LINKS BETWEEN INSULIN RESISTANCE, INFLAMMATION AND SUBCLINICAL MACROVASCULAR DISEASE IN TYPE 2 DIABETES

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Academic dissertation

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Abstract

Background and aims. Type 2 diabetic patients have a three- to four-fold risk of cardiovascular disease. A cluster of cardiovascular risk factors has been identified (high blood pressure, high triglycerides, low HDL cholesterol, obesity, impaired glucose metabolism), which together constitutes the metabolic syndrome. The metabolic syndrome affects up to 80 per cent of type 2 diabetic patients. Inflammation, endothelial activation, and the growth factor system derangement are all novel cardiovascular risk factors. The present studies were undertaken to explore the roles of the insulin-like growth factor system, low-grade inflammation, and endothelial dysfunction in relation to incipient atherosclerosis, traditional cardiovascular risk markers, and the metabolic syndrome especially in type 2 diabetes.

Subjects and methods. Two hundred and thirty-nine type 2 diabetic subjects aged 50 to 75 were recruited from participants of the FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) study at the Helsinki centre. Additionally, a healthy control group (N = 93) was also recruited. The examinations were performed during the placebo run-in phase of the FIELD study for the diabetic patients. An extensive list of lipids and lipoproteins, inflammatory markers, endothelial markers, and IGF system variables was determined. Albumin excretion rate was measured. Carotid arteries were scanned for the determination of intima-media thickness (IMT) as a surrogate marker of atherosclerosis. Pulse-wave analysis (PWA) was performed to determine central arterial augmentation and augmentation index (AIx) to measure arterial stiffness. In a subset (N = 99), carotid scans were reread to determine the severity of local soft and mineralised vessel wall thickening.

Results. Compared with the control group, IMT was thicker in diabetic subjects. Blood pressure, age, gender, the duration of diabetes, dyslipidaemia, and obesity were positively related to IMT in diabetic subjects. In contrast, insulin-like growth factor binding protein-1 (IGFBP-1) was inversely related to IMT in diabetic subjects. In healthy subjects, the determinants of IMT were age, inflammation, endothelial activation, LDL cholesterol, and insulin resistance, but not blood pressure. Among diabetic subjects IGFBP-1 was a marker of insulin sensitivity and inversely related to the severity of the metabolic syndrome. Low-grade inflammation and endothelial dysfunction were enhanced in type 2 diabetes. Among diabetic patients, the severity of the metabolic syndrome correlated strongly with the levels of markers for inflammation and endothelial activation. The concentrations of these markers were not related to clinical cardiovascular disease among diabetic patients. Central pressure augmentation,

AIx, and IMT correlated with each other and with the severity of local vessel wall thickening. Diabetic women had higher augmentation and AIx values than men after controlling for confounders. Determinants of AIx in diabetic subjects were blood pressure, albuminuria, and IMT, and to a lesser extent, obesity and endothelial dysfunction.

Conclusions. 1) IGFBP-1 and AIx could potentially be useful in detecting subjects with cardiovascular risk among diabetic patients. 2) Low IGFPB-1 is a good marker of insulin resistance and the metabolic syndrome. 3) Diabetes has a more detrimental effect on arterial compliance in women than in men. 4) Low-grade inflammation and endothelial dysfunction are enhanced in diabetes, but do not differ between diabetic patients with or without cardiovascular disease in a cross-sectional setting.

Contents

List of original publications

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals.

I Leinonen E, Salonen JT, Salonen R, Koistinen R, Leinonen P, Sarna S, Taskinen M-R. Reduced IGFBP-1 is associated with thickening of carotid wall in type 2 diabetes. *Diabetes Care* 2002; 25:1807-1812.

II Leinonen E, Hurt-Camejo E, Wiklund O, Mattson-Hultén L, Hiukka A, Taskinen M-R. Insulin resistance and adiposity correlate with acute-phase reaction and soluble cell adhesion molecules in type 2 diabetes. *Atherosclerosis.* 2003; 166:387-394.

III Leinonen ES, Hiukka A, Hurt-Camejo E, Wiklund O, Sarna SS, Mattson Hultén L, Westerbacka J, Salonen RM, Salonen JT, Taskinen M-R. Low-grade inflammation, endothelial activation, and carotid intima-media thickness in type 2 diabetes. *Journal of Internal Medicine* 2004, 256:119-127.

IV Westerbacka J, Leinonen E, Salonen JT, Salonen R, Hiukka A, Yki-Järvinen H, Taskinen M-R. Increased augmentation of central blood pressure is associated with increases in carotid intima-media thickness and mineralisation changes in type 2 diabetic patients. In press *Diabetologia*.

In addition some unpublished data are presented.

Abbreviations

AGE	advanced glycation end product
4S	Simvastatin Scandinavian Survival Study
ACCORD	Action to Control Cardiovascular Risk in Diabetes
AFCAPS/TexCAPS	Air Force/Texas Coronary Atherosclerosis Prevention Study
AIx	augmentation index
BMI	body mass index
CAM	cell adhesion molecule
CarDif	the plaque height difference between site-specific maximums and minimums averaged over all scanned carotid sites
CABG	coronary artery bypass grafting
CARDS	Collaborative Atorvastatin Diabetes Study
CARE Study	Cholesterol And Recurrent Events Study
CB IMT	the mean of maximum IMT over all scanned carotid bulb sites
CCA IMT	the mean of maximum IMT over all scanned common carotid artery sites
CHD	coronary heart disease
Chol	cholesterol
CRP	ultra-sensitive C-reactive protein
CV	cardiovascular
CVD	cardiovascular disease
DAIS	Diabetes Atherosclerosis Intervention Study
DPS	Diabetes Prevention Study
e-NOS	endothelial nitric oxide synthase
FIELD	Fenofibrate Intervention and Event Lowering in Diabetes
FW IMT	the mean of mean far wall IMT over all scanned carotid far wall sites
HbA1c	glycosylated haemoglobin HbA1c
HDL	high density lipoprotein
HOMA IR	homeostasis model assessment for insulin resistance
HPS	Heart Protection Study
ICA IMT	the mean of maximum IMT over all scanned internal carotid artery sites
ICAM-1	intercellular adhesion molecule 1

IDL	intermediate-density lipoprotein
IFG	impaired fasting glucose
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
IGT	impaired glucose tolerance
IHD	ischaemic heart disease
IL-6	interleukin-6
IMT	intima-media thickness
LDL	low density lipoprotein
LIPID study	Long term Intervention with Pravastatin in Ischaemic Heart Disease Study
Max IMT	the mean of maximum IMT over all scanned carotid sites
M-CSF	monocyte colony-stimulating factor
Mean IMT	the mean of mean IMT over all scanned carotid sites
MI	myocardial infarction
NGT	normal glucose tolerance
NO	nitric oxide
OGTT	oral glucose tolerance test
PAI-1	plasminogen activator inhibitor
PWA	pulse-wave analysis
SAA	human serum amyloid A
SMC	smooth muscle cell
$sPLA_2$	soluble phospholipase A_2 IIA
TIA	transient ischaemic attack
TNFα	tumour necrosis factor α
t-PA	tissue plasminogen activator
TRL	triglyceride-rich lipoproteins
UAER	urinary albumin excretion rate
UKPDS	United Kingdom Prospective Diabetes Study
VA-HIT	Veterans Affairs High-Density Lipoprotein Intervention Trial
VCAM-1	vascular cell adhesion molecule 1
WHR	waist-to-hip ratio
WISE	Women's Ischaemia Syndrome Evaluation Study
VLDL	very-low-density lipoproteins
VWF	von Willebrand factor

1. Introduction

The number of people with diabetes has risen dramatically during the last decades. The prevalence of diabetes in adults (>20 years) worldwide was estimated to be 135 million in 1995 (King et al. 1998). If the epidemic of diabetes continues as predicted, the estimated global prevalence in adults will rise to 366 million by 2030 (Wild et al. 2004).

People with type 2 diabetes have a three to four -fold increased risk for cardiovascular disorders such as coronary heart disease (CHD), myocardial infarction (MI), transient ischaemic attack (TIA), stroke, peripheral vascular disease, and amputations (Howard et al. 2002). Approximately 75% of deaths among type 2 diabetic patients are accounted for by cardiovascular disease (Laakso and Lehto. 1998). Thus the epidemic of diabetes will be followed by a similar wave of cardiovascular disease (CVD) worldwide.

Age, family history, diabetes, hypertension, smoking, high total and LDL cholesterol, low HDL cholesterol, and obesity are established risk factors for atherosclerosis in the general population (Faxon et al. 2004, Fruchart et al. 2004). More recently, small dense LDL, elevated triglycerides, and homocystein, among others, have become established as cardiovascular risk factors (Fruchart et al. 2004). Overall, the effects of multiple traditional CVD risk factors are additive (Neaton et al 1992).

Recently, several novel cardiovascular risk factors have been identified. Inflammation is an important factor at all stages of atherosclerosis, and has been the topic of extensive research (Hackam 2003). Increasing evidence on the essential role of endothelial dysfunction in atherothrombotic vascular disease is accumulating (Smith et al. 2004).

In diabetic individuals, the CVD risk caused by any individual risk factor or any combination of risk factors rises more steeply than in non-diabetic subjects (Stamler et al. 1993). At any given number of risk factors, diabetic individuals have an approximately 3-fold CV risk. This indicates a specific, diabetes-related risk. Toxic effects of hyperglycaemia, derangement of coagulation and fibrinolysis, platelet hyperaggregability, oxidative stress, and autonomic neuropathy play a role in the excess CV morbidity (Hurst 2003). Data is also accumulating on detrimental postprandial metabolism with hyperglycaemia and elevation of atherogenic lipoproteins in type 2 diabetic patients (Taskinen 2003, Heine et al. 2004, Krauss 2004).

The metabolic syndrome accompanies type 2 diabetes in four out of five type 2 diabetic patients (Isomaa et al. 2001). The underlying metabolic disorder and central feature of the metabolic syndrome is insulin resistance. Insulin resistance is associated with an enhanced inflammatory state and vascular endothelial dysfunction, a tendency to thrombosis, and impaired thrombolysis (Pickup 2004). Therefore, several of the newly identified cardiovascular risk factors are actually features of the metabolic syndrome.

The metabolic syndrome precedes and predicts both CVD and incipient type 2 diabetes (Betteridge 2004). The metabolic syndrome has been reported to be an independent risk factor of cardiovascular disease (CVD), irrespective of the level of glucose tolerance (Isomaa et al. 2001). The concentrations of inflammatory markers identified as cardiovascular risk factors are already elevated in insulin-resistant individuals before the diagnosis of diabetes (Festa et al. 2003). Cardiovascular disease and diabetes seem to develop simultaneously. Diabetes often remains subclinical until presentation of an acute clinical cardiovascular event (Norhammar et al. 2002).

Finally, hormonal factors such as an overactive hypothalamic-pituitary-adrenal axis, deranged insulin-like growth factor system, and altered hormonal secretory activity of the adipose tissue seem to be components of insulin resistance syndrome and risk factors for atherosclerosis (Pickup 2004).

The complex mechanisms underlying the relationship between atherosclerosis, type 2 diabetes and the metabolic syndrome are not well understood yet. One key question that remains unanswered is whether insulin resistance precedes inflammation or vice versa.

The present study has explored these relationships in a cross-sectional setting in type 2 diabetic patients participating in the prospective part of the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study.

2. Review Of The Literature

2.1. Definition, pathogenesis, and epidemiology of type 2 diabetes

According to the criteria of the World Health Organisation (WHO 1999), a fasting plasma glucose concentration of 7.0 mmol/l and above, on two occasions, indicates diabetes. The diagnostic criterion for diabetes in the 75g oral glucose tolerance test (OGTT) is a 2-hour post-challenge value of 11.1 mmol/l or above.

According to the WHO definition, type 2 diabetes *"is the most common form of diabetes and is characterized by disorders of insulin action and insulin secretion, either of which may be the predominant feature. Both are usually present at the time that this form of diabetes is clinically manifest. By definition, the specific reasons for the development of these abnormalities are not yet known".* Disturbance in both insulin secretion and insulin action, i.e. insulin resistance, vary greatly among type 2 diabetic patients (WHO 1999). The relative importance of insulin secretion deficiency and insulin resistance has been debated, but current evidence indicates that both are present early in the natural course of the development of type 2 diabetes (Kahn 2003). Over time, a progressive deterioration of beta-cell function leads to a decline in glucose tolerance (Matthews et al. 1998, Weyer et al. 1999).

The individual's risk to type 2 diabetes is determined by both genetic and environmental factors. Unequivocal evidence for the heritability of type 2 diabetes based on twin studies and also studies on first-degree relatives of type 2 diabetic subjects exists (McIntyre and Walker 2002). Susceptibility genes have been sought by candidate gene, affected sib pair analysis, and genome wide scan approaches. There have been studies conducted on different high risk populations including Pima Indians and inhabitants of Nauru island, as well as in isolated cohorts, e.g. the Amish, etc. (Van Tilburg et al. 2001). Current data suggest a complex genetic background.

According to the "thrifty phenotype hypothesis," originally introduced by Barker and colleagues (Hales et al. 1991) and reinforced by several research groups (reviewed by Barker 2004), poor intrauterine nutrition renders individuals predisposed to type 2 diabetes in adult life. The central element of the hypothesis is that poor foetal and infant nutrition leads to disturbed programming of glucose-insulin metabolism (Hales and Barker 2001).

Lifestyle influences whether a genetically and in utero predisposed individual develops type 2 diabetes. Such lifestyle factors include dietary intake and physical activity, the resulting weight, and the amount and distribution of adipose tissue, especially accumulation of central fat (Wing et al. 2001). There is an interaction between genetic and environmental factors. Specific genes that partly determine the protective effect of lifestyle changes on the risk of diabetes have been identified (Todorova et al. 2004, Shuldiner et al. 2004).

The incidence and prevalence of type 2 diabetes is exploding in most populations, the rate of increase being most marked in the Third World (Diamond 2003). The number of diabetic patients worldwide was estimated to be 135 million in 1995 (King et al. 1998), and 171 million in 2000 (Wild et al. 2004), but is predicted to rise to 366 million by 2030 in people over 20 years of age (Wild et al. 2004). The main reasons for the diabetes epidemic are the growing number of people over 65 years of age together with urbanisation and the increase in the prevalence of obesity in many countries worldwide (Wild et al. 2004).

The number of type 2 diabetic patients in Finland was estimated to be near to 190 000 at the end of 2003. If undiagnosed cases are taken into account, the number would be over 400 000 (Reunanen 2004). By 2030, the number of diagnosed type 2 diabetes in Finland is expected to rise to 400 000, whereas undiagnosed cases may then reach almost one million (Reunanen 2004). The prevalence is one of the highest in Europe, almost the same level as in the United States, and the main reason for the escalating incidence in Finland is increasing obesity (Reunanen 2004).

2.2. Type 2 diabetes and CVD

2.2.1. Type 2 diabetes and the risk of CVD

Type 2 diabetes has been defined as: *"a state of premature cardiovascular death which is associated with chronic hyperglycaemia and may also be associated with blindness and renal failure"* (Fisher 1998). The same traditional risk factors for CVD are operative in type 2 diabetic as in non-diabetic individuals. However, the effect of any given risk factor on the incidence of CVD is greater in diabetic than non-diabetic populations (Stamler et al. 1993).

The three major vascular beds where atherosclerosis clinically manifests are the coronary arteries, lower extremities, and carotid arteries (Beckman et al. 2002). Patients with diabetes but without previous MI have been reported to have a similar risk for subsequent coronary events as nondiabetic patients with previous MI (Haffner et al. 1998). Therefore, type 2 diabetes has been defined as a coronary artery disease risk equivalent by the Adult Treatment Panel III (ATPIII) of the National Cholesterol Education Program (NCEP) (Expert panel 2001).

In addition to the high incidence of coronary heart disease in diabetic patients, both short- and long-term case fatality rate after MI is substantially elevated (Malmberg et al. 2000, Mukamal et al. 2001). In the FINMONICA register, 1-year mortality after first MI for diabetic men was 44 per cent vs. 33 per cent in non-diabetic men, and for diabetic women 37 per cent vs. 20 per cent in non-diabetic women (Miettinen et al. 1998). The 28-day mortality rate of hospitalised MI patients was almost 2-fold in diabetic vs. non-diabetic men and 3-fold in diabetic vs. non-diabetic women (Miettinen et al. 1998). The 5-year mortality rate in diabetic patients has been reported to be up 50 per cent after myocardial infarction, which is more than twice the rate of non-diabetic patients (Beckman et al. 2002). Mortality is even increased before hospital admission. Therefore primary prevention should be strongly emphasized (Haffner 2000). For comparison, the 5-year survival rate for any malignant neoplasm diagnosed in Finland over the 1999 to 2001 period is predicted to be 55 per cent for men and 65 per cent for women (Finnish Cancer Registry 2004). Thus, diabetes is a vascular disease with a poor prognosis.

The risk of stroke ranges from 1.5 to a 4-fold increase in type 2 diabetic patients (Beckman et al. 2002). The risk of claudication was markedly higher in diabetic subjects in the Framingham cohort (Kannel and McGee 1985). The relative risk (RR) for lower extremity amputation is over 12-fold higher in type 2 diabetic vs. non-diabetic individuals in the US (Beckman et al. 2002). In a Finnish study, the risk of amputation in a middle-aged type 2 diabetic cohort followed up for 7 years was over 5 per cent in both male and female patients (Lehto et al. 1996).

2.2.2. Type 2 diabetes and CVD risk factors

Traditional risk factors, such as hypertension, smoking, and high LDL cholesterol, increase CVD risk also in diabetic populations. The presence of microalbuminuria has been identified as a CVD risk factor in both type 1 and 2 diabetic patients and in the general population (MacIsaacs et al. 2004). Several non-traditional, partly overlapping and intertwined, risk factors have been detected during the last decade (Table 1).

Table 1. Non-traditional risk factors for CVD in type 2 diabetes

Hyperglycaemia	Microalbuminuria
Dyslipidaemia	Enhanced inflammation
- elevated triglyceride-rich lipoproteins (TRLs)	Endothelial dysfunction
- small dense LDL particles	Coagulation abnormalities
- decreased HDL cholesterol	Oxidative stress
- small dense HDL particles	Advanced glycation
- postprandial hyperlipidaemia	High homocystein level
- remnant particles	

Chronic hyperglycaemia *per se* is a risk factor for CVD (Laakso 1999). However, the impact of glycaemia is stronger on microvascular than macrovascular end points in type 2 diabetes. In the UKPDS (United Kingdom Prospective Diabetes Study), each 1 per cent point reduction of HbA1c achieved was associated with a reduction of 37 per cent in microvascular complications but with only 14 per cent decrease in the incidence of MI (Stratton et al. 2000). These results may partly be due to a lack of efficient treatment options to maintain good glycaemic control in the long term. Nevertheless, the impact of glycaemia in type 2 diabetic patients has been demonstrated after coronary angioplasty: optimal glycaemic control (HbA1c < 7%) reduced the occurrence of cardiac rehospitalisation and recurrent angina by more than half (Corpus et al. 2004). Potential mechanisms of how hyperglycaemia may induce vascular injury include a decrease in the bioavailability of nitric oxide (NO) and prostacyclin, increased synthesis of vasoconstrictor prostanoids and endothelin, increased production of advanced glycation end products (AGEs), excessive oxidative stress, and activation of protein kinase C (PKC) (Creager et al. 2003).

Among the traditional CVD risk factors, hypertension is twice as prevalent in type 2 diabetic as non-diabetic subjects (Stein et al. 1995, Reunanen et al. 2000). In the UKPDS cohort, 32 per cent of men and 45 per cent of women had a diagnosis of hypertension at baseline (Turner et al. 1998). The level of systolic blood pressure correlated significantly with clinical complications, including CVD, in the UKPDS (Adler et al. 2000). A tight control of blood pressure, compared with a less tight control, resulted in a 32 per cent decrease in deaths related to diabetes, 44 per cent decrease in strokes, and 37 per cent decrease in microvascular end points in the UKPDS (UK Prospective Diabetes Study Group 1998). A recent review on pharmacological and non-pharmacological antihypertensive trials for preventing CV complications in diabetic patients included 15 appropriate trials with data available for analysis derived from 760 references (Fuller et al 2004). The summary odds ratio (OR) for CV mortality from the primary prevention trials was 0.64, short-term secondary prevention trials 0.68, and long-term secondary prevention trials 0.82. The data demonstrated a treatment benefit for all-cause mortality in the secondary, but not in the primary prevention trials.

Type 2 diabetes is also accompanied by a multiple derangement of lipid metabolism, i.e. diabetic dyslipidaemia. Typical features of diabetic dyslipidaemia include elevation of triglyceride-rich lipoproteins (TRL), especially very-low-density lipoproteins (VLDL), lower HDL cholesterol concentration, and small dense LDL particles (Syvänne and Taskinen 1997).

A recently recognised phenomenon is excessive and prolonged postprandial lipaemia (fat intolerance) (Taskinen 2003). This is caused by both increased hepatic secretion of VLDL and impaired clearance of VLDL and intestinally derived chylomicrons (Krauss 2004). The diurnal triglyceride profile consists of a gradual increase after each meal, the peak concentration being reached between dinner and bedtime, and the lowest concentration measured in the morning after

an overnight fast. The major component of TRLs after a fat load, approximately 80 percent, are the VLDL particles (Taskinen 2003). The prolonged retention of VLDL and chylomicrons in circulation results in an accumulation of partially lipolysed remnant particles, including cholesterol-enriched intermediate-density lipoproteins (IDL), which are especially atherogenic (Krauss 2004).

The increase of TRLs also affects the metabolism of LDL and HDL subclasses (Taskinen 2003). The long residence time of the TRLs in circulation favours a change of core lipids between both LDL and TRLs and HDL and TRLs, leading to triglyceride enrichment of LDL and HDL particles. Hepatic lipase, the concentration of which is commonly increased in type 2 diabetes, avidly hydrolyses triglyceride enriched LDL and HDL particles, producing smaller particles (Taskinen 2003). The small HDL particles are rapidly catabolised and cleared from plasma, whereas small dense LDL particles have a longer residence time in plasma due to a reduced affinity for LDL receptors (Krauss 2004).

The trapping of cholesterol-rich, atherogenic lipoproteins within the subendothelial space of the vascular wall is one of the initial events in the cascade leading to atherosclerosis (Williams 2001). The increased atherogenic potential of small dense LDL seem to be related to several properties of these particles: reduced LDL receptor affinity, greater propensity for penetration in the arterial intima, increased binding to arterial wall proteoglycans, and susceptibility to oxidative modification and glycation (Taskinen 2003, Krauss 2004).

HDL particles are important in reverse cholesterol transport, i.e. transport of cholesterol from peripheral tissues via plasma to the liver. HDL particles have additional functions including, among other actions, anti-thrombotic and antioxidant effects, amelioration of abnormal vasoconstriction by stimulation of nitric oxide (NO) production, and inhibition of adhesion of monocytes to the endothelium (Barter et al. 2003). The cardioprotective effects of HDL particles are decreased in type 2 diabetes, due to reduced numbers of HDL particles and to structural changes in the HDL particles (Taskinen 2003).

Land-mark lipid-lowering trials – the 4S (Simvastatin Scandinavian Survival Study), CARE (Cholesterol And Recurrent Events), AFCAPS/TexCAPS (Air Force/Texas Coronary atherosclerosis Prevention Study), and LIPID (Long term Intervention with Pravastatin in Ischaemic heart Disease) – have demonstrated that lowering of LDL cholesterol with HMG-CoA reductase inhibitors reduces the rate of coronary events in diabetic subgroups by 19 to 55 per cent (Kreisberg and Oberman 2002). The Heart Protection Study (HPS) included a subgroup of 5963 diabetic patients, among whom simvastatin intervention reduced the risk for major vascular events (including major coronary events, stroke, and revascularisation) by approximately one quarter (Heart Protection Study Collaborative Group 2003). The Collaborative Atorvastatin Diabetes Study (CARDS) (Colhoun et al 2004) assessed the effectiveness of atorvastatin for primary prevention of CV events in an entirely type 2 diabetic patient cohort (N = 2838). The event rate

was substantially reduced (by 37 percent), and the effect was not related to pre-treatment cholesterol levels.

Fibrates correct most abnormalities in diabetic dyslipidaemia: fibrates lower triglycerides, increase HDL cholesterol, increase the clearance of VLDL and remnant lipoproteins, shift LDL particle size distribution to a larger form, and decrease production of small dense HDL (Watts and Dimmitt 1999). Consequently, fibrates should theoretically be the best treatment option for diabetic dyslipidaemia (Barter 2001).

In a post-hoc analysis of a small diabetic subgroup of the Helsinki Heart Study, gemfibrozil reduced CHD events by 68 per cent, but the result was statistically non-significant due to the small number of patients (Frick et al. 1987). The DAIS (Diabetes Atherosclerosis Intervention Study) demonstrated angiographically documented regression of coronary atherosclerosis by fenofibrate intervention (Diabetes Atherosclerosis Intervention Study Investigators 2001). The Veterans Affairs High-Density Lipoprotein Intervention Trial (VA-HIT) used gemfibrozil for secondary prevention. In that study, gemfibrozil reduced CVD death, stroke and MI by one third in the diabetic subgroup (Rubins et al. 2002). Interestingly, at the VA-HIT, baseline insulin resistance predicted CVD events and the benefit of fibrate intervention more powerfully than HDL cholesterol or triglyceride levels (Robins et al. 2003). The FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) study and the ACCORD (Action to Control Cardiovascular Risk in Diabetes) (Prisant 2004) study will demonstrate if fibrates are actually even more effective than statins in the prevention of CVD in diabetic subjects.

2.3. The metabolic syndrome

2.3.1. Definitions and prevalence of the metabolic syndrome

The metabolic syndrome was recognized in the late 1980's, then called the Syndrome X (Reaven 1988). Rapidly accumulating literature has addressed the metabolic syndrome, recognizing it as both a cardiovascular risk factor and a predictor of type 2 diabetes (Nesto 2003, Grundy 2004).

The metabolic syndrome has been defined by various criteria (Grundy et al 2004a), the main of which are the WHO definition (WHO 1999) and the NCEP (National Cholesterol Education Program) criteria, also called the ATP III (Adult Treatment Panel III) criteria (Expert Panel 2001) (Table 2).

Table 2. WHO and NCEP definitions of the metabolic syndrome.

WHO definition **Diabetes/IFG/IGT + at least 2 of the following:**	**NCEP criteria** **3 or more of the following criteria:**
1. Insulin resistance (under hyper-insulinaemic, euglycaemic conditions, glucose uptake < lowest quartile of background population)	1. Abdominal obesity: Waist circumference > 102 cm in men, > 88 cm in women
2. Raised arterial pressure ≥ 140/90	2. Hypertriglyceridemia > 1.69 mmol/l
3. Raised P-triglycerides (≥ 1.7 mmol/l) and/or low HDL cholesterol (< 0.9 mmol/l in men, < 1.0 mmol/l in women)	3. Low HDL cholesterol < 1.04 mmol/l in men, < 1.29 mmol/l in women
4. Central obesity: (males: WHR > 0.90; females: > 0.85) And/or BMI > 30kg/m^2	4. High blood pressure ≥ 130/85
5. Microalbuminuria (UAER ≥ 20μg/min or albumin : creatinine ratio ≥ 30mg/g)	5. High fasting glucose ≥ 6.1 mmol/l

Both sets of criteria are specific tools to detect low insulin sensitivity among non-diabetic subjects, although the WHO criteria are more sensitive than the NCEP criteria (Hanley et al. 2003). Similar comparisons between the sensitivity of the two criteria sets to detect insulin resistance have not been performed in type 2 diabetic cohorts. Nevertheless, clustering of risk factors typical of insulin resistance have been associated with extreme insulin resistance also among type 2 diabetic patients (Haffner et al 2003).

Two more criteria sets for the diagnosis of insulin resistance syndrome, including features from both WHO and NCEP criteria, have been suggested by the American Association of Clinical Endocrinologists and by the European Group for the Study of Insulin Resistance (EGIR) (reviewed in Grundy et al. 2004a). The key difference from the NCEP criteria are that both of these criteria sets are totally founded on insulin resistance.

Other features of the metabolic syndrome not mentioned in the definitions are hyperuricaemia and gout, non-alcoholic fatty liver disease (NAFLD), abnormalities in fibrinolysis and coagulation, the polycystic ovary syndrome, signs of chronic inflammation, endothelial dysfunction, and increased sympathetic activity (Isomaa 2003).

The estimated prevalence of the metabolic syndrome depends on the population and the definition of the metabolic syndrome and its components. In a cross-sectional US population sample collected over the 1988-1994 period, the age-

adjusted prevalence among 8608 participants aged ≥20 years was 23.9 per cent using NCEP criteria and 25.1 per cent using WHO criteria. In some subgroups the difference was more marked. For example, in African-American men the prevalence was 24.9 per cent according to WHO definition criteria and only 16.5 per cent using the NCEP definition criteria (Ford and Giles 2003). In the year 2000, about 47 million US residents had the metabolic syndrome (Ford et al. 2002). The prevalence of the metabolic syndrome increases with age and is highest in the elderly population. The increase of obesity in the population is paralleled by a rising incidence of the metabolic syndrome also in middle-aged individuals, and even in youths (Grundy et al 2004b, Weiss et al. 2004).

In the Botnia study, metabolic syndrome as defined by the WHO criteria was present in ~ 10 per cent of subjects with NGT, ~ 50 per cent in subjects with IGT/ IFG, and ~ 80 per cent of subjects with type 2 diabetes. (Isomaa et al. 2001). In another Finnish study (Lakka et al. 2002), the presence of the metabolic syndrome in middle-aged men was 14.2 per cent using the WHO criteria and 8.2 per cent using the NCEP criteria. Recently, the population-based FINRISK cohort (aged 45-64 years) and the glucose-intolerant Diabetes Prevention Study (DPS) cohort (aged 40-65 years) where analysed using modified WHO criteria for metabolic syndrome (Ilanne-Parikka et al. 2004). Metabolic syndrome was present in ~ 40 per cent of men and ~20 per cent of women in the FINRISK cohort. The extremely high prevalence in men in the FINRISK cohort was mainly due to abdominal obesity: more than three-quarters of men had a WHR > 0.9. Not surprisingly, in the DPS cohort the prevalence of the metabolic syndrome was 75 per cent.

2.3.2. The metabolic syndrome as a predictor of CVD and type 2 diabetes

Obviously, as the metabolic syndrome is a cluster of several cardiovascular risk factors, it carries a great risk of cardiovascular disease. In the Framingham database, the metabolic syndrome accounted for approximately one fourth of cardiovascular morbidity (Grundy et al. 2004a). Hyperinsulinaemia, which is among non-diabetic subjects a marker of insulin resistance, was associated with an increased CHD risk over a 22-year follow-up in the Helsinki Policemen study (Pyörälä et al. 1998). In the Botnia study, subjects with the metabolic syndrome had a 3-fold increased risk for CHD and stroke, 5-6-fold increased risk of cardiovascular death, and increased all-cause mortality (Isomaa et al. 2001). In the San Antonio Heart Study, where a population cohort was studied between 1984-1988 and followed up 6 - 7 years later, insulin resistance as calculated by the homeostasis model (HOMA IR) was associated with an increased CVD incidence independently of several cardiovascular covariates (Hanley et al. 2002). In Finnish men, insulin resistance and the metabolic syndrome predicted CHD events and both CV and all-cause mortality (Kuusisto et al. 2001, Lakka et al. 2002). In a Dutch study on a cohort of patients with recently diagnosed CHD, the metabolic syndrome was present in 45 per cent, and associated with increased

IMT, albuminuria, and ankle brachial pressure index (Olijhoek et al. 2003). In the Verona diabetes complications study, HOMA IR was an independent predictor of prevalent and incident CVD among type 2 diabetic patients during a follow-up of 4.5 years (Bonora et al. 2002). Trevisan et al. (1998) followed mortality in relation to the components of the metabolic syndrome for a mean of 7 years in a population-based Italian cohort of approximately 20 000 men and women. In that study, an increasing number of features of the metabolic syndrome was associated with increased incidence of CV and all cause death in both genders.

Nine studies on the association between insulin resistance and the risk of stroke were reviewed by Kernan et al. (2002). Six of these studies were methodologically sound and provided substantial evidence that insulin resistance is associated with a 60 per cent to 160 per cent increased risk of stroke.

The metabolic syndrome not only accompanies, but also precedes and predicts, type 2 diabetes (Yki-Järvinen 2000, Kekäläinen et al. 1999, Hanson et al. 2002). In the Framingham cohort, the presence of the metabolic syndrome at baseline as defined by NCEP criteria was considered to be a powerful predictor of newly-onset diabetes, accounting for almost half of the risk for diabetes (Grundy et al. 2004a). The San Antonio Heart Study investigators compared three options to predict the onset of type 2 diabetes: 1) IGT detected by the OGTT, 2) the presence of the metabolic syndrome by the NCEP criteria, and 3) the presence of the metabolic syndrome according to a modified version of the WHO criteria (without OGTT). Of the three, IGT was the best predictor: 43 per cent predictive value vs. 31 per cent by the NCEP criteria and 30 per cent by the modified WHO criteria (Lorenzo et al. 2003). This result indicates that the OGTT remains valuable in screening for diabetes risk. Interestingly, in a study among non-diabetic American Indians, HOMA IR and the metabolic syndrome at baseline were associated with an increased the risk for developing diabetes, but did not predict CVD independently of other CV risk factors (Resnick et al. 2003).

2.3.3. Obesity, inflammation, and the metabolic syndrome

During the last decade, new discoveries elucidated interactions between the adipose tissue and the pathophysiology of the metabolic syndrome, and their links to inflammation, endothelial dysfunction, diabetes, and atherosclerosis.

Insulin resistance is regarded as the fundamental abnormality behind the metabolic syndrome (Haffner et al.1992). Recent data suggest that a high amount of fat and a derangement of the adipose tissue metabolism are in fact the primary factors determining the development of insulin resistance and other components of the metabolic syndrome (Ruan and Lodish 2004). In the Insulin Resistance Atherosclerosis Study (IRAS), the strongest predictor of the metabolic syndrome was waist circumference, thus abdominal obesity may precede the development of the other manifestations/components of the

metabolic syndrome (Palaniappan et al. 2004). Likewise in the San Antonio Study, one third of subjects with both a large waist circumference and high BMI developed the metabolic syndrome during a 8 years' follow-up. Adjusting for fasting insulin concentrations had only a minor effect on the predictive value of the anthropometric indices (Han et al. 2002b).

The crucial role of the fat mass does not rule out the importance of heritability in the development of the metabolic syndrome. Environmental factors, i.e. the obesity epidemic due to the lack of physical exercise and increased caloric intake, are obviously responsible for the current increase in the incidence of the metabolic syndrome worldwide. Yet, the predisposition to gain weight is highly individual and to a great extent determined by genetic factors (Speakman 2004, Bouchard and Perusse 1993).

As early as the late 1980's, adipose tissue was found to be involved in the metabolism of sex steroids and to produce an endocrine factor called adipsin (Flier et al 1987, reviewed in Kershaw and Flier 2004). A variety of adipocyte-derived proteins with humoral functions have been detected, acting both in an autocrine/paracrine fashion and also at a systemic (endocrine) level. These include cytokines and cytokine-related proteins, such as leptin, tumour necrosis factor α (TNFα), and IL-6 (interleukin -6), factors involved with fibrinolysis, such as plasminogen activator inhibitor -1 (PAI-1) and tissue factor, or with the complement system, as well as enzymes involved in steroid metabolism, etc. (Kershaw and Flier 2004). Furthermore, fat cells express receptors that allow them to respond to afferent signals from traditional hormone systems and the central nervous system. Thus adipose tissue is actively involved in energy metabolism, neuroendocrine function, and immune function.

Adiponectin is the only adipokine that is known to increase insulin sensitivity (Bays et al. 2004). In animal studies, the decrease of adiponectin has preceded the development of insulin resistance and obesity (Kershaw and Flier 2004). Low levels of adiponectin are associated with type 2 diabetes and obesity, correlate inversely with insulin resistance, and are predictive of the development of type 2 diabetes (Rajala and Scherer 2003, Chandran et al. 2003, Krakoff et al. 2003, Bays et al. 2004). Improving insulin sensitivity by weight loss or insulin-sensitising medical treatment increases adiponectin levels (Kershaw and Flier 2004). A low level of adiponectin has been shown to be associated with the prevalence of coronary artery disease and to predict the occurrence of myocardial infarction (Nakamura Y et al. 2004, Pischon et al. 2004).

Resistin is an adipocyte-derived protein that has been shown to cause insulin resistance when injected in healthy animals (Bays et al. 2004). Yet, subsequent human studies have failed to provide a consistent link between resistin expression or resistin concentrations with obesity or insulin resistance (Kershaw and Flier 2004).

Leptin is secreted by the adipose tissue in direct proportion to the adipose tissue mass (Kershaw and Flier 2004). Leptin was initially viewed as an antiobesity hormone, but later research has demonstrated that the physiological role of leptin is to act as a metabolic signal of energy sufficiency. Caloric restriction and weight loss are accompanied by a rapid decrease in circulating leptin concentration, associated with increased appetite and a decline in energy expenditure. In obese subjects the leptin concentration is elevated due to increased fat mass, but these supraphysiological leptin levels fail to suppress appetite, consistent with a state of leptin resistance (Kershaw and Flier 2004).

Chronic subclinical inflammation is enhanced in the metabolic syndrome (Festa et al. 2000, Hak et al. 2001, Fröhlich et al. 2000, Fernández-Real and Ricart 2003, Pickup 2004). The degree of inflammation is associated with the amount of fat mass and central adiposity (Lyon et al. 2003). For example, circulating levels of C-reactive protein (CRP) correlate with insulin resistance (Festa et al. 2000, Lemieux et al. 2001) and the amount of adipose tissue (Hak et al. 1999, Festa et al. 2001, Lemieux et al. 2001).

The major cytokine mediator of the acute-phase response, IL-6, is highly expressed and secreted into the circulation by the adipose tissue (Bays et al. 2004). As much as 30 per cent of circulating IL-6 is derived from fat tissue in obese individuals (Mohamed-Ali et al. 1997). IL-6 stimulates hepatic CRP production (Lemieux et al. 2001, Lyon et al. 2003) and may also secondarily augment CRP secretion by its inflammatory-inducing actions (Bays et al. 2004). IL-6 levels are increased in type 2 diabetic patients and are correlated with the severity of glucose intolerance (Bays et al. 2004). The complex mechanisms as to how the insulin-resistant state might induce increased IL-6 production have been reviewed by Fernández-Real and Ricart (2003). Adipose tissue is infiltrated by macrophages in obese rodents; thus obesity-induced insulin resistance may be mediated by macrophage-related inflammatory reactions in addition to adipocyte actions (Xu et al. 2003).

2.4. Endothelial function and its markers

The vascular endothelium physiologically maintains vascular homeostasis by synthesizing and releasing vasoactive substances in reaction to haemodynamic forces and blood borne stimuli (De Caterina 2000, Szmitko 2003). Functional properties of the endothelium include the regulation of vascular tone, as well as active control of haemostasis, leukocyte adhesion and migration, endothelial permeability, medial smooth muscle cell growth, and structure of subendothelial matrix (De Caterina 2000, Sica 2000). A healthy endothelium also exerts antioxidant and anti-inflammatory effects (Bonetti et al. 2003). No universally accepted definition exists for endothelial dysfunction. Disruption of the balance in any or all of the diverse functions of the endothelium including: the disequilibrium between vasodilatation and vasoconstriction, fibrinolytic and

thrombotic properties, derangement of antiadhesive properties, or disturbance in permeability – indicate endothelial dysfunction (Mattz and Andriantsitohaina 2003). Table 3 presents factors that participate in the regulation of endothelial functions (modified from Calles-Escandon and Cipolla 2001).

Table 3. Endothelial functions and examples of mediators.

Vasoconstriction	Endothelin, angiotensin II, thromboxane A_2
Vasodilatation	Nitric oxide, bradykinin
Growth stimulation	Platelet-derived growth factor, fibroblast growth factor, insulin-like growth factor I, endothelin, angiotensin II
Inflammation	Vascular cell adhesion molecule 1, intercellular adhesion molecule 1, selectins, tumour necrosis factor α
Haemostasis	Tissue plasminogen activator, plasminogen activator inhibitor 1, prostacyclin

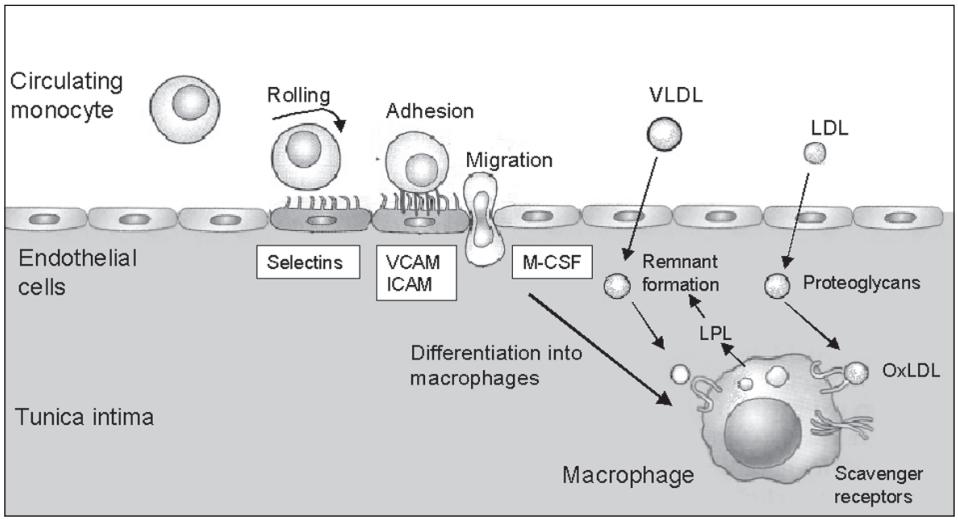
The insulin-resistant state is accompanied by endothelial dysfunction, leading to increased leukocyte adherence and penetration in the arterial intima, accumulation of atherogenic lipoprotein particles in the intima, enhanced thrombosis, as well as to a decline in endothelium-dependent vasodilatation (De Caterina 2000, Wheatcroft et al. 2002,Yki-Järvinen et al. 2003).

Insulin reduces the stiffness of arteries (Westerbacka et al. 1999), and this action of insulin is inversely related to insulin resistance and obesity (Westerbacka et al. 2001). Insulin normally increases the production of NO (Kuboki et al. 2000). In type 2 diabetes, endothelium-dependent vascular relaxation is impaired due to decreased NO bioactivity in the vessel wall. Indeed vascular endothelial dysfunction can be regarded a feature of the insulin resistance syndrome (Yki-Järvinen 2003). Invasive methods for endothelial function testing (Ganz and Vita 2003) are laborious and timeconsuming. Results obtained by non-invasive methods remain partly disputable.

The healthy vascular endothelium is resistant to circulating inflammatory reactants and cells (Libby et al. 2002). Inflammation of the endothelial monolayer leads to secretion of cell adhesion molecules (CAM) that have an autocrine/paracrine effect on the endothelium itself, the surrounding smooth muscle cells, and the blood cells (Calles-Escandon and Cipolla 2001, Hansson 2001). For example, selectins mediate leukocyte recruitment and adhesion at sites of inflammation on the endothelium (Price and Loscalzo 1999). The second major group of adhesion molecules belong to the immunoglobulin supergene family, including among others ICAM-1 and VCAM-1 (Blann and Lip 2000).

The initiation of atherosclerosis requires activation of the endothelium to express CAMs. Figure 1 illustrates the initial steps of atheroma formation: 1) rolling of monocytes on the endothelial surface induced by selectins, 2) adhesion of monocytes to the endothelial layer through interactions with CAMs, 3) penetration of the monocytes to the intima governed by chemotactic factors (e.g. monocyte chemotactic protein-1, MCP-1), 4) differentiation of monocytes into macrophages induced, among others, by macrophage colony stimulation factor (M-CSF), and 5) uptake of modified lipoproteins in the macrophage by way of scavenger receptors, a process regulated by cytokines such as TNFα and interleukins, 6) finally leading to foam-cell formation.

Figure 1. Endothelial dysfunction leading to initiation of atherosclerosis. Modified from Li and Glass 2001.



Local inflammatory reactions are also involved in subsequent smooth muscle cell (SMC) proliferation, atheroma formation, thinning of the fibrous cap of the atheroma, leading to plaque rupture, and thereafter thrombosis at the site of the rupture (Hansson 2001, Libby et al. 2002).

2.5. Inflammation in CVD and diabetes

The importance of systemic low-grade inflammation in the initiation and development of atherosclerosis as well as acute CV events has been extensively studied and firmly established (Ross 1999, Hansson 2001, Libby et al. 2002). Several cytokines and acute-phase reactants have been shown to be associated with and predict cardiovascular disease, among others CRP, IL-6, serum amyloid A (SAA), fibrinogen, white blood cell count, D-dimer, plasminogen activator, TNFα, lipoprotein phospholipase A_2, interleukin-18, metalloproteinase

PAPP-A, and secretory nonpancreatic phospholipase A_2 type IIA ($sPLA_2$) (Pearson et al. 2003, Fichtlscherer et al. 2004, Hurt-Camejo et al. 2001).

Among a wide range of biomarkers CRP is considered to be the most applicable for clinical use. In several studies, CRP has actually predicted cardiovascular disease better than other inflammatory biomarkers including CAM, TNFα, and IL-6, or even LDL cholesterol and other lipoprotein levels (Fichtlscherer et al.2004). In the general population, the level of CRP has consistently predicted incident MI, stroke, peripheral arterial disease, sudden cardiac death, and recurrent ischaemia and death in patients with angina and acute coronary symptoms (Ridker 2003).

Mechanisms as to how CRP might induce atherogenesis include binding to lipids, opsonising native LDL to macrophages, reducing the production of endothelial nitric oxide synthase (e-NOS) and decreasing NO bioavailability (Fichtlscherer et al. 2004), activating CAM expression by endothelial cells, and inducing MCP-1 production (Yeh 2004). Danesh et al. (2000) compared CRP levels in men who died from CHD or suffered a non-fatal MI with those of men who remained CHD free. The odds ratio of CHD was >2 in men in the top tertile of CRP when adjusting for confounders. However, the authors additionally conducted a meta-analysis. According to this it still remains open if CRP is an independent risk factor for CVD (Danesh et al 2000). The AHA recommendations state that the best, but so far inconclusive, evidence supports the use of CRP in clinical assessment of CV risk (Pearson et al. 2003).

Systemic inflammation is also associated with atherogenic changes of lipoprotein metabolism: hypertriglyceridaemia, elevated TRLs, small dense LDL, sphingolipid-enriched lipoproteins, and decreased HDL (Khovidhunkit et al. 2000). HDLs exert anti-inflammatory effects by inhibiting expression of MCP-1 and CAMs in endothelial cells (Barter P 2004, Calabresi et al. 2003). In insulin-resistant states the lowering in HDL cholesterol levels leads to a decline in its protective action against endothelial inflammation. One link between systemic inflammation and atherogenesis may be secretory phospholipase A_2 IIA ($sPLA_2$), an acute-phase reactant, which is able to deplete the phospholipid layer of LDL thus rendering LDL particles smaller, denser, and more proatherogenic (Hurt-Camejo et al. 2000). Small LDL particles are associated with endothelial dysfunction (Vakkilainen et al. 2000).

Elevated concentrations of inflammatory markers have been shown to precede the onset of clinical diabetes in several studies (Pickup 2004). Among over 32 000 participants in the Nurses' Health Study, CRP levels were significantly associated with the risk of developing diabetes during a 10 year follow-up (Hu et al. 2004). In the Atherosclerosis Risk in Communities (ARIC) study, IL-6 independently predicted incident type 2 diabetes, whereas the predictive value of CRP disappeared after adjusting for adiposity, fasting glucose and insulin levels (Duncan et al. 2003). In the Mexico City Diabetes study, CRP was a significant

predictor for the development of the metabolic syndrome – described as incident dyslipidaemia or hypertension or diabetes - only in men, but not in women (Han et al. 2002 a). Using factor analysis in the IRAS cohort, Hanley and co-workers could identify three underlying factors: a "metabolic", "inflammation", and "blood pressure" factor, each of which significantly predicted future diabetes (Hanley et al. 2004).

During endothelial activation, the plasma concentrations of CAMs rise due to increased endothelial production and/or shedding of CAMs into the circulation (Price and Loscalzo 1999). In several studies the concentrations of CAMs have correlated with traditional CV risk factors, acute coronary syndromes, and subsequent progression of atherosclerosis (reviewed by Bonetti et al. 2003). Elevated levels of CAMs have been detected in diabetic patients and individuals with insulin resistance or at high risk for developing type 2 diabetes (Blann and Lip 2000, Calles-Escandon and Cipolla 2001). However, the prognostic value of CAM as predictors of CVD remains controversial (Malik et al. 2001, Bonetti et al 2003). A handful of prospective studies have suggested that inflammation and/or endothelial dysfunction predict CVD risk in type 2 diabetes (Jager et al. 1999, Jager et al. 2000, Saito et al. 2000, Stehouwer et al. 2002, Pickup and Mattock 2003).

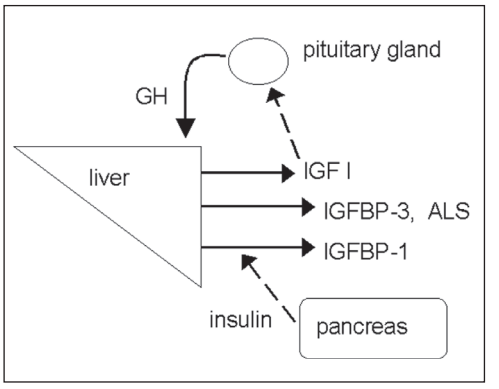
As yet, it is not settled if increased acute-phase reaction and endothelial dysfunction are causally linked to increased CVD risk in type 2 diabetes. Inflammation seems to be a common antecedent of both atherosclerosis and diabetes. Insulin resistance independently predicts CVD in type 2 diabetic patients (Bonora et al. 2002). On the other hand, CRP correlates with the severity of the metabolic syndrome (Fröchlich et al. 2000). The temporal relationship between the initiation of inflammation and insulin resistance still remains unknown.

2.6. The IGF system in CVD and diabetes

2.6.1. The IGF axis

The insulin-like growth factor family (IGF) contains three peptide hormones – (pro)insulin, IGF-I and IGF-II, which share homology in their amino acid sequence (Le Roith 1997). Circulating levels of IGF-I and IGF-II are determined primarily by their production in the liver. IGFs are bound to IGF-binding proteins (IGFBPs), which are mainly of hepatic origin, and which also critically modulate the cell response to IGFs (Bayes-Genis et al. 2000). In addition, many cells in the body (including vascular smooth muscle cells) synthesize IGFs and IGFBPs in an autocrine/paracrine manner (Bayes-Genis et al. 2000, Frystyk et al. 2002).

Figure 2. The IGF axis



Growth hormone (GH) stimulates the hepatic production of insulin-like growth factor I (IGF-I), insulin-like growth factor binding protein 3 (IGFBP-3) and acid-labil subunit (ALS). IGF-I exerts negative feedback on GH secretion. IGF-I bioactivity in plasma is regulated e.g. by the relative amount of IGF-I bound to an inactive complex of IGFBP-3 and ALS, and to IGFBP-1 and other IGFBPs. Insulin regulates (suppresses) the production of IGFBP-1 in the liver.

IGFs bind to a membrane receptor, which is very similar to the insulin receptor (Le Roith 1997). The main reservoir of IGF-I is the inactive trimeric complex with IGFBP-3 and a liver-derived glycoprotein called acid-labil subunit (ALS), binding more than 95 per cent of the IGF in serum (Rehman HU 2000). IGFBP-1 exhibits diurnal variation, in contrast to the more stable IGFBP-2 and -3. It is also one of the major regulators of IGF availability in plasma (Rabkin SW 1996).

Insulin regulates IGF-I bioavailability by suppressing hepatic IGFBP-1 production, resulting in increased circulating free IGF-I concentration (Mohamed-Ali et al. 1999). Insulin circulates at picomolar (10^{-12}) concentrations affecting mainly the liver, muscle and adipose tissue, whereas the IGFs circulate at nanomolar (10^{-9}) concentrations and have a broad range of actions in the body (Le Roith 1997). IGF-I effects may be mediated through high-affinity binding to its own receptor, or through low-affinity binding to insulin receptors (Frystyk et al. 2002). Thus, the end result of IGF-I mediated biological functions depend on the receptor, i.e. differences in post-receptor signalling of IGF-I and insulin receptors. The effects that IGF-I causes by low-affinity binding to insulin receptors may be relevant due to the high plasma concentrations of IGF-I compared with insulin levels.

2.6.2. IGF-I and IGFBP-1 in diabetes and CVD

IGFs mediate most of the effects of growth hormone (Rehman 2000). The multiple physiological effects that IGF-I exerts on the vasculature have been recently reviewed (Delafontaine et al. 2004). IGF-I has endocrine and autocrine/paracrine effects on blood vessels, including proliferative, hypertrophic, cell survival, vasomotor, and metabolic effects, which are modulated by IGFBPs. Growth hormone and/or IGF-I have been shown to be implicated in the development of diabetic micro- and macrovascular complications in several studies reviewed by Rehman (2000). Yet, the role of IGF-I and GH in diabetic complications remains controversial (Frystyk et al. 2002). Paradoxically, cardiovascular risk is increased in both acromegaly (Colao et al. 2001) and growth hormone deficiency (McCallum et al. 2002).

Levels of IGFBP-1 are increased in type 2 diabetic patients (Clauson et al. 1998), and decreased in subjects with impaired glucose tolerance in comparison with healthy subjects (Heald et al. 2001). As insulin regulates (suppresses) hepatic IGFBP-1 production, changes in IGFBP-1 at least partly mirror the change in insulin levels during the deterioration of glucose tolerance, i.e. the compensatory hyperinsulinaemia in subjects with IGT and the following decline in insulin secretion in type 2 diabetes. IGFBP-1 levels are inversely correlated with several cardiovascular risk factors, such as high TG, low HDL cholesterol, hypertension, insulin, proinsulin, and obesity in both type 2 diabetic and healthy subjects (Gibson et al. 1996, Janssen et al. 1998, Mohamed-Ali et al. 1999, Heald et al. 2001). IGFBP-1 is also strongly directly related to insulin sensitivity (Mohamed-Ali et al. 1999, Ricart and Fernández-Real 2001).

2.7. Intima-media thickness (IMT) and CVD and diabetes

Non-invasive imaging techniques to detect preclinical atherosclerosis have been developed during the last decades to identify individuals at an increased risk for clinical CVD. Such techniques are used to monitor the vascular response to different treatment modalities, i.e. to serve as a surrogate end-point in cardiovascular research.

The most commonly used non-invasive method has been the B-mode ultrasound scanning of carotid and/or femoral artery intima-media thickness (IMT) developed in late 1980s (Poli et al. 1988, reviewed by Salonen and Salonen 1993, Lonn 1999, Cheng et al. 2001, O'Leary and Polak 2002, Bots et al. 2003). The IMT is defined by the two parallel echogenic lines which correspond to the interfaces between lumen/intima and media/adventitia (see Methods, 5.3.1. Intima-media thickness (IMT), Figure 3). The thickness of the echogenic line next to the vascular lumen added to the thickness of the adjacent dark layer together compose the IMT both at the near wall and at the far wall.

The relation between ultrasonic scanning and histological IMT measurement has been examined in several studies (reviewed by Cheng et al. 2002). Ultrasonic estimation gives a slightly higher IMT result than histological samples possibly due to post-mortem shrinkage and fixation-caused contraction in the latter method. The near wall sonographic measurement corresponds to approximately 80 per cent of the histological thickness, and a difference of 0.02 mm is present when comparing the near and far wall measurements (Kanters et al. 1997). However, this difference is constant across all measurements. Therefore adding near wall measurement in the protocol has resulted in smaller variation across the measurements (Kanters et al. 1997).

The reproducibility of measurements and their association with CVD has been similar for the near and far wall measurements (Bots et al. 2003). The common

carotid artery (CCA) is the most readily available part of the carotid artery and therefore examined in most studies. The internal carotid artery (ICA) and the carotid bulb (CB) have been studied less frequently, because they are not as close or as parallel to the skin and are therefore more difficult to scan (Kanters 1997). The association of increased IMT with clinical CVD has been stronger, if the carotid scans were bilateral and included multiple parts of the carotid artery (Kanters et al. 1997, Bots et al. 2003).

The validity of the results depends on the repeatability of the scans and reading of scans. The repeatability can be increased by proper training, using one individual scanner and reader, and by using computerised edge detection techniques when reading the scans (Bots et al. 2003).

IMT increases with age and is generally thicker in men than in women (Cheng et al. 2002). The growth rate of IMT depends on the background population (Fathi and Marwick 2001). For example in a population cohort in Eastern Finland, the mean increase was 0.12 mm in two years (Salonen and Salonen 1990). The differences in the growth rates between various populations are obviously determined by the prevalence of risk factors within the populations, as well as their diverse genetic backgrounds. Risk factors predicting IMT in the general population include age, gender, blood pressure, total and LDL cholesterol, low HDL cholesterol, and smoking (Salonen and Salonen 1990, Salonen and Salonen 1991, O'Leary et al. 1996, Crouse et al. 1996, Lakka et al. 1999, Espeland et al. 1999), genetic factors (Cattin et al. 1997, Kakko et al 2000, Zannad and Benetos 2003, Jerrard-Dunne et al. 2003), LDL particle size (Skoglund-Andersson et al. 1999, Hulthe et al. 2000), postprandial hyperlipidaemia (Boquist et al. 1999), fasting and postprandial hyperglycaemia (Yamasaki et al. 1995, Hanefeld et al. 2000, Temelkova-Kurktschiev et al. 2000, Gerstein et al. 2003), the amount and distribution of body fat (Bonora et al. 1997, Takami et al. 2001), and socio-economic factors (Lamont et al. 2000). The correlation of IMT with CV risk factors and events has been variable between different carotid segments (Crouse et al. 1996, Bots et al. 1997, Rosfors S et al. 1998, Ebrahim et al. 1999).

Studies using IMT as a surrogate end point for CVD have been extensively reviewed (Cheng et al. 2002, Redberg et al. 2002). Several large studies (reviewed by Cheng et al. 2001, Fathi and Marwick 2001, O´Leary and Polak 2002, Bots et al. 2003) have demonstrated that increased IMT is associated with prevalent CVD and predicts future CVD events such as myocardial infarction and stroke in the general population. Carotid IMT and its progression rate have correlated with and predicted coronary events, claudication, and stroke in several studies (Cheng et al. 2002). In the Rotterdam study, though, baseline IMT did not substantially add to a calculated risk for CAD when adjusting for other common coronary risk factors (Van Popele et al. 2001). In lipid-lowering trials, attenuation of intima-media thickening has accompanied reduction in clinical CVD incidence (Salonen et al. 1995, Mercuri et al. 1996, Bots et al. 2003).

IMT has nearly always been thicker in type 2 diabetic patients than in non-diabetic subjects (Yamasaki et al. 1995, Yamamoto et al. 1997, Mykkänen et al. 1997, Temelkova-Kurktschiev et al. 1999, Goff et al. 2000, Mohan et al. 2000, Bonora et al. 2000). In the RIAD study, post-challenge hyperglycaemia was related more strongly than fasting glucose to IMT (Hanefeld et al. 2000). In the IRAS (Haffner et al. 2000), IMT was greatest in diabetic subjects with coronary artery disease (CAD) and thinnest in non-diabetic subjects without CAD. Diabetic individuals without CAD had slightly, but not significantly, thicker mean IMT than non-diabetic patients with CAD. In contrast, in elderly Finnish men (70-89 years), glucose tolerance status was not related to carotid IMT (Tuomilehto et al. 1998).

The determinants of IMT among type 2 diabetic patients have been variable. The most constant factors associated with IMT are age, duration of diabetes, and blood pressure. Microalbuminuria has been shown to correlate with IMT in both non-diabetic and diabetic patients (Mykkänen et al. 1997, Yokoyama et al. 2004). The correlations between traditional CV risk factors such as lipids and IMT have been weaker and less consistent in diabetic patients than in the non-diabetic population (Yamasaki et al. 1995, Temelkova-Kurktschiev et al. 1999, Mohan et al. 2000, Goff et al. 2000, Kong et al. 2000). Positive correlations have been demonstrated for Lp(a) (Yamamoto et al. 1997, Velmurugan et al. 2003), LDL and HDL cholesterol (Goff et al. 2000), triglycerides (Temelkova-Kurktschiev et al. 1999, Kong et al. 2000), total-to-HDL cholesterol ratio (Temelkova-Kurktschiev et al. 1999), non-HDL cholesterol (Elkeles et al. 1996) and postprandial hypertriglyceridaemia (Teno et al. 2000). There are discrepancies in the literature as to which lipid parametres have been associated with IMT, although in most studies several parametres have been measured.

The progression of IMT is faster in diabetic than non-diabetic individuals (Wagenknecht et al. 2003). In a Canadian population-based cohort, IMT increased in a linear fashion with increasing HbA1c (Gerstein et al. 2003). Intensive antihyperglycaemic treatment slowed down intima-media thickening in type 1 diabetic patients over a six-year follow-up after the DCCT trial (Nathan et al. 2003). Interestingly, the length of time from diagnosis of type 2 diabetes to the initiation of insulin therapy has correlated positively with IMT in a cross-sectional study (Zheng et al. 2003). Thiazolidinediones (Minamikawa et al. 1998, Koshiyama et al. 2001) and metformin (Matsumoto et al. 2004) have been demonstrated to attenuate intima-media thickening in type 2 diabetic patients. Likewise some antihypertensive drugs have retarded the growth of IMT (Simon et al. 2002, Ludwig et al. 2002).

Carotid ultrasound scanning can be used to measure other aspects of carotid morphology beyond IMT, such as presence and character of plaques. Plaque evaluation has been used less frequently than IMT as a surrogate marker of CVD. Plaque morphology evaluation, as well as scanning protocols and determination of plaque burden vary greatly from study to study. Aortic and carotid plaques

predict cardiovascular mortality (Witteman et al 1986, Sakaguchi et al 2003). The number of plaques markedly contributed to the prognostic value of a predictive model for CVD and all-cause mortality, even though the model included major CVD risk factors such as age, medical and smoking histories, medication, lipoproteins etc. (Störk et al 2004). However, in hypertensive Japanese patients IMT was a better marker of end-organ damage than plaque score (Takiuchi et al 2004).

Calcification of carotid plaques is inversely associated with clinical cerebrovascular symptoms compared with non-mineralised plaques (Kitamura et al 2004, Schaalan et al 2004), indicating that mineralisation is related to plaque stability. Furthermore, the extent of calcification of the plaques correlates inversely with macrophage infiltration of the plaque (Schaalan et al 2004). The power of carotid and femoral ultrasound scanning results for predicting cardiovascular mortality is greatly increased if presence, number and thickness of plaques is evaluated together with IMT (Griffin et al 2002). Recently, carotid IMT, total plaque area and total plaque volume have been demonstrated to be determined by different cardiovascular risk factors. IMT correlated significantly with hypertension, total plaque area with total cholesterol levels and smoking, and total plaque volume with diabetes (Al-Shali et al 2005).

2.8. Arterial stiffness and CVD and diabetes

High blood pressure is a major CVD risk factor. Blood pressure constitutes of mean blood pressure, which equals the steady component of blood pressure, plus the pulsatile component, pulse pressure (Safar et al. 2003). The normal compliance of the arterial tree helps to buffer the intermittent pressure changes caused by the left ventricular ejection of blood into the aorta (Wilkinson et al. 2001). When the arterial elasticity diminishes, both the intra-arterial pressure and the speed of the pressure wave travelling along an artery (pulse wave velocity, PWV) increase (Woodman and Watts 2003).

Several techniques to investigate arterial stiffness have been established (Oliver and Webb 2003). The non-invasive methods can be divided in three main categories: measurements of 1) pulse wave velocity, 2) pulse pressure or blood flow waveform analysis, and 3) arterial distensibility and diameter (Woodman and Watts 2003).

Arterial stiffening is the main cause of increasing systolic and pulse pressure after the age of 40 years (O´Rourke et al. 2002). Arterial stiffness is also an early phenomenon in diabetic vasculopathy, and has been proposed to independently predict CVD incidence (Woodman and Watts 2003). Moreover, it is strongly associated with atherosclerosis (van Popele et al. 2001), and it is an independent determinant of mortality in both diabetic and non-diabetic patients (Cruickshank et al. 2002, Nürnberger et al. 2002, Weber et al. 2004).

Arterial stiffness correlates with IMT in some (Taniwaki et al. 1999, Simons et al. 1999, Mackey et al. 2002, Ravikumar et al. 2002, Fukui et al. 2003) but not all (Oren et al. 2003, Zureik et al. 2002) studies. In the study by Zureik et al. (2002), carotid plaques were independently associated with arterial stiffness measured by pulse wave velocity (PWV), whereas the association between PWV and IMT disappeared after adjusting for age and blood pressure measurements. In that study, IMT was only measured from CCA, which may have affected the sensitivity of the IMT results. Fukui et al. (2003) found a positive correlation between augmentation and both IMT and plaque score using a more profound scanning protocol including CCA, CB, and ICA.

Arterial stiffness increases with the impairment of glucose tolerance (Henry et al. 2003) and is higher in diabetic than non-diabetic subjects (van Dijk et al. 2003, Devereux et al. 2000, Brooks et al. 2001). Factors associated with increased stiffness in different studies have been glucose, insulin and triglycerides (Salomaa et al. 1995), insulin resistance and duration of diabetes (Emoto et al. 1998), age and HbA1c (Ravikumar et al 2002), age, systolic blood pressure, cholesterol and smoking (Duprez et al. 2004, Fukui et al. 2002) and also male gender (Oren et al. 2003).

3. Aims of the Study

The aim of the study was to examine the role of new, non-traditional risk factors to explain the excess of vascular disease in type 2 diabetic subjects. In particular we aimed to ascertain if the CVD risk factors were similar in diabetic subjects with prevalent CVD compared with patients without clinical CVD.

The specific aims of the study were to

1) investigate the role of the IGF system in relation to incipient atherosclerosis, traditional risk markers and the metabolic syndrome in type 2 diabetes (I)
2) examine the connection between inflammation and insulin resistance in type 2 diabetes (II)
3) define determinants of incipient atherosclerosis in type 2 diabetic and healthy subjects (III), and
4) examine correlates and interrelationships of intima-media thickness, plaque occurrence and mineralisation, and arterial stiffness (IV)

4. Subjects and Study Design

We recruited the study subjects from among type 2 diabetic patients participating in the FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) study of the Helsinki study site centre. The FIELD Study is a multinational randomised placebo-controlled double blind study that addresses the effect of fenofibrate in the prevention of cardiovascular morbidity and mortality in patients with type 2 diabetes. It is a combined primary and secondary prevention trial including patients both with and without established cardiovascular disease. The study is being performed in Australia, New Zealand and Finland, and it is administered by the NHMRC Clinical Trials Centre, University of Sydney.

A total of 9795 type 2 diabetic patients were recruited in the study globally (Keech A 2004). In Finland, 2068 patients were screened and 1393 recruited into the FIELD study. At the study site in Helsinki, study subjects were recruited by newspaper advertisements and from among several outpatient clinics in the capital area of Finland. The patients were randomly assigned to receive either placebo or micronised fenofibrate (200mg/d) for 5 years. Type 2 diabetic subjects between 50 and 75 years of age and serum total chol values between 1.0 and 6.5 mmol/l with either serum triglycerides between 1.0 and 5.0 mmol/l or chol/HDL chol ratio over 4 were eligible. In Finland a more tight upper limit for total chol (5.5 – 6.0 mmol/l on screening visit) was followed due to local treatment practice. Subjects with hepatic or renal dysfunction, gallstones, lipid-lowering medication, cyclosporin, alcohol overuse, or other severe mental or physical illness were excluded.

Two hundred and seventy patients were recruited in the FIELD main study in the Helsinki Centre. Of these, 239 (76 female) participants volunteered for this substudy. The main reasons for not participating were: 1) The patient had already passed through the placebo run-in phase of the FIELD study and entered the blinded treatment phase, and was thus not eligible for this substudy, which started several months later than the main study, or 2) The patient's daily schedule did not allow for the time needed for participating the substudy. Seventy-one substudy participants were diagnosed with previous CVD (defined as one or more of the following: angina pectoris, myocardial infarction, previous coronary artery bypass grafting (CABG) or percutaneous coronary intervention, stroke, TIA, carotid endarterectomy, claudication, leg amputation, peripheral arterial reconstruction or balloon dilatation as determined on the basis of clinical history and examination, including resting ECG, and review of all available patient records).

We recruited 93 healthy non-diabetic control subjects (44 female) by letters to the spouses of the study patients, by advertisements in the hospital magazine and intranet, and among retired pilots. The subjects had to be aged between 50-75 years, with no signs or history of clinical CVD except for mild hypertension, and no other major chronic diseases. We performed a 75g oral glucose tolerance test to ensure normal glucose tolerance according to the WHO criteria. Subjects with impaired glucose tolerance (IGT) or impaired fasting glucose (IFG), total chol >6.0 mmol/l, LDL chol >4.0mmol/l, S-triglycerides >2.5 mmol/l, transaminase levels over 2 x upper limit normal, or abnormal thyroid stimulating hormone (TSH) concentration were not eligible.

All patients and control subjects signed informed consent forms. The Ethics Committee of the Helsinki University Central Hospital approved the protocol. In addition we acquired approval by the Ethics committees of Helsinki City health care, Vantaa City health care, Peijas hospital, and Jorvi hospital, as we also recruited patients from these sites.

5. Methods

5.1. Laboratory analyses

All laboratory analyses were performed during the placebo run-in period of the FIELD Study, before fenofibrate intervention. Blood samples were obtained the morning after an overnight fast from both patients and control subjects. Coefficients of inter-assay variation (CV) are presented as per cent after each method, if available.

5.1.1. Glucose, insulin, and C-peptide measurements

We performed a 75 g oral glucose tolerance test on healthy control subjects to ensure normal glucose tolerance according to WHO criteria (WHO 1999). Blood samples were drawn at 0, 30, 60 and 120 minutes for the determination of P-glucose, S-insulin and c-peptide. For diabetic patients, we only measured fasting levels of glucose, insulin, and c-peptide. Fasting and post-load plasma glucose was measured by the hexokinase method (Roche Diagnostic Gluco-quant) using either a Hitachi 917 or a Modular analyser (Hitachi Ltd, Tokyo, Japan). We measured HbA1c by nefelometric inhibition of agglutination (CV 2.6 %) using a DCA 2000 analyser (Bayer Ames Technicon, USA). Serum insulin concentrations were determined by double-antibody RIA (Pharmacia RIA kit, Pharmacia, Uppsala, Sweden, CV 6.5 %) after precipitation with polyethylene glycol. C-peptide was determined by RIA (RIA-coat®C-peptid, Byk-Sangtec Diagnostica, Dietzenbach, Germany, CV 10 %).

5.1.2. Lipid and lipoprotein measurements

Total chol (CV 2 %) and triglyceride (CV 2 %) levels were measured in serum by automated enzymatic procedures (Hoffman-La Roche, Basel, Switzerland). LDL and HDL chol were determined after separating the lipoprotein fractions from fresh fasting sera by sequential ultracentrifugation (Taskinen et al. 1988). Serum HDL chol was additionally quantified by the phosphotungastic acid/magnesium chloride precipitation procedure (Hoffman-La Roche, Basel, Switzerland, CV 2.8 %). In Study IV, we calculated LDL chol using the formula of Friedewald. Apo B was measured by an immunochemical assay from Orion Diagnostica (Espoo, Finland, CV 4 %). Lp(a) was measured by turbidimetric immunoassay (Wako Chemicals GmbH, Neuss, Germany).

We measured LDL peak particle diameters (LDL size) from serum samples that had been stored at -80°C using nondenaturing linear gradient gel electrophoresis, as previously described in detail (Vakkilainen et al. 2002), (CV 1.2 %).

5.1.3. Cytokines, other inflammatory markers, and endothelial activation molecules

Circulating ICAM-1, VCAM-1, E-selectin l, ultra-sensitive CRP, SAA, IL-6, M-CSF and $sPLA_2$ concentrations were determined by commercially available ELISA; the ICAM-1 (CV 7.4%), VCAM-1 (CV 9.2 %), E-selectin (CV 7.3 %), IL-6 (CV 15.1 %) and M-CSF kit (CV 5.8 %) by R&D Systems, MN, USA; the ultra-sensitive CRP kit (CV 12.8 %) by Medix Biochemica, Kauniainen, Finland; the SAA kit (CV 21.9 %) by Biosource International, Camarillo, CA, USA; and the $sPLA_2$ kit (CV 22.1 %) by Cayman Chemical Company, Ann Arbor, USA).

5.1.4. Albumin excretion rate

Urinary albumin excretion rate (UAER) was measured from three sequential overnight urinary collections. The median of the three collections was used to determine normoalbuminuria (<20 ug/min), microalbuminuria (20-200 ug/min) or macroalbuminuria (> 200 ug/min). Interassay repeatability: CV 2.8% at concentration of albumin 191 mg/l, 2.0 % at 96.5 mg/l, and 3.5 % at 18.0 mg/l. Intra-assay repeatability: CV 4.2 %.

5.1.5. The IGF system

IGF-I was determined from the serum samples using the DSL-10-5600 ACTIVETM IGF-I Enzyme-Linked Immuno-Sorbent Assay Kit (CV 8 % at the levels of 135.6 ng/ml). The assay uses a modified version of the standard acid-ethanol extraction procedure.

IGFBP-1 concentration was determined by a two-site immunofluorometric assay as described earlier (Koistinen et al. 1996) using two monoclonal antibodies, F34-15C9 and F36-9G3 (CV 7.5 % at the level of 54 ng/ml).

IGFBP-3 was determined from serum samples using monoclonal antibodies (mAb) generated against recombinant IGFBP-$3^{E.Coli}$ (Koistinen et al. 1994) (CV 8 % and 3.7 % at the levels of 98 ng/ml and 18.8 ng/ml, respectively). The assay uses mAb F42-1B6 as the solid phase antibody and mAb F41-5C11 as the Eu-labeled tracer. The assay had no cross-reactions with the other human IGFBPs or IGFs.

5.2. The homeostasis model (HOMA)

To assess insulin resistance the homeostasis model (HOMA IR) was calculated by (fasting insulin (mU/l) x fasting glucose (mmol/l))/22.5 (Matthews et al. 1985, Haffner et al. 1997, Radziuk 2000).

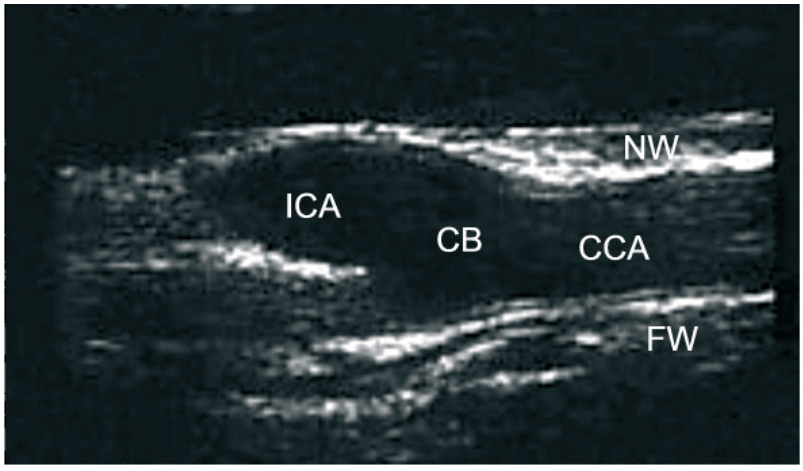
5.3. B-mode ultrasonography of the carotid arteries

5.3.1. Intima-media thickness (IMT)

All ultrasound scans were performed with a Hewlett Packard Image Point M2410A ultrasound system and a high frequency 10 MHz linear array transducer. Relevant parts of the scanning procedures were videotaped with a Panasonic AG-MD830E PAL S-VHS VCR. Both carotid arteries were scanned with the patient being in the supine position with her/his head rotated 45 degrees away (if possible) from the side being scanned.

Longitudinal images were displayed from three projections - the anterolateral, the lateral and the posterolateral - for the common carotid artery (CCA), the carotid bulb (CB) and the internal carotid artery (ICA). The arterial segments assessed were 1) the distal 1 cm of CCA, 2) the entire carotid bulb, and 3) the proximal 1 cm of ICA (Figure 3). Scanning was focused and measurements taken of a total of 28 sites per patient of both the right and the left carotid arteries from three projections for both the far wall (FW) and the near wall (NW) of the CCA and CB, and the one best visualized projection for ICA (Fig. 3). The images were frozen in the diastole, assessed as the phase when the lumen diameter is at it's narrowest and IMT at it's largest. Relevant parts of the scanning were recorded. All scans were performed in Helsinki.

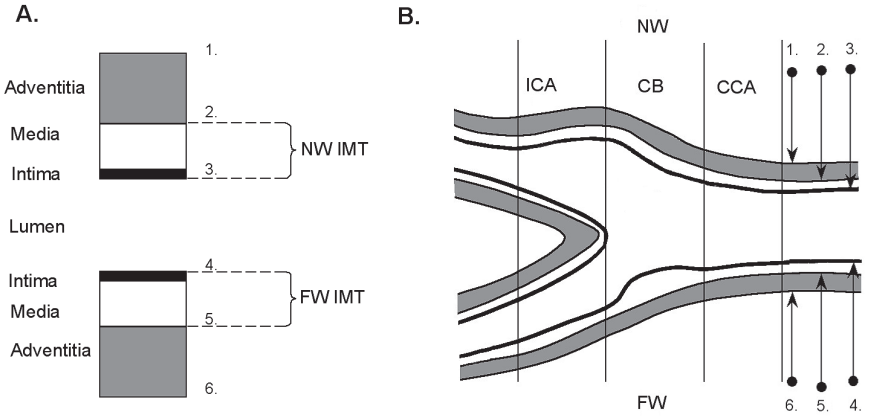
Figure 3. Carotid artery scan



ICA, internal carotid artery. CB, carotid bulb. CCA, common carotid artery. NW, near wall. FW, far wall

Figure 4. IMT imaging sites and projections (modified after Ylitalo 2001 and Soro-Paavonen 2004)

- CCA and CB: anterolateral, anterior and posterior projections bilaterally
- ICA: single best projection bilaterally
- All projections: both near wall and far wall IMT
- A local thickening (plaque) visible in CB FW



Schematic illustration of the B-mode ultrasound image Anatomy of the carotid artery

Computer analysis of the ultrasound images was performed using a PC with a video frame grabber interfaced to a PAL S-VHS VCR, at Oy Jurilab Ltd (www.jurilab.com) in Kuopio. The Prosound software, developed by Robert Selzer (Caltech, Pasadena, CA, USA), was used for the analysis of ultrasound images to measure IMT (Selzer et al. 1994). This software digitises the video ultrasound image, locates the interfaces, and computes the IMTs. The Prosound software enables 100 measurements per 1 cm edge length. IMT is determined as the mean difference at on the average 100 points between intima/lumen and media/adventitia interface. The following variables were derived: the mean IMT, the maximal IMT, and the minimal IMT. All outcome variables were first calculated for each subject.

As the primary outcome variable for each subject we chose the mean of maximal IMT (Max IMT) measurements over all 28 scanned carotid sites, as it corresponds to IMT over the carotid artery from CCA through the carotid bulb to the initial 1 cm of ICA. Secondary outcome variables were 1) the mean of mean IMTs (Mean IMT) over all scanned carotid sites, 2) the mean of mean far wall IMT over all scanned carotid far wall sites (FW), 3) the mean of maximum over all scanned CCA sites, 4) the mean of maximum over all scanned CB sites, 5) the mean of the maximum for all scanned ICA sites, and 6) the plaque height difference between site-specific maximums and minimums averaged for all scanned carotid sites (CarDif).

The ultrasound examination was carried out for 236 of the 239 diabetic study patients and all of the 93 non-diabetic control subjects. One diabetic subject could not be scanned due to neck arthrodesis and two due to impaired visibility of the carotid arteries. The IMT scans of the patients were all read by one ultrasound technician, Arja Malkki. Reading of the scans of the healthy controls was divided between two readers, Arja Malkki and Jarmo Tiikkainen. All readings were performed in Kuopio.

5.3.2. Scoring of plaques

Jarmo Tiikkainen reread 99 videotapes, which were randomly taken from the whole material, according to a different protocol for the determination of local thickenings (plaques) to assess the degree of atherosclerosis. The local changes at every site (bilateral FW and NW of three projections in CCA and CB, FW and NW of the best visualised projection in ICA) were divided into two main categories, soft change and mineralised plaques and graded as follows:

1. Soft change
   - 0 = no change
   - 1 = thickening of artery wall (IMT ≥ 1.5mm at CCA and/or ≥ 2mm at CB or ICA)
   - 2 = protrusion of 25 – 39 per cent of the lumen diameter
   - 3 = protrusion over 40 per cent of the lumen diameter
2. Mineralisation
   - 0 = no mineralisation
   - 1 = solitary mineralisation
   - 2 = several mineralisations or a cluster of mineralisations

For statistical analysis, the results of every single patient of this second ultrasound grading were further categorised so that the "worst" grading of all assessed sites for soft and mineralised changes was chosen to represent the level of soft and mineralised changes for that patient.

Eeva Leinonen (E.L.) performed the carotid scans of all patients and control subjects. E.L. scanned 11 study subjects twice each with one week's interval between measurements to assess the repeatability of the scans. The intra-observer repeatability (R) for Max IMT was 0.994 with a standard error of measurement errors (SE) 0.0152. The reader Arja Malkki's repeatability was assessed earlier in another study with an identical carotid ultrasound protocol (Ylitalo et al. 2002). The intra-reader R for Max IMT for Arja Malkki was 0.996 and SE 0.0082. Arja Malkki and Jarmo Tiikkainen read 10 scans independently from each other to assess inter-reader repeatability. The inter-reader R for Max IMT was 0.977 and SE 0.032.

5.4. Pulse wave analysis (PWA)

When the arterial pressure wave generated in systole travels throughout the arteries, at different points of discontinuity the pressure wave is reflected back, generating a backward travelling reflected wave (Nichols and Singh 2002, Safar et al. 2003). The amplitude and shape of the actual pressure wave is composed by the intensity of these two wave components and the timing between them (Safar et al. 2003). As arterial stiffness increases, the velocity of both waves increases, therefore the reflected wave arrives earlier in the central aorta and augments the arterial pressure during late systole, causing an increase in ventricular afterload (O'Rourke and Mancia.1999). The augmentation of the central pressure divided by the pulse pressure is defined as the augmentation index (AIx), and is a measure of arterial stiffness (Avolio et al. 1985, Kelly et al. 1989, Wilkinson et al. 2001).

We used the technique of pulse wave analysis (PWA) to determine central aortic pressure, augmentation, and the AIx (O´Rourke and Gallagher 1996, Wilkinson et al. 1998, Westerbacka et al. 1999). Pressure waves are recorded by applanation tonometry from the radial artery. A validated and generalised transfer function (Wilkinson et al. 1998) is then used to generate the corresponding central arterial waveform. The aortic waveform in pulse wave analysis is subject to further analysis for the calculation of aortic pressure augmentation, the AIx, central blood pressure and ejection duration (duration of systolic period in milliseconds), and Buckberg's subendocardial viability ratio (SEVR, area of diastole divided by area of systole during one cardiac cycle in the aorta). The measurement of augmentation index is highly reproducible (Wilkinson et al. 1998).

A sole investigator (Jukka Westerbacka) performed all measurements from the radial artery by applanation tonometry using a Millar tonometer (SPC-301; Millar Instruments, Houston, TX).

Data were collected directly into a desktop computer and processed with SphygmoCor Blood Pressure Analysis System (BPAS-1; PWV Medical, Sydney, Australia), which allows continuous on-line recording of the radial artery pressure waveform. We used the integral system software to calculate an average radial artery waveform, and to generate the corresponding ascending aortic pressure waveform using a previously validated transfer factor (Pauca et al. 2001, Karamanoglu et al. 1993).

We measured blood pressure with a calibrated mercury sphygmomanometer after the subject had rested in the supine position for 10 minutes. The average of three measurements was used for analysis.

5.5. Anthropometric variables

We measured waist circumference (midway between lowest rib and iliac crest) and hip circumference (maximum of buttocks) to the centimetre. Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared (kg/m^2). We used the waist circumference and the waist-to-hip ratio (WHR) as measures of body fat distribution.

5.6. Statistical methods

Parametres with skewed distributions (including HOMA IR) were log10-transformed for statistical analysis. Due to significant differences in the age and gender distribution between diabetic patients and healthy controls, all analyses to compare patients and healthy controls were adjusted for age and gender. Pearson and partial Pearson correlation coefficients were used to demonstrate relationships between variables.

Differences between patients without and with CVD and healthy controls were examined by One-Way ANOVA for normally distributed continuous data, by the Kruskal Wallis H -test or the Mann Whitney U -test for continuous data not normally distributed, and by the Chi squared test for categorical variables. We used the GLM Univariate analysis in Study II to compare biochemical characteristics in HOMA IR quartiles, to calculate differences between IMT measures in diabetic and healthy subjects in Study III, to calculate differences between augmentation and AIx in diabetic subjects with or without CVD adjusting for confounders, and to calculate differences between inflammatory and endothelial markers in diabetic and control subjects adjusting for confounders. Dunnett´s two-sided post hoc test was used in Studies II and III and Bonferroni's test in Study IV to calculate significant differences between subgroups. Differences between genders in Study IV were calculated using the Student's T-test.

Biochemical characteristics in HOMA IR quartiles (Study II) and Max IMT tertiles (Study III) were compared by One-Way ANOVA using Dunnett´s two-sided post hoc test.

In Study I, we used linear regression analysis for the whole study cohort and for subgroups to explain variation in Max IMT. To adjust for confounders, we entered these parameters in the regression analyses in step one. Parameters with even a weak correlation ($r > 0.200$) with Max IMT, but independent from each other, were entered in step 2. Parameters were removed until the best fitting model was achieved. Similarly, we used linear regression analysis to investigate determinants of HOMA IR and BMI in Study II, determinants of IMT in the healthy controls in Study III, and determinants of PWA and IMT in Study IV.

In Study III, we used factor analysis to define the main entities determining IMT in the diabetic patients. Only factors with eigenvalues over 1.0 were retained.

Varimax rotation was then performed to achieve factors without intercorrelations. We considered variables with loadings outwith the –0.40 to 0.40 range on a factor to be its major constituents. The factor scores were calculated by the regression method and saved as variables to be used in correlation analyses and linear regression analysis to investigate determinants of IMT.

We used the GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA, www.graphpad.com) when comparing linear regression slopes to study the effect of age on IMT in Study 2. All other statistical analyses were performed with versions 9.0, 10.0 or 11.0 of the SPSS for Windows software (SPSS Inc., Chigaco, USA). A p-value of < 0.05 was considered significant in all analyses.

6. Results

6.1. Study population

The characteristics of our study subjects are presented in Tables 4 and 5. There was a marked preponderance of males among our diabetic cohort, whereas among the controls the gender distribution was almost equal. The control subjects were younger than the patients, and, as expected, also had a significantly lower waist circumference and BMI. Despite the different lipid inclusion criteria for diabetic and control subjects, the mean values for total and LDL chol were comparable. Diabetic patients had lower HDL chol and higher levels of TG, Apo B, and Lp(a) than control subjects, consistent with diabetic dyslipidaemia. The respective modes of diabetes treatments as expressed by the number of participants and (percentage) were: diet-only in 34 (14%), oral antihyperglycaemic agents (OHA) in 149 (62%), insulin only in 11 (5%), and a combination of OHA and insulin in 45 (19%) for the diabetic patients group.

Among the diabetic group, 184 (77%) patients had the metabolic syndrome according to the NCEP criteria. In the 77 diabetic patients with clinical CVD, the metabolic syndrome was prevalent in 56 (79%) patients, and in the 168 diabetic patients without CVD the metabolic syndrome occurred in 128 (76 %) patients. Data was sufficient to calculate the presence of the metabolic syndrome in 83 of the 93 control subjects, seven (8%) of whom had the metabolic syndrome as defined by NCEP guidelines.

Table 4. Subject characteristics.

	DM with CVD N = 71		DM without CVD N = 168		Controls N = 93		Difference (p)	
	Mean	SD	Mean	SD	Mean	SD	Controls vs. patients	Patients without vs. with CVD
Age (years)	63.2	6.5	60.8	6.5	58.8	6.4	0.001	= 0.015
Gender (N male/female)	51 / 20		110 / 58		49/ 44		= 0.016	< 0.001
BMI (kg/m^2)	31.0	5.6	30.4	5.4	25.4	2.8	< 0.001	ns
Waist (cm)	104.1	14.7	101.2	12.5	86.2	11.2	< 0.001	ns
WHR	0.94	0.07	0.93	0.07	0.84	0.09	< 0.001	ns
DM duration (years)	8.7	6.8	7.3	6.1	-	-	-	ns
Pulse pressure (mmHg)	60	17	56	14	52	14	= 0.007	ns
Systolic blood pressure (mmHg)	146	18	144	17	134	16	< 0.001	ns
Diastolic blood pressure (mmHg)	86	10	88	11	82	8	< 0.001	ns
Total chol (mmol/l)	5.1	0.7	5.0	0.7	5.0	0.8	ns	ns
HDL chol (mmol/l)	1.1	0.3	1.2	0.4	1.5	0.4	< 0.001	ns
LDL chol (mmol/l)	3.3	0.6	3.1	0.6	3.0	0.6	ns	ns
Triglycerides (mmol/l)	1.8	0.8	1.8	0.8	1.0	0.3	< 0.001	ns
Apo B (mg/dl)	101	23	104	25	91	17	ns	ns
Lp(a) (mg/dl)	168	138	148	141	144	109	ns	ns
fP-glucose (mmol/l)	8.0	1.7	7.9	2.3	5.4	0.5	< 0.001	ns
HbA_{1c} (%)	7.5	1.4	7.3	1.3	5.1	0.3	< 0.001	ns
C-peptide (nmol/l)	1.2	1.1	1.0	0.8	0.6	0.3	< 0.001	ns
Insulin (mU/l)*	14.2	6.7	13.3	9.3	6.5	2.3	< 0.001	ns
IGF-I (nmol/l)	149.8	62.1	142.2	64.0	163.3	54.5	= 0.001	ns
IGFBP-1 (µg/l)	87.2	70.8	85.4	74.6	108.5	57.9	< 0.001	ns
IGFBP-3 (µg/l)	4719	1558	4462	1623	5643	1652	< 0.001	ns
Smoking (N, never/ex/current)	28/37/6		71/69/28		52/33/7**		= 0.03	ns

* Diabetic patients with insulin treatment excluded.
** Data missing from 1 subject.

Table 5. Prevalent medications and cardiovascular diseases

	DM with CVD N = 71	DM without CVD N = 168	Controls N = 93
Angina pectoris	37 (52%)	-	-
Myocardial infarction	6 (9%)	-	-
CABG	10 (14%)	-	-
Coronary angioplasty	6 (9%)	-	-
Stroke	13 (18%)	-	-
TIA	7 (10%)	-	-
Claudication	23 (32%)	-	-
Peripheral revascularisation	5 (7%)	-	-
Any oral antihyperglycaemic agent (OHA)	63 (89%)	134 (80%)	-
Sulphonylurea	43 (61%)	95 (57%)	-
Metformin	47 (66%)	99 (59%)	-
Other OHA (guargum, acarbose, glinides)	4 (6%)	8 (5%)	-
Insulin treatment	23 (32%)	34 (20%)	-
Combination of OHA and insulin	21 (17%)	25 (15%)	-
Any medication for hypertension	36 (51%)	75 (45%)	16 (17%)
β-blockers	34 (48%)	23 (14%)	8 (9%)
ACE inhibitors	20 (28%)	49 (29%)	3 (3%)
AII inhibitors	2 (3%)	7 (4%)	6 (6%)
Ca channel blockers	19 (27%)	33 (20%)	8 (9%)
ASA	47 (66%)	106 (63%)	13 (14%)
Nitrates	12 (17%)	2 (1%)	-

6.2. The IGF system and atherosclerosis (Study I and unpublished data)

6.2.1. The IGF system, age, anthropometric measurements and insulin resistance

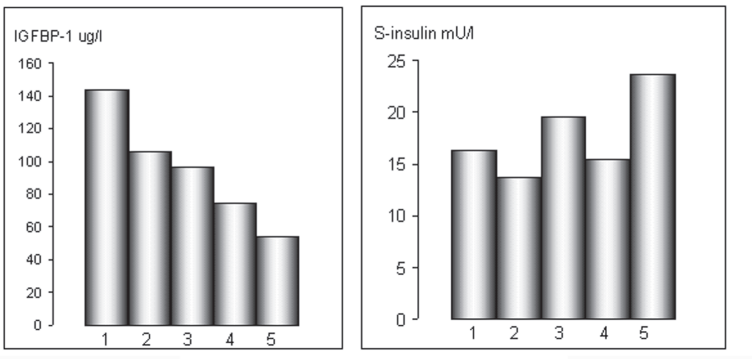
The concentrations of IGF-I, IGFBP-1 and IGFBP-3 were all higher in the healthy controls than in the diabetic patients (Table 4). However, within the diabetic group they were consistently lower regardless of the presence of clinical CVD. In the diabetic patients, the IGFBP-1 concentration increased with age ($r = 0.217$, $p < 0.001$) and correlated inversely with BMI ($r = -0.386$, $p < 0.001$),

waist circumference ($r = -0.438$, $p < 0.001$), and waist-to-hip ratio ($r = -0.371$, $p < 0.001$), whereas IGF-I and IGFBP-3 did not correlate with these parameters.

As expected, there was a strong inverse relationship between the concentrations of insulin and IGFBP-1 both in the diabetic patients ($r = -0.461$, $p < 0.001$) and in the healthy controls ($r = -0.434$, $p < 0.001$).

In the diabetic subjects, the concentration of IGFBP-1 decreased steadily with increasing prevalence of the components of the metabolic syndrome as defined by the NCEP definition (Expert panel 2001); from a mean of 144 μg/l in patients with only diabetes to 54 mg/l in patients with all five components ($p < 0.001$). This inverse relationship between IGFBP-1 and the metabolic syndrome was more consistent than the correlation between concentration of insulin and the metabolic syndrome (Fig. 5), although the relationship between insulin levels and degree of the metabolic syndrome also was highly significant ($p < 0.001$).

Figure 5. Mean concentrations of IGFBP-1 and insulin in categories of diabetic patients having a different number of components of the metabolic syndrome.



We also calculated HOMA IR for the diabetic subjects without insulin treatment (N = 182). There was a strong inverse relationship between IGFBP-1 and HOMA IR ($r = -0.437$, $p < 0.001$). This inverse correlation remained highly significant even after adjusting for age, gender, BMI, and diabetes duration ($r = -0.304$, $p < 0.001$).

In the healthy controls, the concentrations of IGFBP-1, IGF-I, and IGFBP-3 were not related to age. Similar to that found in the diabetic patients, there was a significant inverse correlation between IGFBP-1 and BMI ($r = -0.325$, $p = 0.005$), waist circumference ($r = -0.374$, $p < 0.001$), waist-to-hip ratio ($r = -0.326$, $p = 0.005$), and HOMA IR ($r = -0.465$, $p < 0.001$). The inverse relationship between IGFBP-1 and HOMA IR was also significant in the healthy subjects after adjusting for age, gender, and BMI ($r = -0.457$, $p < 0.001$).

There was an inverse relationship between levels of C-peptide and IGFBP-1 in both diabetic subjects ($r = -0.415$, $p < 0.001$) and healthy controls ($r = -0.401$, $p = 0.038$) in partial correlation analysis adjusting for age, gender, BMI and, for the diabetic subjects, duration of diabetes.

6.2.2 The IGF system and other CVD risk factors

After correcting for age, BMI, gender, and diabetes duration, we found significant positive correlations between IGFBP-3 and total chol ($r = 0.254$, $p = 0.013$), LDL -chol ($r = 0.292$, $p = 0.004$), Apo B ($r = 0.234$, $p = 0.022$), and Lp(a) ($r = 0.235$, $p = 0.021$). There were also similar correlations between IGF-I and LDL chol ($r = 0.226$, $p = 0.027$) and Lp(a) ($r = 0.204$, $p = 0.047$). Other correlations were not significant (Study I). These correlations were not present in the healthy control subjects (data not shown).

6.3. Insulin resistance and inflammation (Studies II and III)

The concentrations of acute phase reactants, cytokines and cellular adhesion molecules were generally higher in diabetic patients than in the healthy control group (Table 6). After adjusting for age, gender, BMI and smoking, the differences between diabetic and control subjects remained significant for CRP ($p = 0.019$), M-CSF ($p < 0.001$), E-selectin, ($p = 0.025$), and ICAM-1 ($p < 0.001$).

Table 6. Inflammatory and endothelial markers in diabetic and healthy subjects

	Diabetic subjects Median, range		**Controls** Median, range		**DM vs. controls** Difference (p)
CRP mg/l	1.7	0.1 - 38	0.9	0.2 - 17	< 0.001
hSAA ug/ml	23	4.8 - 2082	20	5.1 - 153	= 0.006
IL-6 pg/ml	2.4	0.5 - 1687	2.0	0.5 - 25	= 0.008
M-CSF pg/ml	450	128 - 1694	305	71 - 1859	< 0.001
$sPLA_2$ ng/ml	3.1	< 0.01 - 25	2.2	0.6 - 8.2	= 0.002
E-selectin ng/ml	56	15 - 455	45	12 - 110	< 0.001
ICAM-1 ng/ml	259	53 - 549	220	88 - 380	< 0.001
VCAM-1 ng/ml	556	233 - 1400	527	306 - 850	= 0.053

We calculated the correlations between the inflammatory markers and HOMA IR and between the endothelial markers and HOMA IR in the diabetic subjects

without insulin treatment. Adjustments were made for age, gender, and smoking (Table 7). HOMA IR correlated significantly with all of the markers in the diabetic subjects, but with only CRP and E-selectin in the healthy controls. When these correlations were further adjusted for BMI, the correlation remained significant for all markers in diabetic subjects: CRP ($p = 0.013$), hSAA ($p = 0.046$), IL-6 ($p = 0.008$), M-CSF ($p = 0.023$), $sPLA_2$ ($p = 0.025$), E-selectin ($p < 0.001$), ICAM-1 ($p = 0.001$), and VCAM-1 ($p = 0.01$). In the control group, only E-selectin remained significantly correlated ($p = 0.05$) with HOMA after adjusting for age, gender, smoking, and BMI.

Table 7. Correlation between inflammatory and endothelial markers with HOMA IR adjusted for age, gender and smoking.

	Diabetic patients		**Control subjects**	
	r	**p**	**r**	**p**
CRP mg/l	0.324	< 0.001	0.275	= 0.009
hSAA ug/ml	0.237	= 0.002	0.165	ns
IL-6 pg/ml	0.316	< 0.001	0.142	ns
M-CSF pg/ml	0.180	= 0.018	0.127	ns
$sPLA_2$ ng/ml	0.236	= 0.002	0.136	ns
E-selectin ng/ml	0.349	< 0.001	0.337	= 0.002
ICAM-1 ng/ml	0.329	< 0.001	0.055	ns
VCAM-1 ng/ml	0.212	= 0.005	0.137	ns

After adjusting for age, gender and smoking, HOMA IR correlated significantly and positively with BMI, waist-to-hip ratio and waist circumference in diabetic subjects (Study III) and with BMI and waist circumference in the control group (unpublished). The partial correlation coefficients for diabetic and healthy individuals, respectively, were for BMI: $r = 0.388$, $p < 0.001$, and $r = 0.389$, $p < 0.001$; WHR: $r = 0.211$, $p = 0.016$, and $r = 0.265$, $p = 0.062$; and waist circumference: $r = 0.457$, $p < 0.001$, and $r = 0.358$, $p = 0.002$.

Partial correlation coefficients between inflammatory and endothelial markers and waist circumference, adjusted for age, gender, and smoking, are presented in Table 8. Similar to the association between HOMA IR and these markers, only CRP and E-selectin correlated with waist circumference in the healthy group, whereas in the diabetic group the correlation between low-grade inflammation and endothelial activation and waist circumference was significant and constant.

Table 8. Correlation between inflammatory and endothelial markers with waist circumference adjusted for age, gender and smoking.

	Diabetic patients		Control subjects	
	r	p	r	p
CRP mg/l	0.374	< 0.001	0.308	= 0.009
hSAA ug/ml	0.231	< 0.001	0.027	ns
IL-6 pg/ml	0.415	< 0.001	0.059	ns
M-CSF pg/ml	0.134	= 0.043	0.003	ns
$sPLA_2$ ng/ml	0.185	= 0.005	0.003	ns
E-selectin ng/ml	0.314	< 0.001	0.303	< 0.01
ICAM-1 ng/ml	0.230	< 0.001	-0.081	ns
VCAM-1 ng/ml	0.131	= 0.048	0.178	ns

Glycaemic control of the diabetic patients, assessed by HbA1c, was significantly and positively related to CRP, hSAA, ICAM-1 and E-selectin (Study III). In addition, in diabetic subjects HbA1c correlated significantly and independently of age, gender, and BMI, with HOMA IR, CRP, hSAA, and E-selectin (Study III). No consistent relationship existed between HbA1c and inflammatory or endothelial activation variables in the control group.

6.4. Determinants of intima-media thickness IMT (Studies I, III, IV, and unpublished data)

Table 9 shows the carotid IMT results for diabetic patients and healthy controls. All IMT parametres were significantly greater in diabetic than healthy subjects. Furthermore, most of the IMT parameters were greater in diabetic patients with CVD than in patients without CVD. Nevertheless, the differences between IMT measurements in diabetic patients with and without CVD disappeared after adjusting for age, gender and smoking (Study III).

Diabetic men had higher values than diabetic women for Max IMT (1.35 vs. 1.24 mm, p = 0.031), mean IMT (1.07 vs. 1.02 mm, p = 0.027), FW IMT (1.07 vs. 1.02 mm, p = 0.049), CB IMT (1.54 vs. 1.45 mm, p = 0.05), and ICA IMT (1.27 vs. 1.13 mm, p = 0.004). In the healthy controls, there was no gender difference in any of the IMT variables. Unexpectedly, smoking was not related to any of the IMT parameters in either diabetic patients or healthy controls (data not shown). The duration of diabetes correlated with Max IMT in diabetic subjects with CVD, but duration did not correlate with IMT either among the diabetic subjects without CVD (Study I) or in the total diabetic cohort (Study IV).

Table 9. IMT in healthy and diabetic subjects

	DM with CVD N = 71		DM without CVD N = 168		Controls N = 93		Difference (p)	
IMT parametres	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD	Controls vs. patients	Patients without vs. with CVD
Max IMT	1.39	0.22	1.31	0.24	1.19	0.22	< 0.001	= 0.006
Mean IMT	1.09	0.19	1.03	0.17	0.93	0.18	< 0.001	= 0.005
FW IMT	1.11	0.23	1.03	0.20	0.93	0.19	< 0.001	= 0.007
CCA IMT	1.25	0.18	1.17	0.19	1.06	0.17	< 0.001	< 0.001
CB IMT	1.56	0.35	1.49	0.33	1.36	0.32	< 0.001	= 0.111
ICA IMT	1.30	0.45	1.20	0.38	1.10	0.28	= 0.006	= 0.075
CarDif	0.59	0.11	0.56	0.12	0.52	0.11	= 0.001	= 0.046

In Study I, we performed linear regression analysis to explain variation of MaxIMT in the entire diabetic cohort controlling for age, gender, BMI, presence or absence of OHA or insulin medication, and diabetes duration. The main determinants of Max IMT were age, IGFBP-1, pulse pressure, and Lp(a) (Study I). In addition, diabetes duration and insulin treatment remained in the model. The model explained 28.3 % of variation of Max IMT.

When we investigated the blood pressure and compliance effects on MaxIMT, adjusting only for gender and age, the only correlates to MaxIMT were nU-albumin, augmentation and AIx (Study IV).

The presence of the metabolic syndrome, or number of the components of the metabolic syndrome were not correlated with IMT or with the presence of CVD either the diabetic or the healthy subgroup (data not shown). Most of the diabetic subjects had metabolic syndrome (N = 184 (77%); data was missing for one subject). In contrast only seven of the healthy control subjects had the metabolic syndrome, therefore the study probably was not sufficiently statistically powered to detect any difference in IMT parametres between subjects with and without metabolic syndrome.

In Study III, we included the acute phase reactants, cytokines and endothelial activation markers in the analyses to examine determinants of IMT. In Study III we did not include the IGF system parametres, which we had examined in Study I. We chose to perform factor analysis due to the vast number of parametres each of which can have several intercorrelations. Factor analysis yields independent factors, which correspond to entities implicated in IMT thickening.

Factor analysis produced biologically meaningful factors only in the diabetic group but not in the healthy control group. The eight factors derived in the

diabetic cohort were named after the parameters with a high loading for each factor (Table 10): 1) "obesity factor" (high loading of BMI, waist, hip circumference) 2) "dyslipidaemic factor" (triglycerides, LDL size, Apo B, inversely HDL cholesterol), 3) "cholesterol factor" (total and LDL cholesterol, Apo B), 4) "endothelial factor" (M-CSF, E-selectin, ICAM-1, VCAM-1), 5) "blood pressure factor" (systolic blood pressure and pulse pressure), 6) "inflammation factor" (CRP, hSAA, IL-6, $sPLA_2$), 7) "duration factor" (age, DM duration), and 8) "glucose factor" (glucose, HbA1c).

Table 10. Factor scores derived by factor analysis: diabetic patients.

Rotated Component Matrix (a)

Variable	Components, named according to variables with high loading							
	1 Obesity	2 Dyslipi-daemia	3 Chole-sterol	4 Endo-thelial	5 Blood pressure	6 Inflam-mation	7 Dura-tion	8 Glucose
Age	-0.03	-0.04	0.03	0.22	0.27	0.04	**0.70**	0.07
DM duration	0.09	-0.13	-0.08	-0.08	0.14	-0.12	**0.65**	0.24
BMI	**0.91**	0.00	0.03	0.06	0.07	0.20	-0.06	-0.02
Waist	**0.92**	0.17	-0.08	0.09	-0.06	0.06	-0.06	-0.02
Hip	**0.92**	-0.02	0.05	0.11	0.04	0.22	0.06	0.02
Smoking	0.08	0.13	-0.20	-0.22	-0.22	0.03	-0.15	-0.35
Diastolic BP	0.17	0.02	0.13	0.09	0.38	-0.09	**-0.72**	0.17
Systolic BP	0.07	-0.01	0.06	0.05	**0.97**	0.00	-0.11	0.10
Pulse pressure	-0.02	-0.03	-0.01	0.00	**0.89**	0.06	0.30	0.02
Glucose	-0.06	0.07	-0.05	0.04	0.01	0.03	0.01	**0.81**
HbA1c	0.06	0.12	-0.03	0.07	0.05	0.11	0.01	**0.79**
CRP	0.30	0.03	-0.07	0.06	-0.00	**0.76**	-0.15	0.13
HSAA	0.13	-0.04	-0.02	-0.03	-0.10	**0.80**	-0.01	0.17
IL-6	0.34	0.09	-0.27	0.38	0.10	**0.44**	0.05	-0.10
M-CSF	-0.01	0.15	-0.10	**0.69**	0.07	0.09	0.01	0.01
$sPLA_2$	0.05	0.01	-0.00	0.27	0.15	**0.65**	0.11	-0.12
E-selectin	0.23	0.22	0.00	**0.44**	-0.02	0.09	-0.21	0.23
ICAM-1	0.14	-0.07	-0.04	**0.65**	0.12	0.22	-0.07	0.01
VCAM-1	0.05	-0.10	-0.03	**0.81**	-0.13	-0.09	0.20	0.13
Triglycerides	0.04	**0.87**	0.09	-0.00	-0.02	0.03	-0.01	0.09
Total chol	-0.08	0.11	**0.94**	-0.10	0.04	-0.01	-0.01	-0.02
HDL chol	-0.20	**-0.61**	0.32	-0.19	-0.03	0.26	0.16	-0.02
LDL chol	0.05	-0.12	**0.86**	-0.05	0.05	-0.12	-0.11	-0.04
Apo B	0.16	**0.74**	**0.55**	-0.08	-0.12	-0.02	-0.06	0.04
LDL size	0.05	**-0.83**	0.12	-0.04	-0.01	-0.09	0.06	-0.04

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
Variables with loadings outwith the -0.40 to 0.40 are bolded.

In accordance with our previous findings in Study I, only the "duration factor" ($R = 0.298$, $p < 0.001$) and the "blood pressure factor" ($R = 0.318$, $p < 0.001$) correlated significantly with Max IMT. When all factors were entered as variables in the linear regression analysis to explain variation of Max IMT, the "duration factor" ($T = 5.8$, $p < 0.001$), the "blood pressure factor" ($T = 5.6$, $p < 0.001$), and gender ($T = -4.2$, $p < 0.001$) were the main determinants of Max IMT, which combined accounted for 23.6 per cent of the variation. The "dyslipidaemia factor", the "obesity factor", and the "cholesterol factor" also remained in the model. These combined explained 25.2 per cent of the variation in MaxIMT (Study III).

In the healthy control group we performed linear regression analysis to examine determinants of MaxIMT, which according to the final model were age, hSAA, LDL cholesterol, HOMA IR and VCAM-1 (Study III). Interestingly, contrary to that found for the diabetic cohort, there was not even a trend of a correlation between Max IMT and blood pressure variables in the healthy control group (Study III).

6.5. Pulse wave analysis (PWA), intima-media thickness (IMT), and carotid plaques (Study IV and unpublished data) in type 2 diabetic subjects

Study IV analysed associations of aortic pressure, central pressure augmentation and augmentation index (AIx) with carotid IMT and plaques in type 2 diabetic patients.

Determinants of MaxIMT were calculated separately for each gender. Interestingly, while both brachial and aortic systolic and mean blood pressures were similar between the genders, in females the diastolic blood pressure was lower and the pulse pressure was higher than in males for both the brachial artery and the aorta. Accordingly, both central pressure augmentation and the AIx were higher in females than males even after adjusting for height and smoking. In females, central pressure augmentation, and aortic systolic blood pressure, but not brachial systolic blood pressure, were age-independent determinants of MaxIMT as determined by multiple linear regression analysis (R^2 24.9 %, $p < 0.001$).

In males, the central pressure augmentation, and the aortic systolic blood pressure, but not the brachial systolic blood pressure, explained 27.8 per cent of variation of MaxIMT independently of age and weight as determined by linear regression analysis. After adjusting for confounders, albuminuria correlated with augmentation and with Max IMT (Studies I and IV).

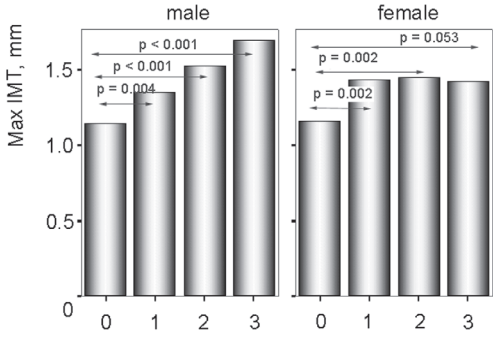
We also entered the factors derived in Study III (Table 10) together with gender in a GLM multivariate model to determine the augmentation and AIx (Unpublished). The model accounted for only 16 per cent of the variation in AIx, and the significant determinants of AIx were gender ($p < 0.001$), blood pressure factor ($p < 0.01$) and – interestingly – the endothelial factor ($p = 0.015$).

The GLM model for augmentation was more fitting and explained 32 per cent of the variation in augmentation. The significant determinants for augmentation were the blood pressure factor ($p < 0.001$), gender ($p < 0.001$), the duration factor ($p = 0.004$), the obesity factor ($p = 0.008$), and the endothelial factor ($p = 0.04$). It is noteworthy that the cholesterol, dyslipidaemia, glucose, and inflammation factors were not associated with either augmentation of AIx.

A total of 99 carotid scans of the diabetic patients were randomly selected for evaluation of focal soft and mineralised changes (= plaques). In Study IV, 97 scans were analysed. Characteristics between these 97 patients and the whole diabetic study cohort were similar (Study IV). Soft plaques were present in 70 subjects (61%), and mineralised plaques in 48 patients (49%).

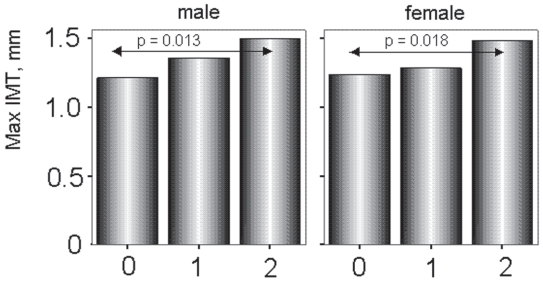
The severity of soft plaques as well as the severity of mineralised plaques correlated significantly with Max IMT for both genders (Figures 6 and 7).

Figure 6. Mean of Max IMT according to the soft plaque score.



0 = no change,
1 = thickening of artery wall (IMT ≥ 1.5mm at CCA and/or ≥ 2mm at CB or ICA),
2 = protrusion of 25 – 39% of the lumen diameter,
3 = protrusion over 40% of the lumen diameter

Figure 7. Mean of Max IMT according to the mineral plaque score.



0 = no mineralisation
1 = solitary mineralisation
2 = several mineralisations or a cluster of mineralisations

Genders combined, the severity grade of the mineralised plaques correlated with an increase in IMT, central pressure augmentation, and increases in both peripheral and central systolic and pulse pressures (Study IV). When the subset with the plaque scores scanned was divided according to gender, the differences between augmentation and the categories for mineralised plaques became non significant. This was obviously due to the lack of power in this study (data not shown).

7. Discussion

7.1. Study subjects

Our diabetic patients represent a wide range of middle-aged and elderly type 2 diabetic subjects with regard to diabetes duration and treatment options. Therefore we consider our study cohort to be relatively representative of people commonly seen at an outpatient diabetes clinic, even though the subjects were recruited by newspaper advertisements and from several different outpatient clinics. The lipid inclusion criteria of the diabetic patients were determined to allow inclusion of subjects with mild diabetic dyslipidaemia, but not patients requiring open-label hypolipidaemic therapy. The inclusion criteria of healthy controls were assumed to allow inclusion of subjects representative of healthy population. The mean level of cholesterol in Finland was 5.5 mmol/l in late 1990´s in both genders (Vartiainen et al 2003). The mean concentrations of total and LDL chol were comparable in diabetic subjects and healthy controls and actually lower than in the general population despite different inclusion criteria for diabetic subjects and healthy controls. Due to inclusion criteria, none of the participants had hypolipidaemic treatment, which excludes any effects by statins on lipids and inflammation.

The lipid values of our diabetic subjects were slightly better than those reported in the UKPDS (Turner et al. 1998) and DAIS (Diabetes Atherosclerosis Intervention Study Investigators 2001), which may give rise to an underestimation of the potential of lipoproteins as determinants of IMT. The imbalance in gender distribution was adjusted for in the statistical analyses.

The presence of CVD was determined on clinical history. All hospital records available were reviewed. The electrocardiograms will be analysed separately in the FIELD main study for detecting silent ischaemia.

Our control group consisted of healthy individuals, with the exception that we allowed for the presence of mild hypertension and its treatment. We excluded from this cohort subjects with derangement of glucose metabolism as well as high total cholesterol, LDL cholesterol, and triglyceride values. Consequently, the number of control subjects with the metabolic syndrome was low in comparison with the general population, and also the anthropometric measurements were more favourable than those found in our diabetic patients. To find control patients matched for age, gender, and anthropometric measurements, but free of glucose derangement and dyslipidaemia, would have been an impossible task. Therefore we strived to compensate for the different confounders in our statistical analyses. Obviously, our results obtained from the healthy control group cannot

as such be extrapolated to the general population, as our "healthy" subjects in all probability are healthier than people in the general population.

7.2. The HOMA model

We used homeostatic model assessment (HOMA) for determining insulin resistance. The HOMA is a method for assessing β-cell function and/or insulin resistance (IR) from the basal concentrations of glucose and insulin using a simple mathematical calculation (Matthews et al. 1985). In a newer computerised modification of the HOMA model C-peptide can also be used as an alternative to insulin (Levy et al. 1998).

The HOMA model has been used in over 500 studies and has been validated against other measures of insulin resistance (euglycaemic clamp and minimal model) and β-cell function (hyperglycaemic clamp, acute insulin response in intra-venous glucose tolerance test, and continuous glucose infusion model assessment) (Wallace et al. 2004). The updated computer model accounts for variations in hepatic and peripheral glucose resistance (Levy et al. 1998), and is therefore recommended when comparing the HOMA model with other models (Wallace et al. 2004). The original HOMA model, which we used, systematically underestimates insulin sensitivity and is thus not optimal for assessing absolute resistance (Wallace et al. 2004). When assessing relative change or comparing groups, the original model functions well, as the underestimation error is consistent.

HOMA IR depends upon both peripheral and hepatic insulin sensitivity. In a recent analysis comparing the HOMA model in NGT, IFG, IGT, IFG/IGT, and type 2 diabetic subjects, the HOMA IR correlated with the M-value obtained during the euglycaemic clamp in NGT and type 2 diabetic subjects (Tripathy et al. 2004). In the IFG and IFG/IGT groups the correlation between HOMA IR and the M value was not significant. In prediabetic individuals the HOMA IR reflected mainly the hepatic insulin resistance instead of the total insulin resistance. The implication of these results for our study is that HOMA IR reliably mirrors insulin resistance in type 2 diabetic and NGT subjects.

The HOMA IR model has been reported to be more biologically variable regarding type 2 diabetic patients than in subjects with NGT (Jayagopal et al. 2002). On the other hand, a study comparing several simple indices of insulin resistance and the glucose disposal rate in euglycaemic hyperinsulinaemic clamping demonstrated that the logarithmic transformation of HOMA IR – which we used – and the Quantitative Insulin Sensitivity Check Index provided repeatable estimates of insulin sensitivity with excellent discriminating potential (Mather et al. 2001).

As a conclusion, the logarithmic transformation of HOMA IR is to be recommended as a measure of insulin sensitivity in large studies when clamp techniques are not feasible.

7.3. Intima-media thickness

7.3.1. The IMT method

Different techniques to measure IMT and reproducibility of results have been thoroughly reviewed by Kanters et al. (1997). Overall the analogy of the far wall IMT with histology is more accurate than that of the near wall: sonographic measurement of the near wall produces an IMT figure that is only 80% of the actual histological thickness (Wong et al 1993). On the other hand this difference is systematic and therefore does not give biased associations (Kanters et al. 1997). Combining near wall and far wall measurements together seems to reduce variability and thus increase reliability of the results (Bots et al. 2003).

The most easily scanned region is the common carotid artery (CCA). There is more variability and missed images in scanning the internal carotid artery (ICA) or the bifurcation/carotid bulb (CB). On the other hand, plaques appear earlier at the bifurcation and in ICA than in CCA. Performing ultrasound scans at different angles and at several sites and calculating the common mean values from these measurements has given results corresponding more closely with CVD than measurements performed at individual sites (Kanters et al. 1997).

A pooled analysis and evaluation of prospective trials using IMT measurements as surrogate end points of CVD (Bots et al. 2003) recommends the mean of maximal IMT measurements from several sites at the carotid artery as a primary outcome measure, and both near and far wall sonographic measurements, to reduce measurement error, increase precision, and estimate the severity and extent of the carotid atherosclerosis in a reliable way. Segment-specific measures can be used as secondary outcomes. Thus our decision at the beginning of the study to apply a complex IMT protocol with scanning at several carotid sites, both NW and FW, at three imaging angles, for both the left and the right carotid arteries, was in line with their recommendations.

7.3.2. IMT and its determinants

We demonstrated for the first time that IMT is thicker in type 2 diabetic patients at all ages between 50 to 75 years (Study III). This is consistent with previous data showing that IMT is thicker in diabetic patients than healthy controls (Yamasaki et al. 1995, Yamamoto et al. 1997, Mykkänen et al. 1997, Temelkova-Kurktschiev et al. 1999, Goff et al. 2000, Mohan et al. 2000, Bonora et al. 2000). It has been reported that IMT is already elevated before the onset of diabetes (Hunt et al. 2003). IMT is potentially partly genetically determined (Lange et al. 2002), and also elevated in glucose-tolerant first-degree relatives of type 2 diabetic patients (Pannacciulli et al. 2003).

In accordance with the findings of previous studies, age, gender, duration of diabetes, and blood pressure were the most important determinants of IMT in our type 2 diabetic patients (I, III). In Study I, Lp(a) remained in the linear

regression model to explain IMT as a significant but only moderate determinant. Similarly, in Study III, the "dyslipidaemia factor" representing diabetic dyslipidaemia with high triglycerides, low HDL and small LDL particle size, as well as the "cholesterol factor" representing high total and LDL cholesterol and high Apo B, remained in the model as weaker determinants of IMT than the "duration factor" and "blood pressure factor". Diabetic patients with lipid values requiring open lipid-lowering therapy were excluded from the FIELD study, therefore the importance of lipids as determinants of IMT might have been underestimated in our study. On the other hand, earlier research suggests that as a rule the association between lipids and IMT is not as strong as in non-diabetic cohorts (Yamasaki et al. 1995, Temelkova-Kurktschiev et al. 1999, Mohan et al. 2000, Goff et al. 2000, Kong et al. 2000).

Against our expectations, we did not detect any association between IMT and the acute phase reaction and endothelial dysfunction markers among the diabetic group, despite thicker IMT and higher levels of these markers in type 2 diabetic subjects than in healthy controls (Study III). Very recently other groups have reported similar observations. Moussavi et al. (2004) compared carotid IMT and the levels of E-selectin, ICAM-1, VCAM-1, CRP, and 8-isoprostane (a marker of oxidative stress) in 40 type 2 diabetic patients and 25 healthy controls. These authors found that in a diabetic group with good glycaemic control and no clinical CVD, the concentrations of E-selectin, ICAM-I, CRP, and 8-isoprostane were significantly higher than in the control group. However they also found that the results in IMT were similar in diabetic and healthy subjects. Their results also show an association between CRP and waist circumference in agreement with our data. A Swedish group (Sigurdardottir et al. 2004) recently published a population-based study on 271 61-year-old men including subsets with established diabetes, newly diagnosed diabetes, and healthy controls. In that study, a composite end-point of IMT and carotid plaque size increased gradually from healthy controls to newly diagnosed diabetic subjects and men with established diabetes. CRP levels were higher in both diabetic subgroups, but did not explain the variation in the composite IMT.

There was no association between low-grade inflammation and IMT in our study. Likewise, the inflammatory and/or endothelial markers were not related to the presence of CVD in our diabetic patients. It has been reported previously that carotid IMT correlates weakly with angiographically determined coronary artery disease (Adams et al. 1995, Zebrack et al. 2002). In the study by Zebrack et al, the correlation between CRP and the severity of CAD was weak, but both factors predicted future CAD events independently and additively. In the ARIC study, baseline levels of CRP were not related to carotid IMT after adjustment for other major risk factors. CRP predicted CAD incidence, though this association became less pronounced after adjusting for confounding factors (Folsom et al. 2002).

The process of increasing IMT differs from the pathogenesis of acute cardiovascular events. Inflammation and endothelial activation are implicated in the initiation

of atherosclerosis, in the prothrombotic state, and in the development of vulnerable plaques prone to rupture (Tedgui and Mallat. 2001). As a matter of fact, nearly two-thirds of all coronary deaths are attributable to plaque rupture, and a substantial number of the rest are due to plaque erosion (Madjid et al. 2004). IMT, on the other hand, is closely correlated to hypertension, and potentially does not reflect only atheromatosis, but also changes in the smooth musculature of the vascular wall (Devynck et al. 2004). The complex biological process ending in the development of an unstable atheroma, thinning of the cap, plaque rupture and presentation of a clinical CVD event conceivably may not be well predictable by IMT. New imaging techniques to identify activated, vulnerable plaques are being developed, such as intravascular ultrasound (IVUS), angioscopy, thermography, spectroscopic methods, etc. (Fayad and Fuster 2001). So far no easily repeatable, safe, non-invasive and reliable methods exist (Schoenhagen et al. 2004).

We could for the first time demonstrate a link between IMT and IGFBP-1, which according to our results (Study I) and previous research is an inverse marker of insulin resistance (Mohamed-Ali et al. 1999, Ricart and Fernández-Real 2001). However, other factors beside insulin resistance must also be operating, as there was no significant association between HOMA IR (calculated for patients without insulin treatment) and MaxIMT in the diabetic subjects whether or not adjustments for the factors of age, gender, BMI and diabetes duration were used.

Almost simultaneously with the publication of our results (I) Heald et al. (2002) demonstrated an association between low IGFBP-1 and the presence of macrovascular disease and hypertension in type 2 diabetic patients. On the other hand, in a general elderly male Finnish population cohort, high IGFBP-1 levels correlated with increased cardiovascular mortality (Harrela et al. 2002). These cross-sectional studies were performed in different populations, which may partly explain the contradicting results.

Recently, prospective studies of the IGF system in regard to CVD have been published. When individuals without ischaemic heart disease (IHD) were followed for 15 years, a low level of IGF-I at baseline was associated with a two-fold increased incidence of ischaemic heart disease (Juul et al. 2002). In a 9-13 year follow-up of the Rancho Bernardo Study cohort, consisting of elderly men and women, low baseline concentrations of IGF-I and IGFBP-1 were independently and jointly related to IHD mortality (Laughlin et al. 2004). In this study, the IGFBP-1 levels were lower in the subset with diabetes or IGT than in those with NGT, and, in accordance with our results, nearly 50 per cent lower among those with insulin resistance than in those without. These authors demonstrated that the association between IHD and the levels of IGF-I and IGFBP-1 existed in all categories of glucose tolerance.

Taken together, low IGFBP-1 seems to be a risk marker for subsequent CVD events probably due to its strong association with insulin resistance.

7.4. The metabolic syndrome

7.4.1. The definition of the metabolic syndrome

For practical reasons, in the present study the diagnosis of the metabolic syndrome was determined by the NCEP criteria. The criterion of the OGTT value, included in the WHO criteria, would not have been relevant in our diabetic patients nor in the healthy control group, the latter only being included if their 2-hour glucose tolerance test was normal. Furthermore, our study cohort was too large to perform clamp studies to investigate insulin resistance. The NCEP criteria have the additional benefit that all criteria are readily available in clinical practice. Thus the obtained results can be transferred into patient treatment.

Hanley et al. (2003) compared the WHO and NCEP criteria in detecting insulin resistant subjects among the non-diabetic participants of the Insulin Resistance Atherosclerosis study, using frequently sampled intravenous glucose tolerance test -derived measures as reference values for insulin sensitivity. Both criteria identified insulin resistant individuals, but the WHO criteria were more sensitive.

It has been speculated that the predictive value of the two sets of criteria (NCEP and WHO) for the metabolic syndrome may depend on the prevalence of the core metabolic components – dyslipidaemia, hypertension, obesity, and glycaemia – in the population investigated (Hunt et al. 2004). Thus the usefulness of the NCEP vs. WHO criteria with a differing emphasis on the components of the metabolic syndrome may vary according to the background population.

7.4.2. The IGF system and the metabolic syndrome

We demonstrated that IGFBP-1 has a strong inverse relationship with the number of metabolic syndrome features in type 2 diabetic patients: the more profound the metabolic syndrome, the lower was the IGFBP-1 concentration. As insulin potently suppresses IGFBP-1 production, this inverse correlation may partly result from an increased concentration of insulin present during insulin resistance. In our diabetic patients the duration of diabetes and thus also endogenous insulin production was highly variable. The treatment modes included diet, different combinations of oral treatment, and insulin treatment, which also modify circulating insulin levels. Therefore it was no surprise that the relationship between serum insulin and the degree of the metabolic syndrome was less consistent (Fig 4). Why IGFBP-1 seems to be a better predictor of metabolic syndrome than insulin cannot be explained in a research setting such as ours, but would require studies on the putative mechanism.

Interestingly, a recent study reported a strong inverse correlation between IGFBP-1 and waist circumference as well as IGFBP-1 and the presence of the metabolic syndrome (Kaushal et al. 2004). CRP, on the other hand, correlated positively with waist circumference and the presence of metabolic syndrome.

When analysed together, subjects with an IGFBP-1 concentration below median and a CRP concentration in the highest tertile, had a 36-fold increased risk of the metabolic syndrome compared with subjects having an IGFBP-1 concentration above median and a CRP concentration in the lowest tertile. The risk was for the most part independent of age, gender, and fasting insulin concentration.

The complex cross-talk between the IGF system and other metabolic systems is so far not fully understood. The IGF system interacts with other growth factor systems, cytokines, lipoproteins, haemodynamic forces etc. IGFs exert various hormonal and auto/paracrine effects, including processes involved in the development of atheroma. IGFBPs and IGFBP proteases not only regulate the amount of free IGFs in serum, but have additionally IGF-independent functions. Understanding the mechanisms requires to be addressed by basic research (Delafontaine et al. 2004).

7.4.3. Inflammation, endothelial activation, insulin resistance, and the metabolic syndrome

We detected a strong association between endothelial activation, inflammation, and the severity of the metabolic syndrome (Study II). These results are in line with those of Weyer et al (2002). They showed that the serum concentrations of CRP, $sPLA_2$, and ICAM-1 in a cohort of 32 non-diabetic Pima Indians were closely correlated with the amount of body fat, BMI, waist-to-hip-ratio, and fasting insulin. Insulin-dependent glucose disposal investigated by the clamp technique was inversely related with the levels of CRP, $sPLA_2$, ICAM-1, E-selectin, and von Willebrand factor (vWF). E-selectin and vWF also correlated with CRP. The authors concluded that the levels of inflammatory markers increase with increasing obesity, whereas endothelial activation mainly increases in proportion to the extent of inflammation and insulin resistance. In prediabetic individuals, the degree of inflammation has been shown to be higher in subjects who are insulin resistant in comparison with subjects with primarily deficient insulin secretion (Festa et al. 2003).

Our finding of a positive relationship between glycaemic control and low-grade inflammation and endothelial activation is in line with previous research, which demonstrated a decrease of inflammatory and endothelial markers by improving glycaemic control (Ryysy et al. 2001, King et al. 2003). Our cross-sectional results could not answer if increased glycaemia causes inflammation or vice versa. Interestingly, Ryysy et al (2001) could demonstrate a correction of E-selectin levels with intensive insulin treatment of type 2 diabetic patients.

We demonstrated that all markers for low-grade inflammation and endothelial activation correlated significantly, most of them strongly, with waist circumference in our diabetic patients. In the healthy and comparatively lean study subset the relationship was weaker, and only CRP and E-selectin were significantly associated with waist circumference.

There is increasing evidence that abdominal fat is a key regulator in the inflammatory process (Tracy 2001). When pre-menopausal women with the polycystic ovary syndrome (PCO) were compared with healthy women paired for BMI, obesity and smoking, there was no difference in the concentrations of several inflammatory markers even though the patients with PCO were more insulin resistant. When both groups were analysed together, the levels of CRP and IL-6 increased significantly with increasing weight (Escobar-Morreale et al. 2003). Omental adipose tissue fragments obtained from obese subjects released IL-6 two- to three-fold more than the same patients' subcutaneous adipose tissue fragments (Fried et al. 1998). This implies that one of the mechanisms why abdominal obesity is detrimental may be that abdominal obesity enhances inflammation more than peripheral obesity.

Obesity and insulin resistance are highly intercorrelated, but a definitive answer as to the question, which is the primary defect in the metabolic syndrome, remains open. In a Chinese cohort including healthy, diabetic, hypertensive, and dyslipidaemic individuals, examined by factor analysis, obesity rather than insulin resistance seemed to be the seminal abnormality behind the metabolic syndrome (Anderson et al 2001). Moreover, in Caucasian cohorts, obesity, especially central adiposity, has been a central disorder behind the metabolic syndrome (Vanhala et al. 1998, Maison et al. 2001, Lemieux 2001). However, some studies provide evidence for the role of insulin resistance over obesity as the unifying factor in the metabolic syndrome (Meigs et al 1997). In a recent prospective analysis by Laaksonen et al (2004), baseline values of CRP predicted the development of both diabetes and the metabolic syndrome, suggesting that inflammation may be the primary defect. However, while the association between CRP levels and the risk of diabetes remained after adjusting for lifestyle factors and other confounders, the relationship between CRP and the incidence of the metabolic syndrome was no longer significant.

7.4.4. The metabolic syndrome and CVD

We could not demonstrate any association between the presence or severity of the metabolic syndrome with either IMT or clinical CVD in our study subjects. This finding contradicts with those of a large number of reports in the literature confirming the importance of the metabolic syndrome as a cardiovascular risk factor (reviewed by Isomaa 2003). Our study was not a population-based epidemiological study. Instead we investigated diabetic subjects, over three quarters of whom had the metabolic syndrome both in the subset with CVD as well as in the subset without clinical CVD. Thus the power of the study probably would not have been sufficient for showing a difference in the prevalence of the metabolic syndrome between subsets divided according to the presence of clinical CVD even if such a difference existed. The cross-sectional nature of our study may also partly explain the apparent lack of association between CVD and the metabolic syndrome.

In the San Antonio Heart Study, NCEP and WHO criteria were compared in regard to their predictive value in the general population for cardiovascular and all-cause mortality (Hunt et al. 2004). Interestingly, the presence of the metabolic syndrome was a stronger risk factor for CVD and all-cause mortality in women than in men. In that study, the predictive value of the NCEP definition was greater than the WHO definition.

The value of the NCEP definition is also highlighted in a cross-sectional analysis of the NHANES study (Alexander et al. 2003). In that cohort, patients with both diabetes and the metabolic syndrome had the highest prevalence of CHD, whereas the prevalence of CHD was low in subjects without the metabolic syndrome regardless of their diabetes status. A subgroup with the metabolic syndrome as defined by NCEP criteria was identified among the prospective Bruneck study (Bonora et al. 2003). Subjects with metabolic syndrome at baseline had a higher incidence and progression of carotid plaques, stenosis, and CVD than individuals without the metabolic syndrome. In the study by Lakka et al. (2002), the presence of the metabolic syndrome according to the WHO definition was associated with CHD, CVD, and all-cause mortality, whereas the metabolic syndrome as defined by the NCEP criteria only correlated with CHD mortality. The Botnia Study used the 1998 WHO criteria (Isomaa et al. 2001). In that cohort the metabolic syndrome carried a markedly increased risk for CV mortality.

Recently, the Women's Ischemia Syndrome Evaluation (WISE) Study investigated and followed 780 women referred for coronary angiography due to suspected ischaemia, for the presence or absence of the metabolic syndrome, diabetes, and prevalence of CAD as well as 3-year risk for CVD (Kip et al. 2004, Marroquin et al. 2004). The metabolic syndrome and BMI were strongly associated with each other, but only the metabolic syndrome correlated with CAD. Furthermore, BMI did not determine 3-year risk of death or major CVD events. However, each unit of BMI increased with the degree of metabolic progression from normal through metabolic syndrome to diabetes and was associated with approximately a two-fold higher risk of death and major CV events (Kip et al. 2004).

In the WISE study, the levels of IL-6 and CRP were higher in women with either diabetes or metabolic syndrome than in women with a normal metabolic state. However, the levels of CRP did not affect the subsequent mortality or CVD incidence in women without angiographic CAD, and in women with angiographic CAD the effect of CRP was marginal (Marroquin et al. 2004). Thus these results support our findings in demonstrating that even though enhanced inflammation is part of the metabolic syndrome, inflammation may not be a major determinant of CVD independently of it.

In a cross-sectional study of a type 2 diabetic cohort, CRP and fibrinogen were intercorrelated; CRP was related to the presence of the metabolic syndrome features but not to macrovascular disease prevalence. However, fibrinogen was higher in patients who had undergone a CV event or revascularisation or had

microalbuminuria (Streja et al. 2003). Therefore CRP may not be as good a predictor of macrovascular disease in type 2 diabetic patients as in the general population. On the other hand, in the same study fibrinogen was found to be a determinant of CVD independent of CRP.

Very recently, inflammation (Amar et al. 2004, Okamura et al. 2004, Kampus et al. 2004, Pirro et al. 2004) and body fat (Ferreira et al. 2004) have been linked with arterial stiffness. Thus arterial stiffness is potentially one of the mechanisms linking low-grade inflammation with cardiovascular disease.

7.5. Pulse wave analysis (PWA)

7.5.1. The PWA method

We chose the practical and reproducible method of pulse wave analysis to estimate central aortic pressure, augmentation and augmentation index. The transfer function used to calculate the corresponding ascending aortic pressure waveform from the radial artery waveform measured by applanation tonometry has been previously validated (Karamanoglu et al. 1993, Pauca et al. 2001).

The concept of using a generalised transfer function validated in a non-diabetic population for assessing diabetic patients has recently been challenged (Hope et al. 2004, Mather et al. 2004). The process of arterial stiffening occurs early in the development of diabetes (Salomaa et al. 1995), and the physical changes induced by diabetes in the vessel wall, including collagen cross-linking and other consequences of advanced glycosylation end product binding, may change the relationship of the waveforms centrally and peripherally (Mather et al. 2004).

The Hoorn study investigators have examined the estimates of arterial stiffness with the glucose tolerance status (Schram et al. 2004). They measured total systemic arterial compliance 1) by the same commercial PWA method, which we used in the present study, and 2) by calculating the ratio of the stroke volume to aortic pulse pressure. Aortic pulse pressure was estimated by two methods: one, which applies a calibration method using distension waveforms at the brachial and carotid arteries to calibrate the pulse pressure at the carotid artery, and the other by applanation tonometry. These authors calculated the AIx (Schram et al. 2004). Furthermore, they measured the carotid-femoral transit time by continuous measurement of the diameter of the carotid and femoral arteries. The calculated total systemic arterial compliance results were comparable by the two methods. It was found that compliance deteriorated gradually by a worsening of glucose tolerance from NGT to IGF/IFG and type 2 diabetes. AIx increased and carotid-femoral transit time decreased with declining glucose tolerance. Measures of glycaemia (HbA1c, fasting and postload glucose) accounted for up to 50 per cent of the decrease in arterial compliance.

A greater error in the generalised transfer function derived central systolic pressure compared with invasive central systolic pressure results has been reported in type 2 diabetic patients than in individuals with NGT (Hope et al. 2004). This should not hinder the use of PWA for detecting relationships between augmentation and AIx and metabolic parameters or measures of vascular disease among diabetic patients. As the potential error is systematic for all diabetic subjects, we consider our results demonstrating an association between the measures of central systolic pressure and carotid IMT and mineralised plaques reliable.

7.5.2. PWA, IMT, and plaque score

We detected a close relationship between IMT and measures of central arterial pressure such as augmentation and augmentation index in study IV. This is in line with our previous findings that pulse pressure and, through factor analysis, the blood pressure factor, were important determinants of IMT (Studies I and III). Interestingly, when we analysed the factors as determinants of augmentation and AIx, we found that the endothelial factor, in addition to the blood pressure and duration factors, was also a determinant of augmentation and AIx. Obviously arterial stiffening includes endothelial damage.

In study IV, pulse pressure and central pressure augmentation were higher in female than male diabetic subjects, even after controlling for smoking and height, which may affect the wave reflections and thus central augmentation. This may indicate that diabetes has a more detrimental effect on large artery stiffness and central haemodynamics in women than men. This reflects the fact that diabetic women are especially predisposed to cardiovascular morbidity (Barrett-Connor et al 1991).

An increasing number of mineralised focal changes was related to age, MaxIMT, and variables reflecting arterial stiffness. These include aortic pulse pressure, brachial pulse pressure, aortic and brachial systolic pressure, and central pressure augmentation. Our results showed a correlation between IMT and large arterial stiffness and are consistent with the study by Fukui et al (2003) who demonstrated that plaque score was associated with augmentation in a Japanese type 2 diabetic cohort. Our results could imply that if used in clinical practice, PWA might reveal the presence of incipient atherosclerosis better than traditional markers such as lipoproteins. Nevertheless, it may be premature to recommend PWA as a substitute for conventional blood pressure measurements in clinical practice while all treatment guidelines so far are as yet based on conventionally measured blood pressure values.

8. Summary of Results and Conclusions

The results of studies I – IV and the presented unpublished data can be summarised as follows:

1. IGFBP-1 was inversely associated with IMT in type 2 diabetic patients. IGFBP-1 was furthermore a marker of insulin sensitivity and inversely related to the severity of the metabolic syndrome among diabetic subjects.

2. Low-grade inflammation and endothelial dysfunction were enhanced in type 2 diabetes. The severity of the metabolic syndrome correlated linearly with the levels of markers for inflammation and endothelial activation. The concentrations of these markers were similar in diabetic patients with or without CVD.

3. Carotid IMT was thicker in type 2 diabetic patients than in healthy subjects over the entire age range in this study. Among diabetic subjects, patients with CVD had greater IMT, but the difference was not independent of recognized CVD risk factors. The main determinants of IMT in diabetic patients were blood pressure, age, gender, and the duration of diabetes. Dyslipidaemia and obesity were weaker determinants of IMT. In healthy subjects, the determinants of IMT were age, inflammation (hSAA), endothelial activation (VCAM-1), LDL cholesterol, and insulin resistance (HOMA IR). Blood pressure was not correlated with IMT in healthy subjects, although it was one of the main determinants of IMT in diabetic patients. Endothelial and inflammatory variables, on the other hand, were only correlated with IMT in the healthy subset.

4. Central pressure augmentation, the AIx (measure of arterial stiffness), and IMT were correlated with each other and with the severity of local vessel wall thickenings (soft and mineralised plaques). Diabetic women had higher augmentation and AIx values than men after controlling for confounders. After adjusting for gender, age, and heart rate, the only determinants of the AIx were blood pressure, albuminuria, and IMT. In factor analysis, also obesity and endothelial dysfunction were weak determinants of AIx in diabetic patients.

Low-grade inflammation has been recognized to predict both CVD and type 2 diabetes in population samples. Some investigators already recommend measuring of ultrasensitive CRP as a CVD risk marker. According to our results and some other recent data, CRP levels cannot among diabetic patients discriminate subjects with clinical or subclinical CVD from patients without CVD. The use

of CRP can therefore not be recommended as a CVD risk marker among diabetic patients. Inflammation seems to be an early feature of the metabolic syndrome occurring before the clinical presentation of diabetes and CVD.

Diabetic women had higher central pressure augmentation and AIx than men. These measures of arterial stiffness correlated strongly with IMT and the severity of the atherosclerotic local thickenings among diabetic patients. Thus diabetes may have a more detrimental effect on arterial compliance in women than men. This fits well with the finding in several studies that diabetes seems to increase the risk of CVD even more in women than in men.

According to our results, the concentration of IGFBP-1 and the measures of central systolic pressure determined by PWA reflect intima-media thickness and potentially incipient atherosclerosis among type 2 diabetic subjects, contrary to markers of inflammation such as CRP. The ongoing prospective part of the FIELD study may, at best, elucidate which variables most accurately predict the incidence of cardiovascular events in this high-risk population.

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