameter voor atherosclerose. De echografisch gemeten waarde van de van

de ECHO-kop verst verwijderde wand ("far wall") heeft een goede relatie met de werkelijke dikte van de vaatwand. De IMT van halsslagaders is dikker in patiënten met DM2 en zou mogelijk ook een grotere progressie vertonen. In de algemene bevolking is de IMT van de halsslagaders een onafhankelijke voorspeller voor hartziekten en herseninfarct, in DM2 is dit echter minder hard bewezen. Uit onderzoeken met statines in patiënten zonder DM2 is gebleken dat progressie van IMT kan worden afgeremd door deze middelen. Bij DM2 waren dergelijke studies nog niet verricht.

De Flow gemedieerde vasodilatatie (engelse afkorting: FMD) van de slagader van de arm is een afspiegeling van de endotheelfunctie. Het endotheel vormt de binnenbekleding van de vaatwand en heeft een belangrijke functie in de bloeddoorstroming, de vaattonus en de permeabiliteit van de vaatwand, in het beperken van ontstekingsreactie en stollingsneiging. Het molecuul stikstofoxide (NO) speelt hierin een belangrijke rol. Echografisch gemeten FMD meet de mogelijkheid van het bloedvat om te kunnen verwijden (door NO) na een periode van zuurstoftekort, de normale verwijding is 5-10%. FMD is beperkt in DM2, volgens sommige studies al in het voorstadium van suikerziekte.

In hoog-risicopopulaties is FMD een voorspeller van hart-en vaatziekten, echter in DM2 is dit niet onderzocht. Onderzoeken naar de effecten van statines op FMD bij patiënten met DM2 laten tegenstrijdige resultaten zien.

Met een ambulant electrocardiogram (AECG) kan in de thuissituatie een continue registratie van de hartactie plaatsvinden terwijl de patiënt zijn gewone dagelijkse bezigheden doet. Zuurstofgebrek van het hart en ritmestoornissen kunnen zo worden opgespoord. Zuurstofgebrek van het hart ontstaat door atherosclerose van de kransslagaderen (coronarialijden) en leidt meestal tot pijn op de borst bij inspanning, angina pectoris. Door aantasting van het centraal zenuwstelsel van het hart (cardiale autonome neuropathie) ervaren patiënten met DM2 deze pijn echter vaak als minder heftig, of voelen het geheel niet: de zogenaamde stille cardiale ischemie (engelse afkorting: SMI). Het zou dus nuttig kunnen zijn om bij patiënten met DM2 SMI op te sporen met bijvoorbeeld AECG. Het vóórkomen van SMI bij verder asymptomatische DM2 patiënten wordt gerapporteerd tussen de 9.1 en 52 %. SMI is een voorspeller van hart-en vaatziekten bij patiënten met DM2. Bij hartpatiënten zonder diabetes leidde een behandeling met statines tot minder SMI, maar studies bij patiënten met DM2 waren nog niet verricht.

In Hoofdstuk 3 wordt het belangrijkste eindpunt van het onderzoek beschreven, namelijk de effecten van 2 jaar statine op de IMT van de halsslagaders bij patiënten met DM2 zonder manifeste hart- of vaatziekten. De 250 patiënten werden gerandomiseerd en kregen dagelijks cerivastatine 0.4 mg of placebo. In augustus 2001 werd cerivastatine plotseling van de markt gehaald na een rapport over de kans op ernstige bijwerkingen op de spieren (rabdomyolyse). Zonder het onderzoek te deblinderen werd cerivastatine 0.4 mg vervangen door simvastatine 20 mg en werd het onderzoek voltooid. Het LDL cholesterol daalde in de statinegroep met

25 % en steeg in de placebogroep met 8 %. Zowel in de statine groep als in de placebogroep veranderde de IMT van de halsslagader in deze 2 jaar niet. Desondanks kwamen in de placebogroep 12 hart-en vaatcomplicaties voor, in de statinegroep kwam dit maar 2 keer voor (p=0.006). We stellen daarom het nut van IMT bij patiënten met DM2 ter discussie.

In Hoofdstuk 4 wordt het effect van 2 jaar statine op FMD gemeten. FMD bleek in ons onderzoek afhankelijk van de duur van de diabetes, van de IMT van de halsslagaderen en van de doorsnede van het bloedvat. De FMD was laag, 1.51 % in de placebogroep en 1.66 % in de statinegroep en veranderde niet significant na 2 jaar. De gunstige effecten van statines op de kans op hart-en vaatziekten zijn dus ook niet gemedieerd door een verbetering van de NO beschikbaarheid.

In Hoofdstuk 5 wordt het effect beschreven van 2 jaar statine op het C-reactive protein (CRP), een parameter voor het onstekingsproces in atherosclerose. Zowel in de statinegroep als in de placebogroep veranderde het CRP niet na 2 jaar. In een hoog risicogroep met het metabool syndroom en een LDL cholesterol boven de streefwaarde van 2.6 mmol/l (40 % van het totaal aantal patiënten) steeg het CRP echter in de placebogroep en bleef het in de statinegroep gelijk, met een significant verschil tussen beide groepen. Dit resultaat suggereert dat statines het ontstekingsproces het best onderdrukken in hoog-risiso patiënten en ondersteunt het concept van risiso-stratificatie in het voorschrijven van statines in de setting van primaire preventie bij patiënten met DM2.

In Hoofdstuk 6 wordt het verband tussen CRP en het metabool syndroom verder onderzocht. Hiertoe worden de gegevens van een ander onderzoek, de DALI studie, gebruikt. In dit onderzoek werden in patiënten met DM2 de effecten van een andere statine, atorvastatine, op lipiden en FMD onderzocht. In de huidige substudie werden de uitgangswaarden van de parameters voor onsteking en stolling en de uitgangswaarden van IMT en FMD gebruikt. Het doel was om de impact van het metabool syndroom en CRP op de parameters van atherosclerose te meten. Het samen vóórkomen van het metabool syndroom en een verhoogd CRP leidde onder andere tot een hogere IMT.

Van de 250 deelnemende patiënten uit ons onderzoek was 19% Hindoestaans, afkomstig uit Suriname, oorspronkelijk uit India. Het is bekend dat deze bevolkingsgroep een hoog risico heeft op hart- en vaatziekten, zeker als er sprake is van DM2. In Hoofdstuk 7 analyseren wij de uitgangswaarden van deze bevolkingsgroep en vergelijken deze met de waardes van patiënten van Europese afkomst van dezelfde leeftijd en hetzelfde geslacht. Het blijkt dat de Hindoestaanse groep gemiddeld langer DM2 had, de DM2 was slechter gereguleerd en er waren meer complicaties. Hindoestaanse en Europese patiënten hadden een zelfde, lage FMD. Ook de CRP waardes waren vergelijkbaar. De IMT waardes waren duidelijk lager in de

Hindoestaanse groep. Dit is mogelijk een gevolg van de lagere diameters van de bloedvaten, maar deze relatie is niet goed onderzocht. Er zijn ook geen goede ras-specifieke IMT waardes bekend voor de Hindoestaanse bevolkingsgroep. Als IMT werkelijk prognostische waarde heeft in patiënten met DM2, dan hebben we in ons onderzoek wellicht juist een relatief gunstige Hindoestaanse groep geïncludeerd, namelijk een groep die ondanks lange diabetesduur en slechte regulatie toch geen hart-of vaatziekte heeft ontwikkeld.

In Hoofdstuk 8 beschrijven we het deelonderzoek naar SMI. Van de patiënten met analyseerbare AECG had 20 % SMI bij de start van de studie. Na 2 jaar was dit 24.5 %, er waren geen verschillen tussen de statinegroep en de placebogroep. Er was geen relatie tussen het optreden van een hart- of vaatcomplicatie en SMI. AECG is dus wellicht geen goede manier om risicostratificatie te verrichten bij patiënten met DM2.

CONCLUSIES

Dit proefschrift beschrijft de effecten van statines op het proces van atherosclerose in patiënten met DM2 zonder manifeste hart- of vaatziekten. We gebruikten verschillende parameters om dit proces te kwantificeren. We vonden geen effect van 2 jaar statinetherapie op de IMT van de halsslagaderen als reflectie van progressie van atherosclerose. We vonden geen effect op de endotheelfunctie, gemeten met FMD. Het effect van statinetherapie op CRP, als marker voor laaggradige ontsteking, was alleen siginificant in een hoog-risicogroep met het metabool syndroom en een hoog LDL cholesterol. Er was geen effect van 2 jaar statinetherapie op het vóórkomen van SMI. Desondanks waren er in de statinegroep minder hart- of vaatcomplicaties dan in de placebogroep, volledig in overeenstemming met de grote klinische onderzoeken. Er zijn verschillende mogelijke verklaringen voor deze bevindingen:

Ten eerste, de vaatwandtechnieken die gebruikt werden waren gevalideerd in niet-diabetische populaties, maar de prognostische waarde voor patiënten met DM2 is voor de meeste technieken onbekend. De vaatwandbiologie in DM2 is anders dan die bij niet-diabeten en de vraag is of vaatwandafwijkingen veroorzaakt door DM2 wel reversibel zijn door statinetherapie. De intima- en mediavaatwanden zijn waarschijnlijk irreversibel veranderd door afzettingen van suikergroepen en kalk.

Een hypothese is dat statines weliswaar niet de vaatwanddikte en de beschikbaarheid van NO verbeteren, maar dat statines een gunstig effect hebben op de stabiliteit van de atherosclerotische plaque. De meeste hartinfarcten ontstaan immers niet door langzaam dichtslibben van een bloedvat (zoals wordt weerspiegeld door IMT en SMI), maar door de vorming van een trombus op een geruptureerde, niet-stabiele plaque. Het is nog niet zeker wat voor rol deze zogenaamde "pleiotrope" effecten van statines hebben in de risicoreductie voor hart-en vaatziekten.

Ten tweede, cerivastatine werd van de markt gehaald toen de inclusie van ons onderzoek al compleet was, waardoor cerivastatine vervangen moest worden door simvastatine. De daling van het LDL cholesterol met 20 mg simvastatine was iets minder dan met cerivastatine 0.4 mg. Bij alle eindpunten hebben wij hiervoor gecorrigeerd, maar de resulaten veranderden daardoor niet. Het lijkt hiermee niet waarschijnlijk dat de switch grote gevolgen heeft gehad voor de resultaten.

Ten derde, in tegenstelling tot de vantevoren gepostuleerde progressie van IMT in 2 jaar, vertoonde de IMT in ons onderzoek geen progressie in de placebogroep. Een verklaring zou kunnen zijn dat onze patiëntenpopulatie een laag risico op hart- en vaatziekten had op basis van een selectiebias. We hebben echter patiënten geïncludeerd met een brede leeftijdsrange en uiteenlopende diabetesduur, met een uitgangs-IMT vergelijkbaar met andere studies. Bovendien zijn het aantal hart-en vaatcomplicaties in de placebogroep vergelijkbaar met de grote HPS studie. Het lijkt hiermee dus niet waarschijnlijk dat onze resultaten beïnvloed zijn door een "gezonde vrijwilliger" effect.

Implicaties voor de klinische praktijk

De niet-invasieve technieken die in dit proefschrift zijn beschreven waren niet in staat de gunstige effecten van statines te detecteren in patiënten met DM2. Tegenwoordig worden met name IMT en CRP veel gebruikt als parameters voor het risico van atherosclerose. Hun voospellende waarde in DM2 staat echter nog niet vast.

Recente richtlijnen ten aanzien van het cholesterol in patiënten met DM2 zonder hart- en vaatziekten gebruiken deze parameters dan ook niet. De ATP III (Adult Treatment Panel III) richtlijn gebruikt de klassieke risicofactoren om een stratificatie te maken voor het aanbevolen LDL cholesterol. De richtlijn van de American Diabetes Association beveelt aan om statines voor te schrijven aan alle patiënten met DM2 zonder hart- en vaatziekten met een LDL cholesterol > 3.5 mmol/L met als doel een reductie van 30 tot 40 % te bereiken. De Joint European Guidelines raden aan het LDL cholesterol te verlagen tot < 2.5 mmol/L in alle patiënten met DM2.

Andere technieken, meer gericht op de karakteristieken van de atherosclerotische plaque, hebben wellicht een betere voorspellende waarde in DM2. De toepassing van niet-invasieve technieken voor risicostratificatie en therapie op maat in de dagelijkse praktijk vergt nog veel nieuw onderzoek.

CURRICULUM VITAE

Edith D. Beishuizen werd geboren op 24 maart 1966 te Amsterdam. Na het behalen van haar eindexamen Atheneum B aan het Christelijk Lyceum te Alphen aan den Rijn in 1984, startte zij in hetzelfde jaar met de studie Geneeskunde aan de Universiteit Leiden. In 1991 behaalde zij Cum Laude het Artsexamen. Van 1991 tot 1993 was zij werkzaam als arts assistent niet-in-opleiding op de afdeling interne geneeskunde van het Rode Kruis Ziekenhuis te Den Haag, tegenwoordig onderdeel van het "HAGA Ziekenhuis" (opleider Dr. D. Overbosch). In 1994 begon zij aan de opleiding tot internist in het Diakonessenhuis Utrecht (opleider Prof. Dr. J.B.L. Hoekstra). De opleiding werd in 1997 voortgezet in het Universitair Medisch Centrum Utrecht (opleider Prof. Dr. D.W. Erkelens†).

In 1999 kon zij in het laatste deel van haar opleiding een aanvang maken met het in dit proefschrift beschreven onderzoek welke in het Ziekenhuis Leyenburg te Den Haag (tegenwoordig onderdeel van het "HAGA Ziekenhuis") werd uitgevoerd (opleider Dr. J.C.M. van der Vijver, promotor Prof. Dr. A.E. Meinders, co-promotor Dr. M.V. Huisman). De registratie als internist vond plaats in 2000. Zij bleef als chef de clinique/onderzoeker tot en met 2002 werkzaam in het Ziekenhuis Leyenburg.

In 2003 en 2004 werkte zij als staflid bij de afdeling Algemene Interne Geneeskunde van het Leids Universitair Medisch Centrum. In deze periode volgde zij het aandachtsgebied Vasculaire Geneeskunde (opleider Dr. M.V. Huisman). In 2005 trad zij toe tot de maatschap interne geneeskunde van 't Lange Land Ziekenhuis te Zoetermeer.

PUBLICATIES

Djaberi R, Beishuizen ED, Pereira AM, Rabelink TJ, Smit JW, Tamsma JT, Huisman MV, Jukema JW.
Non-invasive cardiac imaging techniques and vascular tools for the assessment of cardiovascular disease in type 2 diabetes mellitus.
Diabetologia 2008 Sep;51(9):1581-93.

Alizadeh Dehnavi R, Beishuizen ED, van de Ree MA, Le Cessie S, Huisman MV, Kluft C, Princen HM, Tamsma JT.
The impact of metabolic syndrome and CRP on vascular phenotype in type 2 diabetes mellitus.
Eur J Intern Med. 2008 Mar;19(2):115-21.

Ray A, Beishuizen ED, Misra A, Huisman MV, Tamsma JT.
Vascular phenotype and subclinical inflammation in diabetic Asian Indians without overt cardiovascular disease.
Diabetes Res Clin Pract. 2007 Jun;76(3):390-6.

Tamsma JT, Jazet IM, Beishuizen ED, Fogteloo AJ, Meinders AE, Huisman MV.
The metabolic syndrome: a vascular perspective.
Eur J Intern Med. 2005 Sep;16(5):314-20.

Beishuizen ED, Jukema JW, Tamsma JT, van de Ree MA, van der Vijver JC, Putter H, Maan AC, Meinders AE, Huisman MV.
No effect of statin therapy on silent myocardial ischemia in patients with type 2 diabetes without manifest cardiovascular disease.
Diabetes Care. 2005 Jul;28(7):1675-9.

Beishuizen ED, Tamsma JT, Jukema JW, van de Ree MA, van der Vijver JC, Meinders AE, Huisman MV.
The effect of statin therapy on endothelial function in type 2 diabetes without manifest cardiovascular disease.
Diabetes Care. 2005 Jul;28(7):1668-74.

Hovens MM, Tamsma JT, Beishuizen ED, Huisman MV.
Pharmacological strategies to reduce cardiovascular risk in type 2 diabetes mellitus: an update.
Drugs. 2005;65(4):433-45. Review.

Beishuizen ED, van de Ree MA, Jukema JW, Tamsma JT, van der Vijver JC, Meinders AE, Putter H, Huisman MV.
Two-year statin therapy does not alter the progression of intima-media thickness in patients with type 2 diabetes without manifest cardiovascular disease.
Diabetes Care. 2004 Dec;27(12):2887-92.