Linköping University Medical Dissertations No. 1260

Markers of subclinical atherosclerosis and arterial stiffness in type 2 diabetes

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Linköping 2011

Printed in Sweden by LiU-Tryck, Linköping, Sweden, 2011

ISBN 978-91-7393-075-8
ISSN 0345-0082

To Hanna, Jacob & Rebecca

CONTENTS

ABSTRACT

Background

Type 2 diabetes is a common disease with increased mortality and morbidity due to cardiovascular disease (CVD). This thesis is based on three studies that evaluated traditionally used and emerging risk markers to identify individuals with high-risk of developing CVD in middle-aged men and women with type 2 diabetes. One study was conducted to compare the equivalence between two different ultrasound techniques to measure intima-media thickness since IMT was used to evaluate subclinical atherosclerosis as a surrogate endpoint.

Methods

Data from the cohort study, cardiovascular risk in type 2 diabetes – a prospective study in primary care (CARDIPP) was used in paper I, III and IV. In paper I, baseline data from the first 247 subjects was analysed. Associations between traditionally measured lipids, apolipoproteins, glycaemic control and low-grade inflammation and IMT were analysed.
In paper III, the full baseline cohort, with data from 761 subjects from the CARDIPP study was cross-sectionally analysed regarding correlations between abdominal obesity measured as waist circumference (WC) and sagittal abdominal diameter (SAD), inflammatory markers and IMT and pulse wave velocity (PWV). In paper IV, the associations reported in paper I and III were prospectively investigated with data from the first year of follow-up four years after the baseline investigations in CARDIPP-revisited.
In paper II a study was performed on 24 young healthy subjects, both men and women. IMT was measured in the common carotid artery (CCA) and in the abdominal aorta (AA), by two skilled ultrasonographers, with 2 different ultrasound techniques in a randomised order.

Results

ApoB/apoA-I ratio (r=0.207, p=0.001), apoB (r=0.166, p=0.009) and non HDL-cholesterol (nHDL-c) (0.129, p=0.046) correlated with IMT.
In CCA IMT was equivalent using B-mode- and M-mode respectively. However in AA, IMT was 11.5% thicker using B-mode.
Abdominal obesity were significantly correlated with; IL-6 and CRP (both p<0.001, WC and SAD respectively), IMT (WC p=0.012, SAD p=0.003) and PWV (p<0.001 WC and SAD respectively). Adjusting for age, sex, treatment with statins, systolic blood pressure (SBP), Body Mass Index (BMI), CRP and HbA1c, SAD (p=0.047) but not WC, remained associated with IMT.
There were significant correlations between apoB (r=0.144, p=0.03) and CRP (r=0.172, p=0.009) measured at baseline and IMT measured at follow-up. After adjustment for sex, age, treatment with statins and Hba1c, the associations remained statistically significant. HbA1c, total cholesterol or LDL-cholesterol did not correlate to IMT at follow-up. Baseline body mass index (BMI) (r=0.130, p=0.049), WC (r=0.147, p=0.027) and SAD (r=0.184, p=0.007) correlated to PWV at follow-up. Challenged with sex, SBP and HbA1c, the association between SAD, not WC nor BMI, and PWV remained statistically significant (p=0.036).

Conclusions

There was a significant association between apoB/apoA-I ratio and IMT. The association was independent of conventional lipids, CRP, glycaemic control and use of statins. Both SAD and WC were associated with inflammation, atherosclerosis and arterial stiffness. However, SAD was slightly more robustly associated to subclinical organ damage, compared with WC. Prospectively; apoB and CRP, but not LDL-cholesterol predicted increased subclinical atherosclerosis. Furthermore, SAD was more independent in predicting arterial stiffness over time, compared with WC, in middle-aged men and women with type 2 diabetes.
The two different ultrasound techniques, B-mode and M-mode, measured different IMT thickness in the aorta, emphasizing the importance of using similar technique when comparing the impact of absolute values of IMT on cardiovascular disease.

LIST OF PAPERS

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

I **Dahlén EM, Länne T, Engvall J, Lindström T, Grodzinsky E, Nyström FH, Östgren CJ**. Carotid intima-media thickness and apolipoproteinB⁄apolipoprotein A-I ratio in middle-aged patients with type 2 diabetes. *Diabetic Medicine* 2009: 26, 384-390.

II **Dahlén, EM, Andreasson T, Cinthio M, Nystrom FH, Östgren CJ, Länne T.** Is there an underestimation of intima-media thickness based on M-mode ultrasound technique in the abdominal aorta? *Clinical Physiology and Functional Imaging* 2011. doi: 10.1111/j.1475-097X.2011.01045.x

III **Dahlén, EM, Tengblad A, Länne T, Clinchy B, Ernerudh J, Nystrom FH, Östgren CJ,** Abdominal Obesity and low grade Systemic Inflammation as Markers for Subclinical Organ Damage in type 2 diabetes. Submitted

IV **Dahlén EM, Bjarnegård N, Länne T, Nystrom FH, Östgren CJ,** Sagittal Abdominal Diameter is a more Independent Measure compared with Waist Circumference to predict Arterial Stiffness in subjects with Type 2 Diabetes. Submitted

ABBREVIATIONS

AA	Abdominal aorta
AGE	Advanced glycosylated end products
apoA-I	Apolipoprotein A-I
apoB	Apolipoprotein B
BMI	Body mass index
CARDIPP	Cardiovascular risk in patients with type 2 diabetes- a prospective study in primary care
CARDIPP-R	CARDIPP-revisited
CCA	Common carotid artery
CRP	high sensitive C-reactive protein
CVD	Cardiovascular disease
DCCT	The diabetes control and complications trial
FFA	Free fatty acid
HDL	High density lipoprotein
IDL	Intermediate density lipoprotein
IFCC	International federation of clinical chemistry
IL-1β	Interleukin-1β
IL-6	Interleukin-6
IL-10	Interleukin-10
LDL	Low density lipoprotein
MI	Myocardial infarction
OGTT	Oral glucose tolerance test
PHCC	Primary health care centres
SAD	Sagittal abdominal diameter
SBP	Systolic blood pressure
TG	Triglyceride
TNF-α	Tumour necrosis factor-α
VAT	Visceral adipose tissue
WC	Waist circumference
VLDL	Very low density lipoprotein

INTRODUCTION

Type 2 diabetes

Epidemiology

Type 2 diabetes is a common disease with an increasing prevalence worldwide (1). WHO has predicted that, by the year 2030 the prevalence of diabetes will be doubled from the present 171 million persons with diabetes mellitus (2).
In Sweden approximately 4.3% of the women and 4.5% of the men are currently diagnosed with diabetes (3). A Danish study based on their national diabetes register calculated the lifetime risk of developing diabetes at 30 %, in children born today (4). The incidence of diabetes in Sweden has been reported to stay on the same level for many years even though the prevalence is increasing (3, 5) however there has been one study from Kronoberg reporting on an increase in incidence as well (6).
Apart from a possibly increasing incidence the rise in prevalence might be explained by an aging population with longer lifetime expectancy and a trend that the diabetes diagnosis is set at an earlier stage, probably due to opportunistic screening of persons with risk factors for diabetes.

The risk of developing type 2 diabetes is increasing with age and is more common in men compared with women (7). Overweight and obesity, low education as a marker of socio-economic status and smoking are all known risk factors for developing type 2 diabetes (5, 8-9). There is also a strong genetic component where the risk for developing type 2 diabetes increases with one or both parents with the disease. The concordance rate of type 2 diabetes in identical twins is approximately 90% (10).

Clinical features

Diabetes mellitus is a heterogeneous group of diseases, with the common feature of elevated levels of blood glucose. Diabetes is classified into four different groups based on etiology (11). Diabetes type 1 accounts for 5-10% of all diabetes. The rise in blood glucose is caused by an autoimmune destruction of the β-cells causing insulin deficiency. Type 2 diabetes is the most common type and represents 85-90% of all diabetes.

In Sweden, most patients with type 2 diabetes are treated in primary care by general practitioners and by nurses especially educated in diabetes care. This thesis is confined to type 2 diabetes. The third category refers to other specific types of diabetes that are either caused by pancreatic diseases or rare genetic disorders. Finally, gestational diabetes is a temporary disease that occurs during pregnancy. After giving birth the hyperclycaemia is usually reversed. Gestational diabetes confers a high risk of developing type 2 diabetes (12) and these women are usually monitored with glucose testing annually.

Diabetes is diagnosed either by two fasting blood glucose levels ≥ 7.0mmol/l or by performing an oral glucose tolerance test (OGTT), where the fasting test subject receives an oral glucose load of 75g and the venous blood glucose is ≥ 11.1mmol/l after two hours (11).

Etiology

Insulin action

In healthy subjects, insulin is secreted from the β-cells of the pancreas due to various signals where glucose in the bloodstream is the most important signal. Insulin is an anabolic hormone that enhances uptake of glucose in the peripheral tissues and reduces production of glucose in the liver. Insulin increases the storage of energy, in adipose tissue, muscles and in the liver. Minutes after a rise in blood glucose there is a secretion of insulin and uptake of glucose and at the same time a decrease in the production of glucose and degradation of glycogen in the liver, and the blood glucose is subsequently lowered. Hence the blood glucose homeostasis is tightly regulated. Insulin stimulates formation of glycogen, triglycerides and synthesis of proteins.

Insulin resistance

The onset of type 2 diabetes is usually gradual and without early symptoms (13). Type 2 diabetes is a condition characterized by a relative insulin deficiency (14). There is also a dysfunction and a progressive degeneration and apoptosis of the β-cells (15-17), that eventually impairs the secretion of insulin, probably caused by high levels of blood glucose that is toxic to the β-cell (18) and by inflammation and autoimmune response (19-20).

At early stages of the disease the insulin production is generally high but still the blood glucose level rises due to peripheral insulin resistance (21-22), where the glycogen storage pathway is defective (23) and the insulin effect in the liver is decreased, causing lack of suppression of the production of glucose by the liver (24). The mechanisms of insulin resistance are still poorly understood, but there is a close relationship between insulin resistance and abdominal obesity in particular (25-26).

Abdominal obesity and the metabolic syndrome

Insulin resistance is a part of the metabolic syndrome (27) characterized by hypertension, abdominal obesity and high circulating triglyceride levels. Subjects with the metabolic syndrome are prone to develop type 2 diabetes (28) and are at high risk of developing cardiovascular disease (CVD) (29).
Abdominal obesity is a sign of increased visceral adipose deposits. The visceral adipose tissue (VAT) is a potent producer of pro-inflammatory cytokines, for example interleukin-6 (IL-6), which promotes production of c-reactive protein (CRP) in the liver, and tumor necrosis factor α (TNF-α). Compared with subcutaneous adipose tissue there is also an endocrine excretion of metabolically active hormones such as angiotensin II and leptin (30-31). The free fatty acids (FFA) released from the VAT are more likely to enter the general circulation and readily reaches the portal vein and the liver, where it further induces production of glucose (32) and plays a central role in the hepatic insulin resistance. The FFA in the liver also affects the lipid metabolism by reducing transport of cholesterol via high density lipoproteins (HDL) and promoting formation of very low density lipoproteins (VLDL) via re-esterification into triglycerides and low density lipoproteins (LDL) and increasing circulating triglycerides and LDL-cholesterol (19).

Macro-vascular complications

Type 2 diabetes confers an increased morbidity and mortality due to micro- and macro-vascular complications. The micro-vascular complications are diabetes specific; retinopathy, nephropathy and neuropathy (33). Generally these complications are correlated to the duration of the disease and treatment of hyperglycemia reduces these symptoms (34). Macro-vascular complications such as myocardial infarction (MI) stroke and peripheral arterial disease develops in younger age in a diabetic population and is often more severe.

Subjects with type 2 diabetes has at least a two-fold risk of premature death, and the most common cause of death is cardiovascular disease (CVD) that accounts for approximately 80% of all deaths. (35-38). Previously, it has been stipulated that a person with type 2 diabetes has the same risk for cardiovascular events as someone who has already experienced an MI (39) This opinion has been somewhat revised but still, compared to a non-diabetic population the risk of MI, stroke and peripheral arterial disease is approximately twice as high in subjects with type 2 diabetes compared to a non-diabetic population (40). There is an association between metabolic control and CVD but intensive glucose lowering therapy has not proven to reduce the risk of macro-vascular complications (41). The therapeutic targets to prevent CVD in the diabetic population are, beside glycaemic control, similar to all high risk populations; lifestyle intervention; treatment targeting blood pressure and lipid control (42-43).

Arterial stiffness

Age and blood-pressure are the two most important determinants of arterial stiffness (44). The stability of the vascular wall is depending on the balance between two prominent proteins, collagen and elastin. Due to inflammation there is a shift towards more collagen content and less elastin and the vessel becomes less elastic. Increased luminal pressure due to hypertension also stimulates the shift towards more collagen (45). Increased sympathetic tone is also a contributor to the process.
Diabetes is associated with increased stiffening (46) of the arteries due to multiple reasons, for example low-grade inflammation and cross-linking's of proteins in the arterial wall (47), where glucose forms glycosylated products with proteins known as advanced glycosylated end-products (AGE). Hyperglycaemia and hyperinsulinemia induces the renin-angiotensin-aldosterone system (48) and up-regulates the expression of angiotensin 1 receptor that promotes proliferation and fibrosis in the vessel wall (49).

Pulse wave velocity

Pulse wave velocity (PWV) measured by tonometry provides a non-invasive estimate of arterial stiffness (50-52) and is an independent predictive risk factor for all-cause mortality and cardiovascular mortality (53-55).

In this thesis PWV was measured by applanation tonometry over the carotid and femoral arteries. The distance between these reference points was measured and the transit time for the aortic pulse wave was measured by electrocardiogram-gated recordings of the femoral and carotid pulse waves.

Atherosclerosis

The term atherosclerosis refers to a condition in which an artery wall thickens as a result of accumulation of lipids and structural changes with increased collagen in the wall. Continuous deposition of oxidized lipoproteins in the vascular intimal wall leads to an inflammatory response from the endothelial cells and endothelial dysfunction which signals to monocytes that transforms into macrophages. The macrophages scavenge the lipoproteins and form foam cells that leads to fatty streaks in the vessel wall. This is the initiation of the cascade of events that eventually leads to atheroma formation and atherosclerotic plaques in the arterial wall (56-57).

Intima-media thickness

Measuring intima-media thickness (IMT) represents the combined layers of the intima and the media in the vessel wall. The intima, closest to the lumen of the vessel, consists of a thin layer of endothelial cells and a surrounding layer of connective tissue. The media is the thickest layer in the vessel wall and consists of elastin and collagen fibers as well as smooth muscle cells. B-mode ultrasound in the common carotid artery (CCA) has emerged during the last 20 years as a well validated and reproducible non-invasive measurement of subclinical atherosclerosis (58), that is an independent predictor of MI and stroke (59-60). Atherosclerotic disease seems to appear at an earlier stage in the aortic wall compared to smaller arteries (61). IMT in the abdominal aorta (AA) would therefore be valuable in early diagnosis of atherosclerosis, but it has previously been assumed that the AA is located too deep to achieve reliable IMT readings. However some studies have shown reliable results from AA with B-mode technique (62). In this thesis, IMT was used as a measure for subclinical atherosclerosis since the study population was middle-aged and in most cases had not developed endpoints such as MI or stroke. In paper II IMT was measured on relatively young subjects.

The IMT is defined as the distance between the inner echogenic line representing the intima and blood interface and the outer echogenic line representing the junction between the adventitia and media layers of the arterial vessel wall (63).

Lipoproteins and apolipopoteins

The atherosclerotic process is initiated when the endothelium of the blood vessels are presented with oxidized LDL. Subjects with type 2 diabetes often present with a typical form of dyslipidaemia, which is characterized by elevated serum triglycerides (TG), reduced HDL and low or normal LDL and with increased amounts of small dense LDL compared to a non-diabetic population (64). The LDL penetrates the intima of the vascular wall and become biologically modified.

Measuring or calculating LDL provides no information on the other lipoprotein particles or the atherogenicity of the LDL particles. As each proatherogenic VLDL, Intermediate Density Lipoprotein (IDL) and LDL particle has one apoB attached to the surface, the serum apoB concentration yields the number of atherogenic particles. Apolipoproteins are surface proteins and receptor ligands at the outer layer of the lipoproteins. ApoB acts as a ligand to the LDL receptor. ApoB is produced in the intestine wall after a meal and serves as a part of the TG rich chylomicron which transports TG and cholesterol, via the lymphatic system to the bloodstream through the thoracic duct. The chylomicron transports TG and cholesterol to the peripheral tissues, where the TG is hydrolyzed into FFA that is utilized as energy in the muscles or is stored in the adipose tissue. The remnants of the chylomicron serve as building blocks in the formation of lipoproteins VLDL, IDL and LDL that carries the fat-soluble lipids in the water-based blood stream.
Small dense LDL particles are considered to be at least as atherogenic as larger LDL particles (65). Serum apoA-I, which is a protein attached to the HDL particle, is an overall equivalent to the concentration of HDL in plasma. HDL particles transport cholesterol from peripheral tissue and vessels to the liver. Thus, the balance between the proatherogenic apoB and the antiatherogenic apoA-I, has been suggested to be more predictive of cardiovascular risk compared to conventional lipids (66-67).

Inflammatory markers

Inflammation has a key role in the atherosclerotic process and it has previously been shown that patients with type 2 diabetes have higher levels of inflammatory markers compared to subjects without diabetes (68). Certain markers of inflammation, such as CRP (69-70) and IL-6 (71), have previously been repeatedly studied and shown to be associated with insulin resistance and atherosclerosis. CRP is an acute-phase reactant produced mainly in the liver and is promoted by elevated levels of IL-6. IL-6 is produced by many different cells in response to possible threats to the organism, such as microorganisms, signaling danger to the cells within the immune-system. Adipocytes in visceral adipose tissue (VAT) produces higher levels of IL-6 compared to the adipocytes in the subcutaneous fat (72).

A cascade of events that leads to atheroma formation in the arterial wall is initiated with a deposition of oxidized lipoproteins in the vascular wall. An inflammatory response from the endothelial cells signals to monocytes that transforms into macrophages. The macrophages, scavenges the lipoproteins and form foam cells and fatty streaks. (56). The macrophages not only scavenge lipid cells, they also secrete pro-inflammatory cytokines (73).

Abdominal obesity

Development of type 2 diabetes is closely associated with obesity and abdominal obesity is the best obesity-related predictor of type 2 diabetes (74). Anthropometric measures are clinically useful since they are both non-invasive and cheap. Waist circumference (WC) is currently the most commonly used measurement for abdominal obesity, and highly associated with CVD (75). However, recent studies suggest that sagittal abdominal diameter (SAD) to be the best measurement to assess an adverse metabolic profile (76-78) and to estimate the metabolically active VAT (79-80).

Visceral fat also known as intra-abdominal fat is different from subcutaneous fat. Excess visceral fat is in contrast of subcutaneous fat linked to insulin resistance, and the development of type 2 diabetes (81). SAD can be measured in different ways. Either by images produced preferably by magnetic resonance (79) or by anthropometric measurements (77). In this thesis SAD was measured with the subject in the supine position, with knees slightly bended, with a sliding calliper at the maximum height of the abdomen (82).

CARDIPP

CARDIPP, Cardiovascular risk in patients with type 2 diabetes- a prospective study in primary care, was launched in November 2005 and by the end of 2008 761 subjects with type 2 diabetes were consecutively recruited from 25 different primary health care centers (PHCC) in the counties of Östergötland and Jönköping, Sweden. CARDIPP comprises data on an extended annual follow up on patients, aged 55-66 years.

The centres were located in different demographic areas and differed in size. However, the model of treatment and care of type 2 diabetes, was organized similarly and all centres adhered to the same national guidelines of diabetes care (83). The annual follow was performed at the PHCCs and was conducted by nurses especially dedicated to treatment of diabetes.

The investigation included a standard medical history, including data on diabetes duration and on-going medication. The nurses measured blood pressure, height and weight. At the visit to the PHCC the patients were booked in for examinations at the Department of Physiology either at Linköping University Hospital or at Ryhov County Hospital in Jönköping. Blood samples were drawn after a 10 hour over-night fast, for routine lab analyses at the PHCC and were also frozen for later analyses at the Centre for Laboratory medicine, Linköping University Hospital.

The investigations at the departments of physiology included measurement of SAD, IMT measurements of the common carotid artery and applanation tonometry over the carotid, the femoral and radial arteries.

CARDIPP-Revisited

The CARDIPP-R comprises a re-investigation of the cohort four years after the completion of the baseline examination. In CARDIPP-R, all participants from the baseline study were invited to the re-investigation conducted at the Department of Physiology, Linköping University Hospital and at County Hospital Ryhov, Jönköping. The CARDIPP-R study protocol for the carotid ultrasonographic investigations and tonometry for measurements of the carotid, femoral and radial pulse pressure wave form and pulse wave velocity followed the study protocol from the baseline investigation. The routine laboratory tests were performed at the different PHCCs as described in the baseline protocol.

AIMS

General aim

To cross-sectionally and prospectively analyze the associations between clinical characteristics, markers of dyslipidemia and inflammation with subclinical atherosclerosis and arterial stiffness, in type 2 diabetes.

Specific aims

- To explore the association between the carotid IMT and apoB/apoA-I ratio compared to conventional lipids in middle aged patients with type 2 diabetes.

- To compare B-mode and M-mode ultrasonography techniques when measuring IMT, in terms of reproducibility and to explore if the results from the different techniques are equivalent in both the common carotid artery and the abdominal aorta.

- To explore the association between measurements of abdominal obesity, inflammatory markers, lipids and subclinical organ damage defined as carotid IMT and aortic PWV.

- To prospectively analyze the association between lipids, abdominal obesity, inflammation and subclinical organ damage after four years defined as carotid IMT and aortic PWV.

STUDY POPULATIONS

In paper I, we analyzed baseline data from patients who participated in the first phase of a community-based cohort study, CARDIPP. During the first year, 316 subjects were enrolled. In a first stage apoB and apoA-I was analyzed in 250 subjects. Due to missing values in 3 cases, data from the first 247 (156 men and 91 women) recruited subjects was analyzed. Sixty-eight (27.5%) patients were treated with diet and exercise only. The remaining patients were treated with oral hypoglycemic agents (n=88, 35.6%) or insulin alone or in combination with oral hypoglycemic agents (n=91, 36.8%). 127 (51.4%) patients were treated with statins.

In paper III the full baseline cohort of CARDIPP was included. 761 patients were recruited until the end of 2008 when CARDIPP was completed. Due to missing data on IMT the study was confined to 740 subjects. There were 481 men and 259 women, mean age was 60.7 years. For glucose control 402 (54.3%) subjects were treated with statins. 213 subjects (28.8%) were treated with diet and exercise only. Remaining subjects were treated with oral hypoglycemic agents, n=296 (40%) or with insulin alone or in combination with oral hypoglycemic agents, n=231 (31.2%).

Paper IV comprises data from the first year of follow-up investigation four years after the baseline investigation in the CARDIPP-R study. 255 patients were included. Four years after baseline investigations the number of subjects treated with statins had increased to 175 (68.6%).

Paper II was based on a study with 24 young healthy Caucasian subjects (12 females and 12 males, range 21-31 years). All were non-smokers without history of cardiovascular disease.

METHODS

Paper I, III and IV

Anthropometric measurements

Nurses especially dedicated to treatment of diabetes at the primary health care centers, measured height (to the nearest cm) and weight (to the nearest 0.1 kg) with the patients wearing light indoor clothing. WC was measured according to WHO's recommendations (84) with the patient standing, after a regular expiration, to the nearest cm, midway between the lowest rib and the iliac crest. SAD was recorded with the patient in the supine position and with bent knees, with a standardized sliding beam calliper at the highest point of the abdomen.

Laboratory tests

Blood specimens were drawn in the morning after a 10 hour over-night fast. Routine tests such as HbA1c, plasma glucose and serum lipids were analysed according to routines at the primary health care centres. HbA1c was analysed according to the Swedish Mono-S HPLC. In paper III and IV, HbA1c values were converted to DCCT standard values using the formula: HbA1c DCCT = 0.923 x HbA1c (Mono-S) + 1.345 (R^2=0.998) (85) and data on HbA1c was also reported in IFCC units.

Blood samples were frozen for later analysis of CRP, apoB and apoA-I, IL-1β, IL-6 and IL-10 at the Centre for Laboratory medicine, Linköping University Hospital, Linköping, Sweden. CRP values above 10 mg/ml were excluded from the analyses according to current guidelines since higher values probably reflect active infection, acute inflammatory response or trauma (86).

Levels of CRP, apoB and apoA-I were measured by immunoturbidimetric assays, Bayer healthcare and Siemens Diagnostic Medical Solutions.

Plasma levels of IL-1β, IL-6 and IL-10 were measured with an ultrasensitive cytokine bead kit (Invitrogen Co, Carlsbad, CA, USA) according to the manufacturer´s instructions and analysed on a Luminex® 100™ system (Austin, TX, USA). The limit of detection was 0.19, 0.84, 0.68 pg ml^{-1}, for IL-1β, IL-6 and IL-10, respectively. Intra-assay coefficient of variation (CV%) was 5-12 % and inter-assay was 17-20 %.

Physiological vascular examinations

The blood pressure measurements, carotid ultrasonographic investigations, PWV and SAD were performed at the Department of Physiology, Linköping University Hospital, Linköping, Sweden and at the County Hospital Ryhov, Jönköping, Sweden. Non-invasive upper arm blood pressure (BP) was recorded with oscillometric technique (Dinamap PRO 200 Monitor, Critikon, Tampa, FL, USA).

IMT

IMT of the carotid arteries were evaluated using a B-mode ultrasound. A digital ultrasound system (ATL HDI 5000, Bothell, WA, USA) equipped with a broadband linear transducer (L12-5) was used for scanning the carotid artery in longitudinal section. ECG leads were connected. For lumen diameter (LD) and IMT determination, during diastole, three consecutive frozen images with special focus on lumen-intima echo and media-adventitia echo of the far arterial wall were saved for later analysis. The digital B-mode images were subsequently transferred to a PC, where software for off-line measurement of LD and IMT is installed (Artery Measurement System II, Image and Data Analysis, Gothenburg, Sweden). Calibration and subsequent measurement was performed by manually tracing a cursor along the leading edge of the intima-lumen echo of the near wall, leading edge of the lumen- intima echo and media-adventitia echo of the far wall. A 10 mm long section of the common carotid artery in the proximity of the carotid bulb was selected to obtain mean LD and far wall IMT. During analysis, the measurement window was hidden for the reader and values were saved in a text file. Mean values of IMT and carotid LD from both the right and the left sides were used in all analyses.

PWV

Aortic PWV was measured with applanation tonometry (SphygmoCor® system, model MM3, AtCor Medical, Sydney, Australia) over the carotid and femoral arteries. The aortic pulse wave transit times were measured by electrocardiogram-guided readings of the femoral arterial pulse waves, using the carotid arterial pulse wave as the reference site. The surface distances were estimated from the suprasternal notch to the carotid and femoral measurement sites, respectively. PWV was calculated by dividing the surface distance with the pulse wave transit time yielding $m \times s^{-1}$.

CARDIPP-Revisited

The CARDIPP-R study protocol for the blood pressure measurements, the carotid ultrasonographic investigations and tonometry for measurements of the carotid, femoral and radial pulse pressure wave form and pulse wave velocity followed the study protocol from the baseline investigation. The routine laboratory tests were performed at the different health-care centers as described in the baseline protocol. In the follow-up investigation, CRP was not measured with an identical method as at baseline, thus our results regarding CRP values are confined to baseline data only.

Paper II

IMT measurements

The right CCA was examined 1 cm proximal to the bifurcation and the AA was examined at the midpoint between the renal arteries and the aortic bifurcation. All twenty-four subjects were examined by two skilled ultrasonographers at one single occasion, at Linköping University Hospital, Sweden. The examinations were performed after at least 15 minutes of rest, and all subjects were examined in a supine position. Two different ultrasound techniques were used for measuring IMT, B-mode technique, according to the described method in paper I. Philips ATL HDI 5000 (Philips Ultrasound, Seattle, USA) and M-mode technique, Wall track system (WTS, Pie Medical, Maastricht, Holland). In both cases a 4–7 MHz linear transducer was used. In the B-mode technique IMT was measured semi-manually on a 10mm section of the vessel with the aid of the digitizer (87).

In the M-mode technique IMT was measured in one single line. On each subject, each ultrasonographer examined the infra-renal AA twice and the CCA twice with both ultrasound techniques (B-mode: ATL HDI 5000 and M-mode: WTS). The ultrasonographer lifted the probe prior to each image acquisition, and the first two readings were performed by one ultrasonographer and the last two by the other, in a randomized order.

Statistical Methods

Paper I

SPSS for Windows 14.0 was used for statistical analysis. Serum triglycerides and CRP was log transformed because of skewness before statistical analyses. Pearson correlation coefficients were calculated between the different laboratory measurements and IMT using bivariate correlation analysis. Table 2 presents the results of independent 2-sample T-test, with modification for unequal variances and Fishers exact test. Analyses using cross-product interaction terms were used to investigate possible modifying factors such as gender and treatment with statins. Statistical significance was assumed when $p < 0.05$. Logistic regression analyses were used to further explore the association between IMT and apoB/apoA-I ratio compared to conventional lipids and other risk factors by stepwise models and by models where all variables were entered all together.

Paper II

SPSS for Windows 16.0 (SPSS inc. Chicago, IL, USA) was used for statistical analyses. Data were presented as mean ± SD. The coefficient of variation (CV) was used to calculate the inter-observer and intra-observer variability, CV (%) = $s \times 100/\tilde{x}$. To visualize potential differences between B-mode and M-mode in AA IMT the Bland and Altman method was used (88).

Paper III and IV

SPSS for Windows 16.0 (SPSS inc. Chicago, IL, USA) was used for statistical analyses. Variables with skewed distribution IL-6, IL-10, CRP were log transformed. Pearson correlation coefficients were calculated between the different measurements, using bivariate correlation analysis. Statistical significance was assumed when $p < 0.05$. In multiple linear regression analyses with IMT or PWV, as dependent variables, the increase of one unit for each of the variables explored, conferred a change in IMT or PWV respectively expressed as the regression coefficient (beta) with 95 percent confidence intervals (CI). In stepwise linear regression criteria for entry were $p<0.05$ and for removal $p>0.1$

Ethics

The studies which complied with the declaration of Helsinki were approved by the Regional Ethical Review Board in Linköping, Sweden.

RESULTS AND DISCUSSION

Paper I

Carotid intima-media thickness and apolipoprotein B / apolipoprotein A-I ratio in middle aged patients with type 2 diabetes

Results

ApoB/apoA-I ratio (r=0.207, p=0.001), apoB (r=0.166, p=0.009) and non HDL-cholesterol (nHDL-c) (0.129, p=0.046) correlated with IMT.
Conventional lipids, CRP, HbA1c and systolic blood pressure were not significantly correlated to IMT

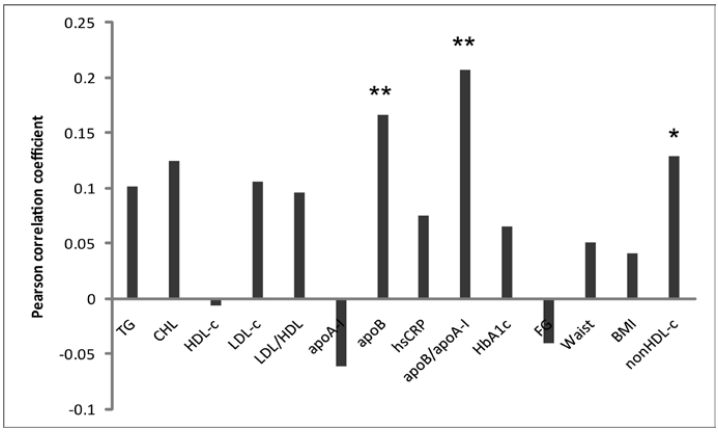

Figure 1. Pearson correlation coefficient between IMT, TG, total-cholesterol (CHL), HDL-c, LDL-c, LDL/HDL ratio, apoB, apoA-I, apoB/apoA-I ratio, HbA1c, hsCRP, Fasting plasma glucose (FG), WC, BMI, Non-HDL-cholesterol

*correlation is significant at the 0.05 level

**correlation is significant at the 0.01 level

A stepwise logistic regression analysis was conducted with IMT as the dependent variable and apoB/apoA-I ratio, HbA1c, CRP, LDL-c, total cholesterol, nonHDL-c and treatment with statins as independent variables. Following adjustment for age and gender, only the apoB/apoA-I ratio remained significantly associated with IMT. In table 1 the odds ratio (OR) is given for elevated apoB/apoA-I and increased IMT, adjusted for age and gender, in different settings

Table 1. Association between dichotomized carotid IMT with a cut off level of > 0.9 as dependent variable and apoB/apoA-I ratio adjusted for age and gender in different settings.

Variable	OR	95% CI	p
ApoB/apoA-I ratio	3.9	1.7-9.2	0.002
ApoB/apoA-I ratio, CRP	4.3	1.8-10.2	0.001
ApoB/apoA-I ratio, statin treatment	3.6	1.5-8.7	0.004
ApoB/apoA-I ratio, total cholesterol	3.2	1.2-8.4	0.022
ApoB/apoA-I ratio, total cholesterol	3.2	1.2-8.4	0.022
ApoB/apoA-I ratio, nonHDL-c	3.8	1.2-11.8	0.021
ApoB/apoA-I ratio, systolic BP	3.9	1.7-9.1	0.002

The OR values are given for elevated apoB/apoA-I ratio. Analyzes were made with logistic binary regression.

Discussion

In spite of a relatively small number of study subjects, we were able to show that the association between the apoB/apoA-I ratio and carotid IMT was independent of conventional lipids, CRP and use of statins. This was further investigated with gender and statins as modifying factors and remained statistically significant. Compared to nonHDL-c, the apoB/apoA-I ratio showed a significant association, after adjustment, with increased IMT in patients with type 2 diabetes.

Paper II

Is there an Underestimation of Intima-Media Thickness based on M-mode Ultrasound technique in the Abdominal Aorta?

Results

The intra-observer variability of IMT in CCA and AA, using B-mode showed a coefficient of variation (CV) 8% and 9%, and with M-mode 11% and 15% respectively. Inter-observer variability of IMT in CCA and AA using B-mode was 6 % and 12%, and with M-mode 11% and18 % respectively. CCA IMT was 0.53±0.07mm and 0.53±0.09 mm using B-mode- and M-mode, respectively. However in AA, IMT was 0.61±0.05 mm and 0.54±0.10 mm using B-mode and M-mode, respectively. Thus AA IMT was 11.5% thicker using B-mode ($p<0.01$). Figure 2 and 3 views the difference from the two methods in AA.

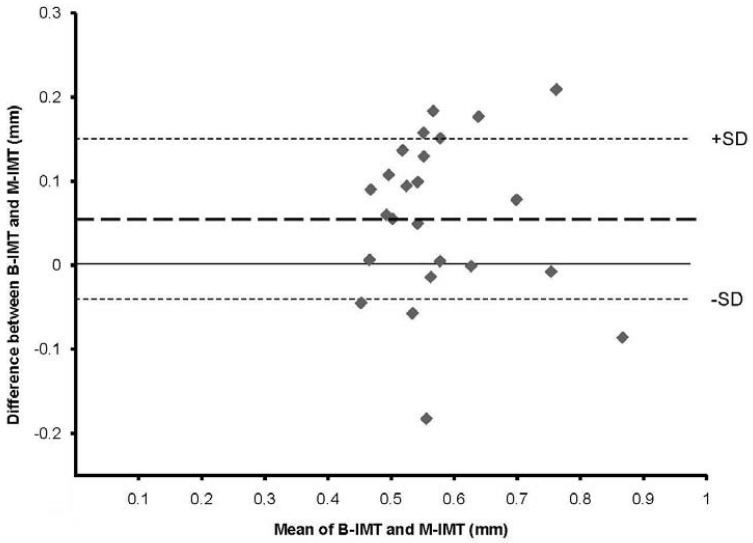

Figure 2. Bland and Altman plot; comparing intima-media thickness (IMT) in the abdominal aorta, measured with B-mode (B-IMT) and M-mode (M-IMT) Note the difference in mean value between the two techniques with an underestimation of the IMT measurement by the M-mode technique of 11.5 %, $p<0.01$.

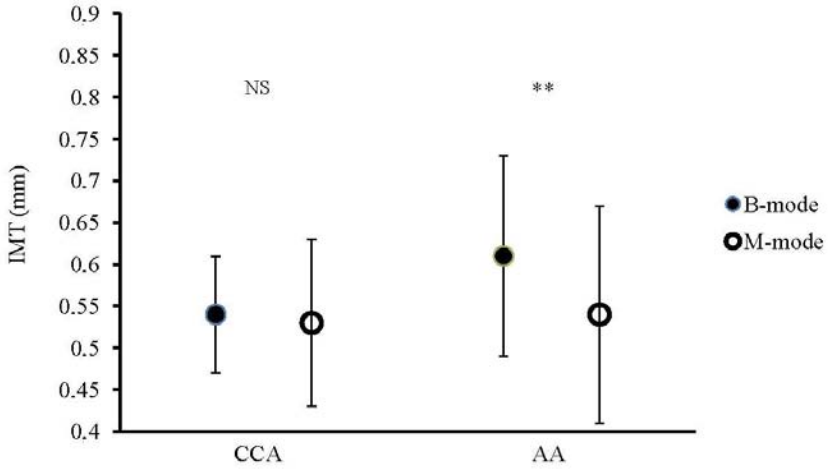

Figure 3. IMT in the AA and CCA measured with B-mode and M-mode ultrasonography (mean ± SD). Note the differences between the techniques found in the AA but not in CCA ($p<0.01$).

Discussion

Although there was no difference in IMT comparing the two techniques in the common carotid artery, they gave significantly different IMT values in the aorta. Previous studies on IMT in the AA are congruent with our data, suggesting differences between absolute values using B- and M-mode techniques in measuring the IMT of the aorta. The results of this study emphasize the importance of using similar technique when comparing the impact of IMT on cardio-vascular disease.

A possible explanation of the differences might be differences in timing of the reflected pulse waves between the CCA and AA, a larger fraction of the diameter change will occur in the late part of the systolic phase in the aorta. IMT changes during the cardiac cycle in relation to the pulsatile diameter change, it is thickest at end-diastole and quickly compressed 5-15% during peak-systole, and at late systole it slowly thickens again (89-94). This might induce a relative reduction in IMT with M- compared to B-mode in the AA that is not seen in the CCA. However to our knowledge, no data are available regarding the IMT variation during the cardiac cycle of the AA at present.

Paper III

Abdominal Obesity and low grade Systemic Inflammation as Markers for Subclinical Organ Damage in type 2 diabetes

Results

Abdominal obesity were significantly correlated with; IL-6, CRP (both p= <0.001, WC and SAD, respectively), IMT (WC p=0.012, SAD p=0.003) and PWV (p<0.001, for WC and SAD, respectively).

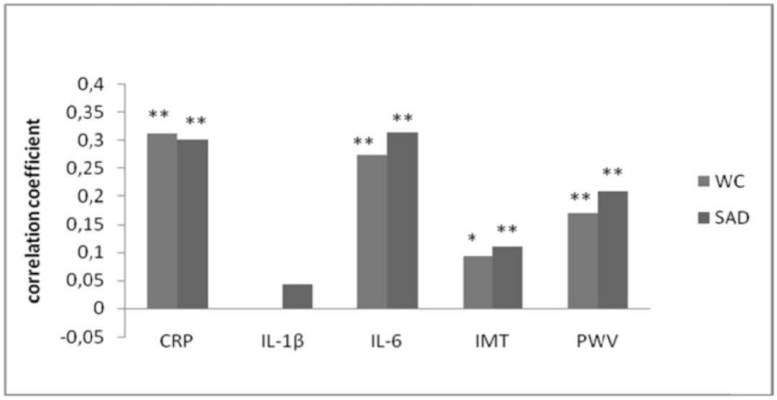

Figure 4. Pearson correlations between SAD and WC, and inflammatory markers, IMT and PWV. * correlation is significant at the 0.05 level, ** correlation is significant at the 0.01 level

Table 2. Pearson correlation coefficients (r) in relation to IMT and PWV

Variable	IMT		PWV	
	r	*p*	*r*	*p*
Age, year	0.133	<0.001	0.186	<0.001
HbA1c	0.033	0.377	0.167	<0.001
Diabetes duration	0.032	0.401	0.204	<0.001
LDL-cholesterol	0.038	0.316	-0.012	0.765
nonHDLcholesterol	0.048	0.204	0.016	0.677
ApoB/apoA-I	0.083	0.030	-0.043	0.270
IL-6*	0.089	0.021	0.098	0.001
IL-10*	0.051	0.182	0.087	0.027
CRP*	0.016	0.688	0.045	0.271
Systolic BP	0.092	0.009	0.239	<0.001
BMI	0.037	0.320	0.127	0.001
SAD	0.111	0.003	0.209	<0.001
WC	0.093	0.012	0.171	<0.001

*Geometrical mean was used in analyses

In multiple linear regressions with IMT as dependent variable and age, sex, systolic blood pressure (SBP), BMI, CRP and HbA1c, as independent variables, SAD (p=0.047) but not WC, remained associated with IMT. When adding previous MI to the adjustments the results remained unchanged (not in table).

Table 3. Multiple linear regression with IMT (a) and PWV (b) as dependent variables and IL-1β, IL-6, IL-10, SAD and WC as independent variables in different settings

a.		IMT		
Variable	(Unit)	Beta coefficient	95 % CI	*P*
IL-1β	(pg ml^{-1})	0.14	(0.053-0.18)	<0.001
IL-6	(pg ml^{-1})	0.03	(-0.017-0.074)	0.23
IL-10	(pg ml^{-1})	0.03	(-0.019-0.083)	0.30
SAD	(cm)	0.006	(0.000-0.012)	0.047
WC	(cm)	0.002	(0.001-0.004)	0.14
b.		PWV		
IL-1β	(pg ml^{-1})	0.46	(-0.24-1.16)	0.20
IL-6	(pg ml^{-1})	0.58	(0.08-1.1)	0.024
IL-10	(pg ml^{-1})	0.77	(0.15-1.39)	0.015
SAD	(cm)	0.10	(0.03-0.17)	0.005
WC	(cm)	0.17	(0.017-0.076)	0.001

Multiple linear regression analyses of, in a. IMT and in b. PWV, as dependent variables adjusted for age, sex, treatment with statins, BMI, systolic blood pressure, CRP and HbA1c. The increase of one-unit for each of the variables confer a change in IMT (a) mm and in PWV (b) m s^{-1}, expressed as the regression coefficient (Beta) with 95 percent CI.

In stepwise linear regression, entering both SAD and WC, the association between SAD and PWV was stronger than the association between WC and PWV.

Discussion

In this study, we found no association between conventionally measured lipids (LDL-c and HDL-c), glycaemic control or CRP and subclinical atherosclerosis in the carotid arteries. However, we confirmed the findings from paper I from a small sample (n=247) of this study reporting on an association between the apoB/apoAI ratio and IMT. Since classical risk factors, such as lipid levels and smoking status show poor correlation to IMT there is a need for gaining further knowledge about clinically feasible determinants of subclinical atherosclerosis. We found significant associations between abdominal obesity, measured both as SAD and WC, and subclinical atherosclerosis as well as arterial stiffness. However, in most analyses SAD was more robustly associated to IMT and PWV compared to WC. There were also significant associations between both abdominal obesity and inflammatory markers, as well as between inflammatory markers and markers of subclinical organ damage. The inflammatory markers may contribute to the explanation of the association between abdominal obesity and atherosclerosis.

Paper IV

Sagittal Abdominal Diameter is a more Independent Measure compared with Waist Circumference to predict Arterial Stiffness in subjects with Type 2 Diabetes

Results

In table 4 the change between baseline characteristics and characteristics at follow-up is displayed. After four years from the baseline investigation, there were significant changes in abdominal obesity, lipids, IMT and PWV. Table 5 show correlations between baseline variables and IMT and PWV measured at the follow-up investigation after four years. There were significant correlations between apoB (r= 0.144, p=0.03), CRP (r=0.172, p=0.009) at baseline and IMT measured at follow-up. After adjustment for sex, age, treatment with statins and Hba1c, the associations remained statistically significant. HbA1c, total cholesterol or LDL-cholesterol did not correlate to IMT at follow-up. Baseline BMI (r=0.130, p=0.049), WC (r=0.147, p=0.027) and SAD (r=0.184, p=0.007) correlated to PWV at follow-up. Challenged with sex, SBP and HbA1c, the association between SAD, not WC nor BMI, and PWV remained statistically significant (p=0.036), table 6. When adding previous MI to the adjustment the results remained unchanged. (Not in table.)
In a stepwise linear regression, entering both SAD and WC, the association between SAD and PWV was stronger than the association between WC and PWV.

Discussion

In this study we were able to prospectively confirm some of our previous results from paper I and III. Baseline apoB, but not the ratio apoB/apoA-I, were associated with subclinical atherosclerosis, over time.
The reliability of measurements is an important factor to consider in clinical practice. SAD has a high reliability in both lean and obese subjects (95). WC can be measured in various ways and there is no consensus about which is the best measurement protocol (96). Self reported WC measurements have not been considered reliable (97).

Table 4. Characteristics at baseline investigation (2006) and follow-up four years later (2010), in 172 men and 83 women with type 2 diabetes. In the Cardiovascular Risk factors in Patients with Diabetes – a Prospective study in Primary care, (CARDIPP)

	Baseline			Follow-up			
	All	Men	Women	All	Men	Women	Δ
Characteristics	*mean (sd)*	*mean (sd)*	*mean (sd)*	*mean (sd)*	*mean (sd)*	*mean (sd)*	*mean (sd)*
Age (years)	61 (2.8)	61 (2.9)	61 (2.6)	65 (2.9)	65 (2.9)	65 (2.7)	4
BMI (kg/m^2)	29.6 (5.0)	29.3 (4.7)	30.2 (5.5)	29.8 (5.1)	29.4 (5.0)	30.6 (5.3)	0.2 (2.2)
Sagittal Abdominal Diameter(cm)	25.2 (4.1)	25.4 (4.1)	24.8 (4.0)	25.9 (4.5)	26.0 (4.6)	25.8 (4.3)	0.6 (2.9)**
Waist circumference (cm)	102.4 (12.5)	103.2 (12.3)	100.5 (13.3)	105.6 (12.4)	106.1 (12.4)	104.5 (12.6)	3.3 (6.6)**
Systolic Bloodpressure (mmHg)	131 (17)	132 (16)	130 (17)	131 (16)	131 (15)	132 (21)	0.3 (17)
Diastolic Bloodpressure (mmHg)	75 (9)	77 (8)	70 (8)	73 (9)	75 (21)	69 (9)	-1.7 (8.5) **
HbA1c (% units)	7.0 (1.0)	7.0 (1.0)	7.0 (1.0)	7.2 (0.9)	7.2 (0.9)	7.3 (0.8)	0.23 (1.0)**
HbA1c (mmol/mol)	53.2 (11.6)	52.9 (11.6)	53.7 (11.8)	56.1 (9.8)	55.5 (10.2)	57.2 (8.9)	2.6 (10.9)**
Total cholesterol (mmol/L)	4.7 (1.0)	4.6 (0.9)	5.0 (1.0)	4.4 (1.0)	4.3 (1.0)	4.4 (0.9)	-0.4 (1.1)**
LDL cholesterol (mmol/L	2.6 (0.8)	2.6 (0.6)	2.3 (0.9)	2.4 (0.8)	2.4 (0.9)	2.3 (0.7)	-0.3 (0.9)**
HDL cholesterol (mmol/L)	1.4 (0.3)	1.3 (0.3)	1.5 (0.3)	1.2 (0.3)	1.2 (0.3)	1.3 (0.4)	-0.1 (0.2)**
Non HDL cholesterol (mmol/L)	3.4 (1.0)	3.3 (0.9)	3.6 (1.1)	3.1 (0.9)	3.1 (0.9)	3.1 (0.9)	-0.3 (1.1)**
ApoB/ApoA-I	0.73 (0.17)	0.73 (0.16)	0.71 (0.19)	0.66 (0.20)	0.68 (0.20)	0.61 (0.18)	-0.08 (0.18)**
ApoB	0.95 (0.19)	0.94 (0.18)	0.97 (0.20)	0.88 (0.21)	0.89 (0.21)	0.86 (0.21)	-0.08 (0.21)**
Serum Triglycerides (mmol/L)	1.7 (1.0)	1.7 (1.0)	1.7 (0.9)	1.7 (1.0)	1.7 (1.0)	1.7 (0.8)	-0.04 (0.79)
Pulse wave velocity (m/s)	10.2 (2.2)	10.3 (2.2)	10.0 (2.2)	11.0 (2.4)	11.1 (2.4)	11.0 (2.5)	0.84 (1.9)**
Intima-Media Thickness (mm)	0.70 (0.17)	0.70 (0.18)	0.67 (0.14)	0.78 (0.20)	0.79 (0.23)	0.77 (0.15)	0.09 (0.2)**
Treatment with statins n (%)	121 (47.5%)	81 (47%)	40 (48.2%)	175 (69%)	111 (61%)	64 (78%)	54 (21%)**
Smoking n (%)	41 (16%)	24 (14%)	17 (20.5%)	35 (14%)	19 (11%)	16 (19.5%)	-6 (-2%)**

** Difference at p<0.01 level

Table 5. Pearson correlation coefficients (r) of baseline characteristics in relation to intima media-thickness (IMT) and pulse wave velocity (PWV) after four years from baseline investigation.

	IMT		PWV	
Variable	*R*	*P*	*R*	*P*
Diabetes duration	-0.013	0.841	0.250	<0.001
HbA1c	-0.018	0.772	0.125	0.058
Total cholesterol	0.097	0.126	-0.037	0.575
LDL-cholesterol	0.067	0.300	-0.078	0.252
nonHDLcholesterol	0.080	0.207	-0.011	0.867
ApoB	0.144	0.030	-0.053	0.453
ApoB/apoA-I	0.072	0.280	-0.046	0.515
CRP*	0.172	0.009	0.116	0.096
Systolic BP	0.170	0.007	0.258	<0.001
Diastolic BP	0.003	0.967	0.081	0.223
BMI	-0.064	0.310	0.130	0.049
SAD	-0.012	0.861	0.184	0.007
WC	-0.054	0.395	0.147	0.027

Table 6. Associations between PWV and SBP, BMI, SAD and WC as independent variables one by one in different settings

		PWV		
Variable	(Unit)	Beta coefficient	95 % CI	*p*
SBP*	(mm Hg)	0.034	(0.015-0.053)	<0.001
BMI	(kg m^2)	0.046	(-0.022-0.114)	0.181
SAD	(cm)	0.092	(0.006-0.179)	0.036
WC	(cm)	0.020	(-0.007-0.048)	0.143

Multiple linear regression analyses of PWV measured 2010, as dependent variable adjusted for, sex, HbA1c and systolic blood pressure. The increase of one-unit for each of the variables confers a change in PWV m s^{-1}, expressed as the regression coefficient (Beta) with 95 percent CI. * Not adjusted for SBP.

GENERAL DISCUSSION

The main findings in this thesis were firstly; that apoB and apoB/apoA-I ratio was associated with subclinical atherosclerosis. Secondly; abdominal obesity, measured as SAD, was an independent predictor for arterial stiffness. These results were in contrast to traditionally used markers for evaluating risk for cardiovascular disease and organ damage in patients with diabetes such as LDL- and HDL-cholesterol, HbA1c and BMI that were weakly or not at all associated with subclinical atherosclerosis and arterial stiffness.

In paper I we found that the commonly used risk markers BMI, LDL- and HDL cholesterol, HbA1c for evaluating cardiovascular risk was not associated with subclinical atherosclerosis measured as IMT. However both apoB and the ratio apoB/apoA-I were associated to IMT and remained associated after several adjustments for possible confounders. This result is in concordance with results from previous large studies on more heterogenic groups in the Amoris and Interheart studies where cardiovascular events were used as endpoints (67, 98).
In paper III the apoB/apoA-I ratio remained associated to IMT in the full baseline cohort. However in paper IV the association was not significant. The only lipid measurement that came out as significantly associated to IMT was apoB. The reason for this incongruence is unclear, but may partly be explained by the increase in statin treatment that has given significantly lower levels of both apoB/apoA-I and apoB. Since the prospective part of the cohort is relatively small, subgroup analyses could not be performed. The prospective analyses of the full study-cohort will eventually give more conclusive answers.

The apoB and apoB/apoA-I ratio has been debated and several studies have been performed in smaller cohorts (99-102). The results of these studies were not conclusive. Measuring apoB or apoB/apoA-I has not been added in the Swedish national guidelines for diabetes care or cardiovascular disease. However, in a consensus document from the American Diabetes association and the American College of Cardiology Foundation for lipoprotein management in patients with cardiometabolic risk, has suggested treatment goals for apoB (103). Interestingly apoB/apoA-I ratio has been proven to be more associated to VAT compared to subcutaneous fat supporting the idea that VAT is metabolically active and atherogenic (104).

In a relatively healthy and middle-aged cohort of men and women with type 2 diabetes the rate of cardiovascular events is quite low. The main purpose with the CARDIPP project was to explore impact from commonly used and relatively new markers of atherosclerosis on the risk for CVD. To be able to draw conclusions within reasonable time after the launch of the study there was a need for intermediate endpoints. Hence PWV and IMT were chosen since they are non-invasive and well validated (58, 105). Eventually within the coming five to ten years there will be interesting data on hard endpoints as cardiovascular events and mortality in the CARDIPP cohort.

Since ultrasonographic measurement of IMT was used as an endpoint in all papers of this thesis, we added a study on IMT to earn deeper knowledge about the technique and the usefulness and possible drawbacks of IMT. Paper II is based on a methodological study of IMT. The main finding in this study was that there was a difference in the absolute values of IMT in the AA measured with two commonly used ultrasound techniques. This implicates that there is a need to use the same technique when comparing the impact of absolute values of IMT. In CARDIPP the B-mode technique in the CCA was used. According to our study and others, the B-mode technique in the CCA is a reproducible measure with low variability (106-110).

Obesity is an increasing health issue all over the world and obesity increases both morbidity and mortality (111) and the risk for developing type 2 diabetes (74). However BMI has showed to have limitations in risk assessment of disease. A very low BMI is as related to high morbidity and mortality as a very high BMI (112). The role of visceral fat and abdominal obesity has been shown to be a stronger predictor of all-cause mortality compared to subcutaneous fat (113). Visceral fat is a potent source for expression of pro-inflammatory, atherogenic cytokines and is closely related to levels of inflammatory markers (114). Recent studies have also shown that reducing the visceral fat improves endothelial dysfunction, which is an early predictor for cardiovascular disease (115).

There are many ways of measuring abdominal obesity, for example WC or waist to hip ratio (WHR). In Sweden, WC is the most commonly used measure of abdominal obesity. However WC can be measured in various ways and there is no consensus about which is the best measurement protocol (96).

In paper III and IV, we report that SAD was an independent predictor for increased arterial stiffness and a marker of low-grade inflammation.

In the cross-sectional study in paper III, with approximately the triple amount of subjects compared to paper I, there was also an association between SAD and IMT that was independent of BMI, low-grade inflammation and glycaemic control.

SAD has previously been showed to predict insulin resistance and levels of CRP in immigrant women from the middle-east (76) as well as in obese men (77) and that SAD is closely related to visceral adiposity (116). However in paper III and IV, the difference between WC and SAD was quite subtle. In our study WC and SAD correlated at $r=0.83$, $p<0.001$. This implicates that both measurements are in most cases equivalent and measuring abdominal obesity is an important and clinically feasible tool in predicting the individual patient's risk of developing CVD.

Methodological considerations

Study design

Paper I, II and III are based on cross-sectional observational studies. This has the advantage of describing the clinical reality in this cohort. The only excluding criteria in the CARDIPP study was inability to understand Swedish and severe disease with short expected survival. Subjects with previous MI or stroke were not excluded. The major drawbacks of the design are that definitive conclusions on causality and effects cannot be drawn. The results from these studies needs to be confirmed in future prospective research in more controlled settings.
In paper IV the results were investigated prospectively in 255 subjects, approximately one third of the total CARDIPP cohort. This relatively small number of subjects precludes subgroup analyses and the result needs to be confirmed and analyzed with subgroup stratification in larger cohorts.
The choice of variables studied in paper III and IV were not random. We chose lipids, markers of inflammation and abdominal obesity in the light of previous knowledge on the pathogenesis of atherosclerosis.

Representativity of the study population

The CARDIPP study was planned to give a representative picture of middle-aged patients with type 2 diabetes in the south of Sweden. The study subjects were recruited from healthcare centers localized in urban and rural areas, as well as from areas with both high and low socioeconomic statuses. The aim, when planning the study, was to include all patients with type 2 diabetes in the age-span 55-65 at the selected health care centers excluding as few subjects as possible according to criteria previously described. However the coverage of patients did differ from center to center due to local conditions. Considering the size of the study with a relatively large number of subjects, we believe that the cohort is representative for middle-aged men and women with type 2 diabetes in Swedish primary care.

There were considerably more men compared to women included in the CARDIPP study. The main reason for this phenomenon could probably be explained by the fact that there are more men compared to women diagnosed with type 2 diabetes, and men in general tend to develop type 2 diabetes at an earlier age compared to women. The relatively young age of the study cohort was chosen for different reasons. Firstly; to be able to find markers of subclinical disease, we wanted to include relatively healthy subjects. Hopefully results from the CARDIPP study will identify markers of subclinical disease that will be useful in clinical practice, to individually identify subjects at high risk of developing CVD and adjust preemptive actions and treatment. Subclinical organ damage could not have been studied in an older cohort where the majority would already have developed cardiovascular disease. Secondly; to make the best use of all recourses invested in the study we wanted a cohort that can be followed prospectively for many years.

Statistical analyses

Most results in this thesis are based on correlation analyses and multiple linear regression analyses. The correlation coefficients were generally low and hence also the coefficients of determination. This could in some ways be explained by the fact that CVD are caused by a complex of diseases with multifactorial causes and pathogenesis. One single risk factor only explains a small piece of the puzzle. The low values of the correlation coefficients are better understood when compared to the even lower ones.

Clinical implications

ApoB and apoA-I are measured by direct techniques with coefficient of variation (CV) errors < 5%, while LDL is in most cases calculated according to the Friedewald formula which leads to larger errors than with directly measured apoB and apoA-I. Furthermore, triglyceride values usually have a larger biological variation and too high values invalidate the use of the formulae for calculation of LDL (117). However there are methods for direct measurement of LDL and HDL but not in use at any larger scale in Sweden. The measurements of apoB/apoA-I can be made in a non-fasting state, which is not the case for lipids or lipoproteins. Another advantage over conventional LDL measurements is that the methods for apoB/apoA-I are internationally standardized with a universal scale.

The main reason for using SAD in clinical practice is that SAD is a clinically feasible measurement with higher reproducibility compared to WC (118-119), which gives a good approximation of the atherogenic visceral fat. Measuring abdominal obesity can easily be used in clinical care when examining the patient. It serves as one piece of the puzzle when adding up the individual patient's risk of future organ damage and CVD. In addition to this it can give positive feed-back to the individual patient to adhere to physical exercise, since abdominal obesity often is reduced by exercise even though the weight might be unchanged (120).

In Sweden, patients with type 2 diabetes are treated in primary care by general practitioners and nurses especially educated in diabetes care. Patients with type 2 diabetes represent a group of resource demanding, aging patients with a lot of co-morbidity. There is a need to perform studies in primary care by primary care physicians and nurses in order to use feasible tools and markers in the context of primary care and the complex patient.

Future research

To once and for all decide whether or not to use apolipoprotein measurements in clinical practice, it is necessary to perform randomized clinical trials comparing target levels for apolipoproteins and conventionally measured lipids.

The value of measuring abdominal obesity needs to be further studied prospectively to get a better understanding of which is the best method of measurement and its value in cardiovascular risk-assessment alone or included in commonly used risk-assessment scores.

CONCLUSIONS

- There were significant associations between apoB and the apoB/apoA-I ratio and carotid IMT, in middle aged patients with type 2 diabetes. The association was independent of conventional lipids and lipoproteins, CRP, glycaemic control and the use of statins.

- We obtained adequate IMT readings from the carotid artery as well as the abdominal aorta using two commonly used B-mode and M-mode techniques. B-mode technique seemed to show less variability especially in the abdominal aorta. More importantly, the two techniques measured different IMT thickness in the aorta.

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- SAD and WC are feasible measures of obesity that provides information on inflammation, atherosclerosis and arterial stiffness in type 2 diabetes. However, SAD was somewhat more robustly associated to subclinical organ damage compared with WC.

- ApoB and CRP, but not LDL-cholesterol predicted subclinical atherosclerosis measured as increased IMT after four years. Furthermore, SAD was more independent in predicting arterial stiffness measured over time, compared with WC, in middle-aged men and women with type 2 diabetes.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Typ 2-diabetes är en folksjukdom som blir vanligare över hela världen. Oftast har sjukdomen ett smygande debut och den drabbade har inga symtom tidigt i förloppet. Diabetes typ 2 är en kronisk sjukdom och personer som drabbas har betydligt ökad sjuklighet och dödlighet jämfört med friska individer. Främsta dödsorsaken är hjärt-kärlsjukdom och personer med typ 2-diabetes har åtminstone fördubblad risk att drabbas av hjärtinfarkt och stroke och sjukdomen är oftast mer aggressiv hos dessa patienter.

Hjärt-kärlsjukdom orsakas av åderförkalkning, så kallad ateroskleros och även en tilltagande stelhet i kärlen. Utvecklingen av ateroskleros är en komplicerad sjukdomsprocess som startar relativt tidigt i livet. Flera faktorer som blodfetter och inflammation spelar stor roll. För att kunna påbörja och individuellt anpassa förebyggande behandling och åtgärder är det viktigt att ha kliniskt lättillgängliga och användbara riskmarkörer för hjärt-kärlsjukdom.

Traditionellt har man använt mått på blodfetter, kolesterol, och BMI, som ett mått på övervikt och fetma, som riskmarkörer. Studier har visat att bukfetma är mer relaterat till utvecklingen av typ 2-diabetes och hjärtkärlsjukdom jämfört med BMI. Fettväven inne i buken är mer metabolt aktiv än underhudsfett och leder även till låggradig inflammation. Hittills har man mätt midjeomfång för att mäta bukfetma men nyligen introducerades mätning av bukhöjd som ett bättre mått på fett i buken.

Syftet med den här avhandlingen var att utvärdera traditionellt använda och nya riskmarkörer för hjärtkärlsjukdom hos medelålders män och kvinnor med typ 2-diabetes. Tre av fyra ingående arbeten baseras på en studie i primärvården som genomfördes på 25 vårdcentraler i Östergötland och Jönköping. 761 medelålders patienter med typ 2 diabetes genomgick en utökad årlig kontroll hos diabetessköterska och mätning av tjockleken på de två innersta lagren, intima-media (IMT), av kärlväggen med ultraljud som ett mått på subklinisk ateroskleros och pulsvåghastighet (PWV) som mäter kärlstelheten.

Fyra år efter den första undersökningen utfördes undersökningarna igen och resultaten från första årets återundersökningar presenteras i delarbete IV
Delarbete I visade att apoB och apoB/apoA-I kvoten var associerat till IMT oberoende av blodsockerkontroll, inflammation, blodtryck och behandling med blodfettsänkande medicin (statin). Konventionella kolesterolvärden och andra riskfaktorer var inte associerade till subklinisk ateroskleros.

Delarbete II baseras på en jämförande studie av två olika ultraljudstekniker, B-mode och M-mode, för att mäta IMT i halspulsådern och i stora kroppspulsådern (aorta) i buken. Metoderna visade olika resultat i buk aorta. Vilket talar för att man bör vara noga med att använda samma metod när man jämför absoluta mått på IMT.

Delarbete III visade att både bukhöjd och midjemått var associerade till inflammation, subklinisk ateroskleros och kärlstelhet. Justerat för BMI, systoliskt blodtryck och blodsockerkontroll, kvarstod enbart associationen mellan buk höjd och kärlstelhet.

Delarbete IV baserades på en prospektiv uppföljande studie, fyra år efter de första undersökningarna. ApoB och CRP, men inte LDL-kolesterol predikterade IMT. Buk höjden var en mer oberoende prediktor för kärlstelhet jämfört med midjemåttet hos medelålders män och kvinnor med typ 2-diabetes.

Sammanfattningsvis tycktes apoB och apoB/apoA-I kvoten vara starkare relaterade till subklinisk ateroskleros jämfört med konventionellt uppmätta blodfetter och andra riskmarkörer. Bukhöjden jämfört med midjemått var mer prediktiv avseende utvecklingen av ateroskleros och kärlstelhet hos medelålders patienter med typ 2-diabetes.

ACKNOWLEDGEMENTS

I would like to express my warmest gratitude to everyone who helped me and contributed to this thesis. Especially thanks to:

Professor **Carl Johan Östgren**, my supervisor, who got me started with a small project that eventually grew. Somehow you made me think that research is really fun and interesting. Your encouragement and high expectations made this work possible!

Professor **Toste Länne,** my co-supervisor, who patiently guided me in cardiovascular physiology and explained the same things over and over until I finally (?) got the picture.

Professor **Fredrik Nyström,** my co-supervisor, who has scrutinized every line in our manuscripts. I appreciate that you found both big and small errors and asked questions that made me think in new directions.

Anna-Karin Schöld, at FoU enheten, for your friendly co-operation and all the hard work with the CARDIPP database and freezers.

The **CARDIPP project group** for good collaboration between departments and across professions.

My **colleagues** and **friends** in Ödeshög, Skänninge and Ekholmen Health Care Centers.

My children, **Hanna, Jacob** and **Rebecca** and their father **Fredrik** for giving me the time and space.

These studies were supported from grants from the Medical Research Council of Southeast Sweden, the Center for Medical Image Science and Visualization (CMIV), Linkoping University, Futurum, GE Healthcare and the Swedish Heart-Lung Foundation and the Swedish Research Council Grant 12661.

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