Atherosclerosis in 64-year-old women with diabetes mellitus and impaired glucose tolerance

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A doctoral thesis at a university in Sweden is produced either as a monograph or as a collection of papers. In the latter case, the introductory part constitutes the formal thesis, which summarises the accompanying papers. These papers have already been published or are in manuscript at various stages (in press, submitted or in manuscript).

This thesis is dedicated
to the women who have participated
in the DIWA-study.

"Måttet på livets krav är endast din egen kraft.
Och din eventuella bragd – att inte ha deserterat."

Dag Hammarskjöld, Vägmärken 1963

Abstract

Atherosclerosis in 64-year-old women with diabetes mellitus and impaired glucose tolerance

The incidence of obesity and type-2 diabetes (t2D) is rising around the world. T2D is accompanied by an increased risk of macrovascular, atherosclerotic diseases. The timing of the disease process and exact mechanism underlying the association between diabetes and atherosclerotic disease are not known. High-resolution B-mode ultrasound is a non-invasive technique to measure very early atherosclerotic disease as the intima-media thickness (IMT) and to assess occurrence, size and echogenicity of atherosclerotic plaques in superficial arteries such as the carotid arteries. Remodelling, i.e a change in vascular diameter in response to atherosclerosis may also be examined.

The overall aim of this thesis was to examine the occurrence of early atherosclerosis in t2D and impaired glucose tolerance (IGT). Our hypothesis was that there is a gradual enlargement of IMT and an increased occurrence of plaques, especially echolucent plaques, with worsening glucose tolerance, accompanied by a remodelling that might be visible already in subjects with IGT.

In order to summarise current knowledge a systematic reviews were made in order to identify cross-sectional studies using the ultrasound method. The differences between IMT in t2D or IGT and control subjects were calculated. Meta-analysis using random-effects model was used to calculate summary measures. In a cross-sectional study, the entire cohort of 64 years old women in Göteborg were invited to take part in a screening examination. Of these, 2595 participated and underwent anthropometric measurements and an oral glucose tolerance test (OGTT) that was repeated within two weeks. Ultrasound examinations were made in a cohort of women with known (n=99) and new diabetes (n=106), IGT (n=205) and NGT (n=188).

In the systematic review of 23 studies we found that t2D was associated with an 0.13 mm increase in IMT compared to controls. There was a considerable heterogeneity between the studies that complicated the interpretation of the meta-analysis. This difference can be interpreted as if the diabetic patients were ten years older than the control group and that they had an elevated risk for stroke and myocardial infarction. In patients with IGT, the increase in IMT was about 25% of that observed in diabetes.

The screening examination showed that 10% of the women had diabetes of whom half were newly detected. Without repeated OGTTs 37% of the new diabetes would have been missed. The women with t2D had larger IMT and plaqueareas compared to women in IGT and NGT groups. The plaques were most frequenty echolucent. In the IGT group, no increase in atherosclerosis was observed, but the result was within the confidenceinterval of the meta-analysis, indicating that IGT is associated with a small increase in IMT in the CCA (0.03mm). Carotid vascular remodelling with enlargement in IMT and lumen diameter had however occurred already in the women with new t2D. This remodelling process was seen only in women with atherosclerotic plaques and was not associated to t2D per se.

In conclusion, already in screening detected diabetes sub-clinical atherosclerosis with IMT enlargement and occurrence of plaque with vascular remodelling are observed, indicating that a powerful intervention must be initiated in such cases.

Keywords; systematic review, atherosclerosis, type-2 diabetes, IMT, vascular remodelling, IGT.

List of publications

This thesis is based on the following papers, which will be referred to in the text by their roman numerals I-IV.

I. **Carotid artery intima-media thickness in patients with Type 2 diabetes and impaired glucose tolerance: a systematic review**
   Brohall G, Odén A, Fagerberg B
   Diabetic medicine 2006;23:609-16

II. **Prevalence of diabetes mellitus and impaired glucose tolerance in 64-year-old women. Experiences of using repeated oral glucose tolerance tests**
   Brohall G, Behre CJ, Hulthe J, Wikstrand J, Fagerberg B
   Diabetic Care 2006;29:363-67

III. **Atherosclerotic plaques and carotid remodelling in a population sample of 64-year-old women with known and newly diagnosed diabetes**
   Brohall G, Schmidt C, Prahl-Abrahamsson U, Holdstedt P, Hulthe J, Gustavsson T, Wikstrand J, Fagerberg B
   Submitted

IV. **Impaired glucose tolerance and subclinical atherosclerosis in carotid arteries: Results from a study of 64-year-old women and a meta-analysis of available studies**
   Brohall G, Schmidt C, Behre CJ, Hulthe J, Wikstrand J, Fagerberg B
   Submitted

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Abbreviations

ADA   American Diabetes Association

BMI   Body Mass Index

CCA   Common Carotid Artery

CI   Confidence Interval

CAD   Coronary Artery Disease

CVD   Cardiovascular disease

ECG   Electrocardiogram

DM   Diabetes Mellitus

FBG   Fasting Blood Glucose

FH+   Family history of diabetes in any of the parents

FH-   No family history of diabetes in any of the parents

GAD   Glutamic Acid Decarboxylase

HDL   High Density Lipoprotein

IFG   Impaired Fasting Glucose

IGT   Impaired Glucose Tolerance

IMT   Intima Media Thickness

LDL   Low Density Lipoprotein

NGT   Normal Glucose Tolerance

NGTm   NGT, matched

NGTr   NGT, random

OGTT   Oral Glucose Tolerance Test

SD   Standard Deviation

TG   Triglyceride

T2D   Type 2 Diabetes

WHR   Waist Hip Ratio

Introduction

Recently UN declared that diabetes mellitus is a chronic, debilitating and costly disease associated with severe complications, which poses severe risks for families, Member States, and the entire world (General Assembly – 61st session – Agenda 113). Obesity and physical inactivity are the main underlying causes. Diabetes is a strong risk factor for development of atherosclerosis and cardiovascular diseases [*Meigs* 2002, *Wei* 1998]. Even when adjusted for conventional risk factors, diabetic individuals still exhibit a two- to fourfold increased relative risk in comparison to non-diabetic subjects [*Stamler* 1993]. This risk is even higher among diabetic women compared to non-diabetic women [*Niskanen* 1998]. It is a huge challenge to prevent this threat to public health and to better understand the underlying disease mechanism.

Onset and diagnosis of diabetes mellitus

Diabetes mellitus has several causes, ranging from autoimmune disease processes (type-1) to a combination of insulin resistance and pancreatic beta-cell insufficiency as a result of different mechanisms (type-2). There is also an overlap between type-1 and type-2 diabetes [*Groop* 1998, *Groop* 1988, *Tuomi* 1999]. Environmental and life style factors are important, especially obesity and a sedentary life style, that explain the steady raise in the incidence of type 2 diabetes. The current concept is that there is a gradual development of glucose intolerance with insulin resistance, and deteriorating beta-cell function leading to impaired glucose tolerance and finally diabetes[*Stumvoll* 2005]. The diagnosis is arbitrary as shown by the changing cut-off levels for definition of diabetes [*WHO* 1999]. It should be kept in mind that the diabetic complications that have been used in the attempt to decide the blood glucose level that is associated with increased risk are related to micro- and not macrovascular diseases [*Engelgau* 1997, *McCance* 1997]. OGTT is the golden standard in diagnosing diabetes mellitus. According to the latest guidelines, fasting glucose values have been

harmonised with OGTT values [*ADA* 2006, *Engelgau* 1997, *Finch* 1990, *WHO* 1999]. However, the OGTT is characterised by a high degree of intraindividual variability. [*Feskens* 1991, *McDonald* 1965, *Riccardi* 1985].

Type -2 diabetes is the most common form of diabetes and a worldwide epidemic increase is expected during the first quarter of the 21th century [*Amos* 1997, *King* 1998]. This increase is, however not so apparent in the Scandinavian countries [*Drivsholm* 2001, *Eliasson* 2002, *Jansson* 2007, *Vilbergsson* 1997] even if overweight is becoming more common [*C Berg* 2005].

Figure 1 demonstrates the prevalence of diabetes mellitus in European countries [*Ryden* 2007]. As seen in the figure there is a steep rise in diabetes prevalence after the age of 50 in men and after the age of 60 in women. For each decade the prevalence increases and this is most explicit in women. Other data indicate that the onset of type-2 diabetes occurs several years before clinical diagnosis [*Harris* 1992].
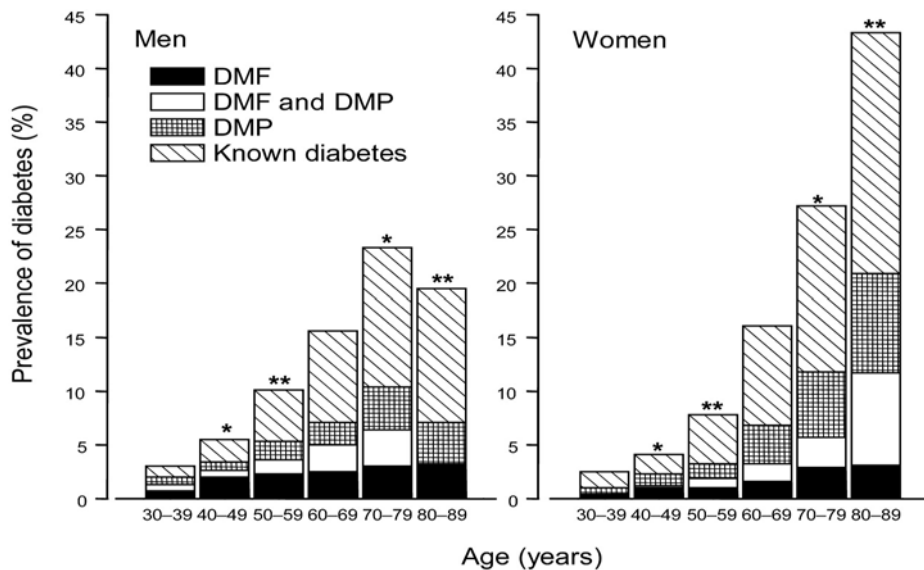

Figure 1. Age-and gender-specific prevalence of diabetes (DM) in 13 European population-based cohort included in the DECODE study. DMF = DM determined by FPG≥7.0 mmol/l and 2 h plasma glucose < 11.1 mmol/l. DMP = DM determined by 2 h plasma glucose ≥ 11.1 mmol/l. (L.Rydén 2007) Reprinted with permission from European Heart Journal.

Impaired glucose tolerance (IGT) was defined in 1979 in USA by the National Diabetes Data Group as a state of disturbed glucose metabolism. IGT is viewed as a transitional stage in the development from normal glucose tolerance to type-2 diabetes. This concept that IGT is a road to diabetes is supported by results from two studies showing that in subjects with IGT, life style treatment with weight reduction and increased physical exercise prevents the transition to diabetes [*Knowler* 2002, *Lindstrom* 2003, *Lindstrom* 2006, *Tuomilehto* 2001].

Impaired glucose tolerance (IGT) often coexists with a cluster of metabolic abnormalities such as; hypertension, central obesity, dyslipidemia, insulin resistance [*Kylin* 1923, *Reaven* 1988]. It is known that IGT is associated with a risk of future cardiovascular disease (CVD) [*Coutinho* 1999, *Fuller* 1980, *Meigs* 2002]. It is not clear if high "non-diabetic" glucose levels are independently related to atherosclerosis [*Bonora* 1999] Data indicate, however that IGT is a risk factor for CVD even when IGT is not followed by the subsequent development of diabetes [*Qiao* 2003].

The prevalence of IGT varies between different populations, just as the prevalence of diabetes. In a large population-based study in Sweden the prevalence of IGT among women at the age of 60 was 11% [*Lindahl* 1999].

(Against this background it is of interest to know the prevalence of IGT and diabetes in high risk groups such as women in late middle-age using repeated OGTT as the most sensitive diagnostic method.)

Atherosclerosis and diabetes

Atherosclerosis is an insidious process with specific predilection sites in the arterial tree. Atherosclerosis is found in young adults and increases steadily in parallel with aging [*Dahl-Jorgensen* 2005, *Zieske* 2002]. Manifest atherosclerotic diseases typically start in middle age and increase steeply in parallel with aging as shown in the Oxvasc-study [*Rothwell* 2005]. As previously mentioned diabetes is associated with an increased cardiovascular risk, also when

conventional risk factors have been taken into account. Many patients already have atherosclerotic disease when the diagnosis of diabetes is made [*Uusitupa* 1985].

Mechanisms involved in atherosclerosis

The mechanisms of atherogenesis in atherosclerotic disease are not fully known. The most popular hypothesis explaining the atherosclerotic process is the response to retention hypothesis [*Camejo* 1998, *Carew* 1984, *Falcone* 1984, *Schwenke* 1989]. The infiltration and retention of LDL-cholesterol in the arterial intima initiate an inflammatory process in the arterial wall where the modification of LDL through oxidation or other mechanisms take place [*Leitinger* 2003, *Skalen* 2002]. This process includes the activation of endothelial cells preferentially at sites of hemodynamic strain in the artery wall [*Leitinger* 2003] [*Nakashima* 1998]. A critical step in the development of atherosclerosis is taken when cytokine and cell adhesion molecules, produced in the inflamed intima, induce the monocytes to enter the plaque and differentiate into macrophages [*Smith* 1995]. The macrophages is thereafter further transformed to cholesterol-laden foam cells [*Haberland* 1984].
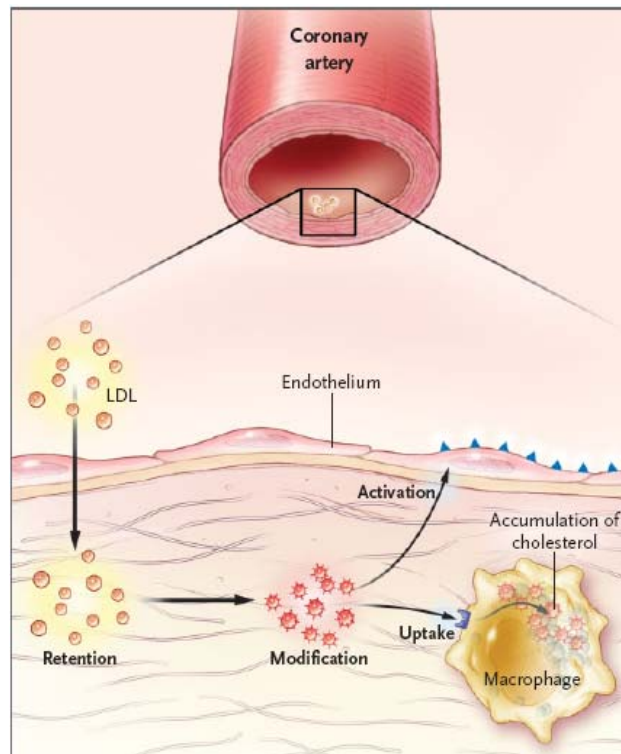
Figure 2. LDL infiltrates the artery and is retained in the intima, particularly at sites of hemodynamic strain. The modified LDL particles are taken up by macrophages, which evolve into foam cells. (G Hansson.2005) Reprinted with permission from N Eng J Med.

Accumulation of macrophages, migration and proliferation of smooth-muscle cells and formation of fibrous tissue lead to further enlargement and restructuring of the lesion. The lesion becomes covered by a fibrous cap that overlies a core of lipid and necrotic tissue. An advanced, complicated lesion is created [*Ross* 1999].

The hallmark of atherosclerosis is the fibrous plaque. Such plaque increase by age and among 60 year old subjects more than half have plaques in the carotide arteries [*Joakimsen* 1999]. In the further process some lesions develop into vulnerable plaques which in general are characterised by large lipid pool, a thin overlying cap and an abundance of inflammatory cells in the shoulder regions of the plaque [*Mann* 1996].
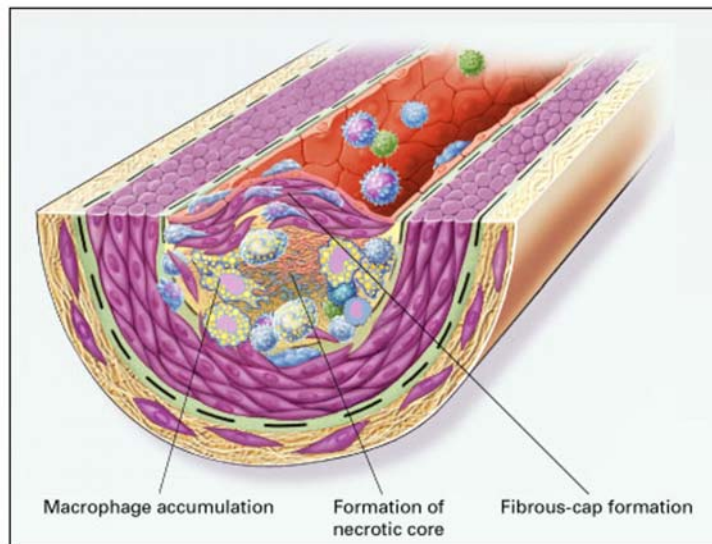
Figure 3. The formation of the vulnerable plaque with the lipid-core and the thin fibrous cap. The fibrous cap covers a mixture of leukocytes, lipids and debris, which may form a necrotic core.(Ross, R 1999) Reprinted with permission from N Eng J Med.

Some of these plaques rupture or have proportions that lead to thrombosis, resulting in end organ damage[*Fuster* 1990, *Rothwell* 2005]. Plaque vulnerability and plaque rupture are also mechanisms related to symptomatic plaques in the carotid arteries (*Rothwell,* 2005).

In the coronary circulation other mechanisms than plaque rupture are involved in acute coronary syndrome.

Ultrasound diagnosis of atherosclerosis in the carotid arteries

Non-invasive ultrasound techniques have been developed, enabling studies of atherosclerosis during the long clinically silent phase. B-mode ultrasound is a method that allows quantification and qualification of atherosclerosis in terms of intima-media thickness (IMT) and the occurrence and characteristics of atherosclerotic plaques [*Gronholdt* 1997, *Schmidt* 1999]. The lumen-intima and media-adventitia echoes in carotid ultrasound images were first identified by

Pignoli and co-workers [*Pignoli* 1986]. Our group also showed that the far wall IMT and lumen diameter could be measured in a valid way [*Wendelhag* 1991].

Several large epidemiological studies have demonstrated an association between carotid artery IMT and CVD risk factors in both men and women [*Bonithon-Kopp* 1991, *Mathiesen* 2001, *Mitsuhashi* 2002, *O'Leary* 1999]. Studies have also showed a relationship between carotid IMT and the angiographic presence of coronary artery disease (CAD) [*Hulthe* 1997, *Wofford* 1991] and the number of coronary vessels with lesions [*Wofford* 1991]. In addition, prospective studies have demonstrated a relationship between carotid IMT and future CAD [*Bots* 1997, *Chambless* 1997, *Hodis* 1998, *O'Leary* 1999]

The number of plaques and the degree of stenosis also predicts risk for CVD events [*Hollander* 2002, *Mathiesen* 2001]. Plaque occurrence in the carotid arteries has shown a positive correlation with coronary atherosclerosis [*Hulthe* 1997] and stroke [*Hollander* 2002]. In subjects with stabile angina, plaques are more common in patients with subsequent CVD-death or myocardial infarction compared to event-free subjects [*Held* 2001]. Studies have shown that age, systolic blood pressure, cholesterol and smoking are associated with plaques [*Ebrahim* 1999].

However, most atherosclerotic plaques never lead to clinical disease and the development of vulnerable plaque with plaque rupture. There is a need to identify vulnerable plaques and the ultrasound technique can be used for this purpose. Carotid plaques can be classified into those with low-level echoes (echolucent plaques) and those with medium and high-level echoes (echogenic plaques) [*Gray-Weale* 1988, *Reilly* 1983]. In the carotid artery the echolucent plaques seem to be lipid-rich, whereas echogenic plaques have a high content of fibrous tissue and calcifications as assessed by ultrasound related to histology [*El-Barghouty* 1996, *Gronholdt* 1997].

1 **2**

3 **4**
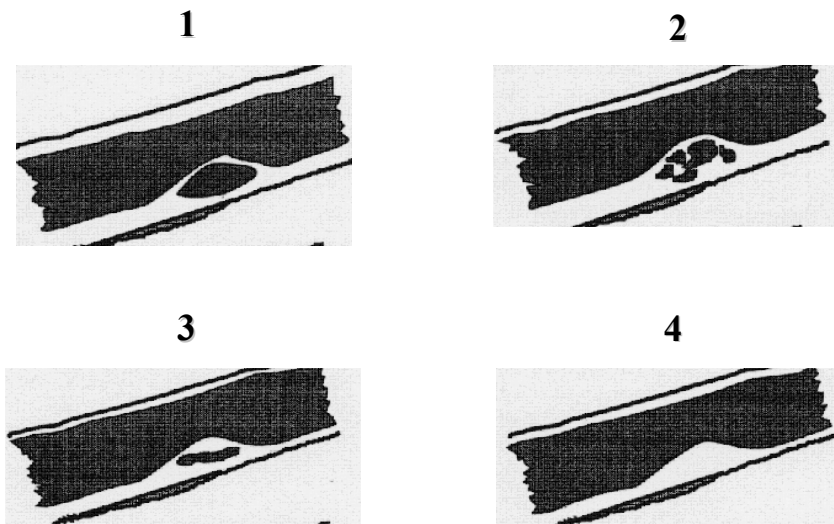
Figure 4. Plaque echogenicity; **1**) Dominantly echolucent with thin echogenic cap. **2**) Substantially echolucent with small areas of echogenicity. **3**) Dominantly echogenic lesions with small areas of echolucency. **4**) Uniformly echogenic lesions.

Echolucent plaques are associated with cardio-vascular events, silent coronary ischemia [*Belcaro* 1993, *Geroulakos* 1993] and also with ischemic cerebrovascular events [*Honda* 2004, *Mathiesen* 2001, *Schmidt* 2003]. Also the degree of echolucency in non-stenotic plaques is related to the future risk of CVD[*Honda* 2004].

(More knowledge is needed to better understand the involved mechanisms behind the development of vulnerable plaques and to identify the risk factors correctly. There is also a need for better imaging and information regarding the prevalence of echolucent plaques in t2D.)

Diabetes, IGT and atherosclerosis.

Atherosclerotic disease is a major complication of diabetes mellitus. A number of known risk factors for coronary disease such as hypertension and abnormal lipids are also more common in diabetics than the general population. However, no more than 25% of the excess coronary atherosclerotic from diabetes can be

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attributed to these known risk factors [*Keaney* 2000]. The mechanism behind the relation between diabetes and atherosclerosis is not fully known. Oxidative stress, through the production of reactive oxygen species (ROS) has been proposed as the root cause underlying insulin resistance, type 2 diabetes and vascular complications [*Ceriello* 2004]. Apart from inflammation and diabetic dyslipidemia [*Haffner* 2003], the impaired glucose metabolism and "advanced glycation end products" (AGE) [*Bansilal* 2007, *Brownlee* 1992, *Cipollone* 2003] might contribute to the atherosclerotic process.

There are many studies indicating that type-2 diabetes mellitus (t2D) is associated with thicker intima-media in the carotid artery [*Folsom* 1994, *Wagenknecht* 1998]. Some studies have, on the other hand, not showed any significant difference between t2D and controls [*el-Barghouti* 1997, *Tuomilehto* 1998].

Studies examining whether IGT is associated with increased carotid IMT show inconsistent results [*Ishizaka* 2003, *Temelkova-Kurktschiev* 2000, *Wagenknecht* 1998]. However, all these studies were based on one single OGTT, and it is known that glucose tolerance is normal at a second OGTT in half of the cases [*Riccardi* 1985].

(Hence, a more strict diagnosis of IGT using repeated OGTT may show more consistent association with IMT. Taken together it is unclear when the atherosclerotic process starts in relation to the level of glucose intolerance.)

Remodelling

Remodelling is a process when the artery geometry changes as a response to the atherosclerotic process [*Pasterkamp* 2002, *Ward* 2000]. In histological sections of coronary arteries Glagov et. al., demonstrated that coronary arteries enlarge in response to the development of atherosclerotic plaques [*Glagov* 1987]. According to the analyses of Glagov et al the compensatory remodelling process maintained or even enlarged luminal dimensions during early atherosclerosis.

These investigators also reported that the capacity for compensatory enlargement diminished as plaques reached a cross-sectional area stenosis of ~ 40 % and plaque growth beyond this point resulted in a reduction of luminal area.(Figure 5) [*Glagov* 1987].

Glagov S et al, N Engl J Med 1987
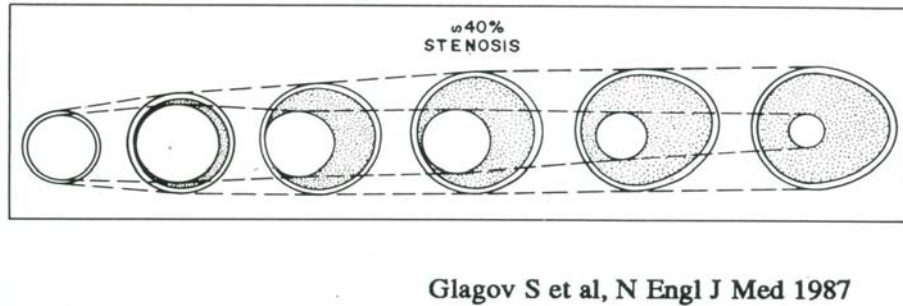
Figure 5. In early atherosclerosis a compensatory remodelling process is maintaining or even enlarging the lumen dimension. (Glagov et al, N Eng J Med 1987)

It has been suggested to take lumen diameter and remodelling into account when examining the importance of IMT as a surrogate marker of atherosclerotic disease [*Bots* 2005]. One previous study has indicated that remodelling occurs among patients with known diabetes but the relation to plaques is unknown [*Henry* 2004]. Artery size is also related to other factors. Male sex, height, weight and blood pressure are positively associated with carotid artery lumen diameter [*Bonithon-Kopp* 1996]. An increased IMT may also be a non-atherosclerotic response to increased stress in order to keep the wall tension normal [*Bots* 2005].

(There is a lack of knowledge regarding the onset of atherosclerosis assessed as increase in IMT, plaque occurrence and remodelling in relation to the gradual deterioration of glucose tolerance, leading to diabetes.)

Aims of the study

The main aim of this thesis was to examine the occurrence of early atherosclerosis in type 2 diabetes (t2D) and impaired glucose tolerance (IGT).

The specific aims were:

I. To perform a systematic review and a meta-analysis of the literature in order to clarify whether t2D and IGT are associated with increased carotid artery IMT, and to relate differences in IMT to clinical correlates such as age and risk for myocardial infarction and stroke. (Paper I)

II. To describe the prevalence of diabetes mellitus and IGT in a cohort of 64-year-old women in Gothenburg, Sweden and to examine the variability and practical use of the OGTT in the screening for IGT and diabetes. (Paper II)

III. To investigate the occurrence of echolucent plaques in diabetic patients; to examine if carotid artery remodelling in women is present already at the diagnosis of new t2D, and if it is similar to that in known t2D, and to investigate if non-atherosclerotic remodelling may occur in t2D. (Paper III)

IV. To examine if IGT, as diagnosed by double OGTT, is associated with sub clinical carotid atherosclerosis assessed as IMT and occurrence of echolucent plaques. A post-hoc aim was to clarify if carotid IMT is thicker in groups of IGT compared to groups of NGT by performing an up-dated meta-analysis, adjusted for differences in age. (Paper IV)

Materials and Methods

Paper I

Study design of the systematic review

Inclusion criteria

Inclusion criteria were 1) cross-sectional studies of patients with type 2 diabetes mellitus or impaired glucose tolerance (IGT), and controls; 2) use of the ultrasound method to examine the carotid artery IMT. Type 2 diabetes was defined by the authors concerned, and IGT was based on the WHO definition[*WHO* 1999]. Both epidemiological, cross-sectional studies based on random population samples and case-control studies were included. If a study was reported in more than one publication we could use data from other publications to obtain necessary information, but only one report was included.

Search strategy

MEDLINE, EMBASE, and Cochrane were the primary sources of identification of included studies. We started our searches with the keywords "diabetes", "impaired glucose tolerance", "atherosclerosis" and "intima-media". The searches spanned the previous 20 years. The references in the identified or related articles were manually reviewed in the search for other published studies. Articles in languages other than English were accepted. Personal contacts with researchers in the field were used to identify unpublished studies.

Data extraction

A data abstract form was created and two of the authors (GB, BF) independently extracted the requested information. The tabulated data included study design, recruitment and number of patients and control subjects, ethnicity, sex and age distribution, definition of diabetes and IGT and description of the ultrasound

method to measure carotid IMT. In case of discrepancy of assessments, consent was reached after discussion. The main outcome was carotid artery IMT in the different study groups. The analysis was restricted to studies which contained known numbers of participants and in which carotid artery IMT measurements were presented in figures. Attempts were made to obtain complementary data in studies lacking necessary data.

Updated review

In paper IV an updated meta-analysis of the difference between IMT in IGT and NGT was performed with the addition of the present and two new studies [*Mohan* 2006, *Zhang* 2006]. Adjustment for differences in age was also performed as age was higher in IGT than in NGT groups in seven studies[*Henry* 2004, *Ishizaka* 2003, *Keven* 1999, *Snehalatha* 2001, *Wagenknecht* 1998, *Zhang* 2006]. In previous studies it has been reported that an age difference of one year corresponds to a 0.01 mm difference in CCA IMT [*Hedblad* 2000, *Howard* 1993, *Le Gal* 2003]

Paper II-IV

Study population and study design

DIWA study
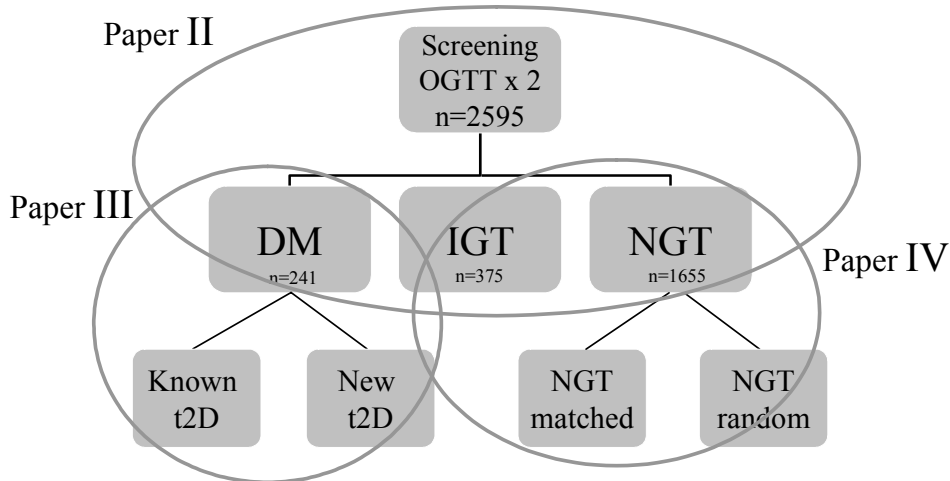
Figure 6. Overall design of the DIWA-study; paper II - the screening procedure; paper III - atherosclerosis in t2D; paper IV - atherosclerosis in IGT.

Inclusion and exclusion criteria

The inclusion criteria were 64-year-old woman registered at the County Register in Gothenburg Sweden. Exclusion criteria for all women were recent cancer disease, severe mental disorder, chronic inflammatory disease or other diseases demanding longer use of anti-inflammatory drugs. Women with drug addictions or other severe illnesses were excluded. Women who could not understand Swedish were also excluded. For women with normal glucose tolerance (NGT) additional exclusion criteria were coronary heart disease, previous stroke or

transient ischemic attack, intermittent claudication, treatment or need for treatment for hypertension or dyslipidemia.

The study was approved by the Ethical Committee of Sahlgrenska University Hospital. The subjects received both written and oral information before they gave their consent to participate.

Screening procedure

All the 64-year-old women identified through the County Register in Gothenburg were invited to take part in a screening that took place 2001-2004 and included women born 1937-1940.

In all, 4856 women were sent an invitation and reply form in which they accepted or did not accept participation in the screening examination. Those 2893 who accepted completed a brief questionnaire that was enclosed. Subject who in this first reply letter reported diseases according to exclusion criteria were excluded. As a reminder to non-responders a second letter was sent. An attempt was made to contact a sample of these non-responders for a telephone interview concerning their medical conditions including diabetes.

The screening examination included an oral glucose tolerance test (OGTT). Women with known diabetes treated with oral anti-diabetic drugs or insulin were directly included in the study and examined without preceding OGTTs, whereas women with diet treated diabetes and fasting blood glucose (FBG) < 7.5 mmol/l were examined with OGTTs. The OGTT was repeated within two weeks if impaired glucose tolerance (IGT) or diabetes was found.

Glucose tolerance groups

The women were stratified into three groups; type-2 diabetes, IGT and NGT. In the NGT group of 1651 women, 1231 women had no history of cardiovascular disease and were without medication for hypertension and hyperlipidemia. Out of this healthy group, two groups were recruited. The first group (NGTr) of 99

women was randomly selected from the entire NGT group. The second group (NGTm) consists of 96 women matched to the IGT group for body mass index (BMI) and waist-hip-ratio (WHR).

Sample size calculations

The preliminary sample size was calculated as the number of subjects necessary to include in order to show a significant difference in the common carotid artery (CCA) intima-media thickness (IMT) between women with type-2 diabetes and normal glucose tolerance ($\alpha < 0.05$ and $\beta = 0.20$). The assumptions were based on data obtained from a large population-based study of 3800 women 55-67 years old in Malmö Sweden using almost similar ultrasound technique (personal communication Hedblad). These Malmö women with NGT had a mean CCA IMT of 0.76 ± 0.13 mm and it was 0.04 mm higher IMT in the diabetes group. The calculations indicated that 200 women with type-2 diabetes and 200 with normal glucose tolerance would be included in the study.

Examinations

At both entry and at the ultrasound examination the subjects were examined with anthropometric measurements and blood pressure. The first visit to the Wallenberg Laboratory included completing of questionnaires, anthropometric measures, measurement of blood pressure, recording of ECG and examination with OGTT. The second visit, within a few weeks included the ultrasound examination.

Questionnaires

Self-administered questionnaires were used to obtain information on previous and present disease, current medication, smoking habits and family history of diabetes[*CM Berg* 2005].

Anthropometry

Body weight was measured in underwear on a balance scale to the nearest 0.1 kg and height to the nearest 1.0 cm. Waist and hip circumference were performed with the patient standing and in accordance with current guidelines. Waist-hip-ratio (WHR) and body mass index (BMI) were calculated. BMI was defined as weight in kilograms divided by the squared height in meters.

Blood pressure

Blood pressure was measured in the right arm with the patient in supine position, using a cuff of appropriate size after at least 5 minutes of rest. The mean of two recordings was used.

Oral glucose tolerance test (OGTT)

A 75g OGTT (WHO) was performed in the morning (before 11 a.m.), fasting- and 2-h post load capillary blood glucose were measured. The participants had been asked to fast overnight, to avoid heavy physical activity during the previous day and to avoid smoking in the morning before the test. Women who reported a current infection had the examination postponed two weeks. Women fulfilling the criteria for DM or IGT were re-examined within 2 weeks with a repeated OGTT. If fasting glucose was in the diabetic range at the second examination, OGTT was not performed.

Ultrasound measurements of the carotid arteries

Examination was performed with an ultrasound scanner (Acuson Sequoia 512 Siemens, Mountain View, CA) with a linear 8-MHz transducer aperture of 42 mm. An electrocardiographic signal (lead II) was simultaneously recorded to synchronize the image capture to the top of the R wave in order to minimize variability during the cardiac cycle. The right and left carotid arteries were scanned at the level of the bifurcation. At the position of the thickest part (visually judged), frozen longitudinal images of the common carotid artery

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(CCA) and the carotid bulb were recorded. A short real-time sequence was also recorded to assist in interpreting frozen images.

The ultrasound images were analyzed in an automated analyzing system, based on automatic detection of the echo structures in the image but with the option to make manual corrections by the operator [*Wendelhag* 1997]. IMT was defined as the distance from the leading edge of the lumen-intima interface of the far wall to the leading edge of the media-adventitia interface of the far wall. The measurement of IMT in the carotid artery was made in two separate segments (Figure 7): along a 10-mm-long segment in the CCA, and along a 10-mm-long segment distal to the beginning of the carotid artery bulb, (i.e. where the echoes from the near and far walls are no longer parallel).
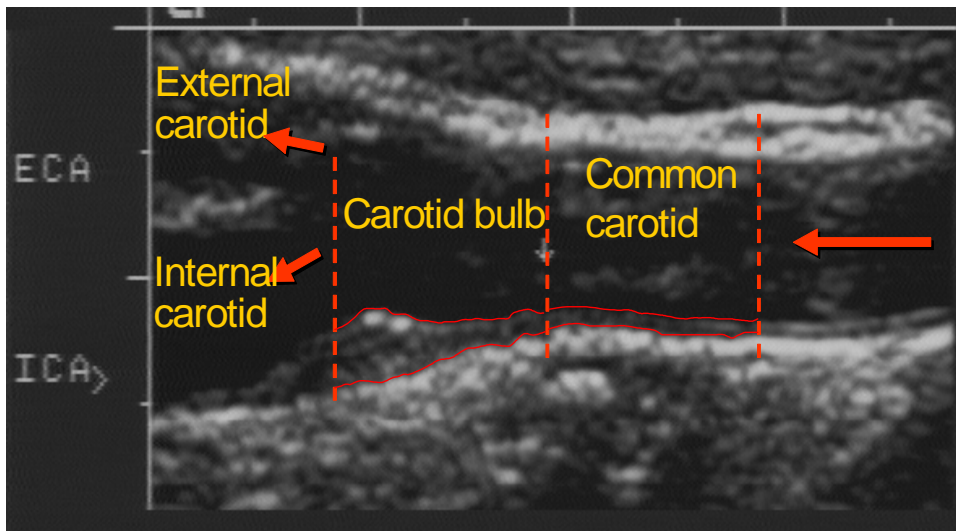
Figure 7. Ultrasound picture of carotid artery.

The computer program calculated the mean thickness of the intima-media complex of the far wall. Lumen diameter was defined by the distance between the leading edges of the intima-lumen interface of the near wall and the lumen-intima interface of the far wall. A composite measure of IMT in the carotid artery was calculated as the mean IMT in the CCA and the carotid bulb from the right

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and left side. The interobserver variability for measurement of IMT in the CCA and the carotid artery bulb is 5.3% and 6.0%, respectively, and 7.6 % for the composite measure [*Schmidt* 1999].

The screening for plaques was performed over a larger area of the carotid arteries starting 40 mm proximally to the bulb and extending 10 mm in to internal carotid artery and external carotid artery, respectively. The right and left carotid arteries were scanned both longitudinally and transversely to assess the occurrence of plaques. A plaque was defined as a distinct area with an IMT >50% greater than neighbouring sites (visually judged). This analysis included plaques in the near wall as well as the far wall in both the carotid arteries. The total numbers of plaques were calculated. A computer program was used to calculate the area of each plaque and the total areas were computed. The assessment of plaque echogenicity was based on the version of classification proposed by Gray-Weale et al [*Gray-Weale* 1988], modified in the present study to two classes; 1) dominantly or substantially echolucent plaques, or 2) dominantly or uniformly echogenic. Two examiners simultaneously classified the plaque echogenicity, which has previously been shown to have good reproducibility (К=0.95) [*Schmidt* 2003]. Echogenicity was defined in each plaque and the ratio between the numbers of echolucent and all plaques was calculated.

Biochemical measurements

At the visit with ultrasound examination blood samples for biochemical analysis were collected and serum and plasma were frozen in aliquots at -70ºC within 4 hours.

Serum lipids

The cholesterol and triglyceride levels were determined by fully enzymatic techniques (Thermo Clinical Labsystems, Espoo, Finland). All analyses were performed on a Konelab 20 autoanalyser (Thermo Clinical Labsystems) at the Wallenberg Laboratory. High-density lipoprotein (HDL) was determined after

precipitation of apolipoprotein (apo) B-containing lipoproteins with magnesium sulfate and dextran sulfate (Thermo Clinical Labsystems). Low-density lipoprotein (LDL) was calculated as described by Friedewald et al. [*Friedewald* 1972].

HbA1c and blood glucose

HbA_1c was determined with high pressure liquid chromatography on a Mono S HR 5/5 column (Amersham Biosciences, Piscataway, N.J., USA and Pharmacia, Uppsala, Sweden) [*Eckerbom* 1994]. Fasting capillary blood glucose was measured immediately with the glucose oxidase technique by using HemoCue® (Hemocue AB, Ängelholm, Sweden).

GAD-antibodies

Antibodies against glutamic acid decarboxylase (GAD) were measured by an autoantibody ELISA kit (RSR Ltd, Cardiff, UK) according to WHO standard. Women with serum GAD antibodies ≥4.6 were defined as suffering from type 1 diabetes[*Bingley* 2003].

Definitions

The WHO criteria [1999] for capillary blood glucose cut-off values were used. IGT was defined as fasting-blood-glucose less than 6.1 mmol/l and 2 h post glucose load ≥ 7.8 - < 11.1 mmol/l measured at two occasions. Diabetes was defined as FG ≥ 6.1 and/or 2 h post glucose load ≥ 11.1 mmol/l measured at two occasions. Impaired fasting glucose (IFG) was defined as fasting-blood-glucose ≥ 5.6 - < 6.1 mmol/l and 2 h post glucose load < 7.8mmol/l.

Subjects with normal glucose tolerance at the first OGTT were defined as normal glucose tolerance (NGT); women with IGT at both the first and second examinations were defined as IGT; women fulfilling diabetes criteria at the first examination but IGT at the re-examination, or the reverse were also classified as

IGT. Women with IGT or DM at the first examination and (NGT) at the second examination were defined as IGT/NGT.

Family history of diabetes was defined as having either a parent or a sibling with diabetes.

Statistical analysis

The statistical analysis was carried out using SPSS version 9.0 for Windows. Results are presented as means and standard deviations for continuous variables if nothing else is indicated, and as percentage for categorical variables. P<0.05 (two-sided) was regarded as statistically significant.

Paper I and IV

For each study in the systematic review, the differences between diabetes patients or subjects with IGT and controls in carotid artery IMT were calculated, as well as the 95 % confidence intervals. A random effect model (an iterative maximum likelihood estimation) was used to perform the meta-analysis, resulting in an overall estimate of the mean difference (and 95% confidence interval) in IMT between diabetes patients and controls, and between patients with IGT and controls, respectively. Homogeneity was studied by the weighted mean μ of the differences Δ_i between the diabetes and control group in n studies. The estimated standard error SE_i of the difference within each group was used to calculate the sum of squares $\sum[(\Delta_i - \mu)/ SE_i]^2$, which under the hypothesis of homogeneity has a chi-square distribution with n-1 degrees of freedom. Possible bias was also checked by creating a funnel plot with the difference in IMT plotted versus the number of subjects included in each trial. Similar analyses were performed in an up-dated meta-analysis after addition of the present and two new studies, and after adjustment for age (Paper IV).

Paper II

Mann-Whitney and ANOVA were used for comparisons between groups, and a linear model was used for testing of trend between groups. Pearson's correlation coefficient was calculated. Multiple regression was used to create an algorithm that was based on fasting glucose and waist girth from the first examination in order to predict fasting glucose at the second examination for eleven women who abstained from undergoing a second examination. (This was done in order to have the diagnoses of diabetes based on two elevated values.)

Paper III and IV

Unpaired Student's t-tests were used for comparison of continuous variables. In paper III multiple regression was used and β-coefficients are presented as 95% confidence intervals. Covarianse analysis was used to calculate partial correlation coefficients in paper IV.

Results

Paper I

(*Aim: To perform a systematic review and a meta-analysis of the literature in order to clarify whether t2D and IGT are associated with increased carotid artery IMT and relate differences in IMT to clinical correlates such as age and risk for myocardial infarction and stroke*.)

Twenty-one studies of patients with t2D and control subjects were included in the review. Nine studies of subjects with IGT were identified. In all studies IMT of the common carotid artery was measured. Nearly all studies used the far wall for measurements. One study examined only the near wall[*Guvener* 2000]. In all 21 studies, except one study[*Tuomilehto* 1998], the diabetic patients had larger IMT than the subjects in the control groups. The differences were statistically significant in all except four studies [*el-Barghouti* 1997, *Rajala* 2002, *Tuomilehto* 1998, *Visona* 1995](figure 7). The estimated mean difference in IMT calculated by a random effect model was 0.13 (95% CI: 0.123-0.144) mm.

Three [*Henry* 2004, *Temelkova-Kurktschiev* 1999, *Wagenknecht* 1998] out of nine studies showed, with statistical significance, that carotid IMT was thicker in the IGT group compared with the control group (figure). The estimated mean difference in IMT calculated by a random effect model was 0.04 (95% CI: 0.014-0.071) mm.

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Carotid Artery IMT in Diabetes vs Controls

Study	Number		Mean	95% CI
Folsom WW (17)	4348		0.05	(0.05 to 0.05)
Folsom BW	1727		0.06	(0.059-0.061)
Folsom WM	3490		0.07	(0.069-0.071)
Folsom BM	1016		0.05	(0.049-0.051)
Yamasaki (18)	294		0.41	(0.404-0.416)
Pujia (30)	108		0.08	(0.074-0.086)
Geroulakos (23)	194		0.17	(0.165-0.175)
Visona (36)	87		0.01	(-0.002-0.022)
Niskanen (25)	182		0.14	(0.130-0.150)
Wei (22)	867		0.05	(0.049-0.051)
El-Barghouti (35)	484		0.02	(0.017-0.023)
Bonora (38)	114		0.25	(0.243-0.257)
Tuomilehto (27)	144		-0.08	(-0.122- -0.038)
Wagenknecht (29)	1079		0.10	(0.099-0.101)
Keven (37)	41		0.22	(0.200-0.240)
Temelkova (31)	142		0.13	(0.122-0.138)
Mohan (24)	243		0.21	(0.204-0.216)
Taniwaki (28)	356		0.36	(0.357-0.363)
Guvener (21)	122		0.18	(0.171-0.189)
Hanefeld (33)	712		0.13	(0.127-0.133)
Rajala (19)	111		0.09	(0.076-0.104)
Ishizaka (30)	904		0.06	(0.057-0.063)
Malmoe men (16)	2303		0.07	(0.069-0.071)
Malmoe women	3291		0.07	(0.069-0.071)
Sigurdardottir (32)	271		0.10	(0.096-0.104)
All	22630		0.12	(0.11 to 0.13)

Difference in carotid artery IMT, mm (95% CI)

Carotid Artery IMT in Impaired Glucose Tolerance (IGT) vs Controls

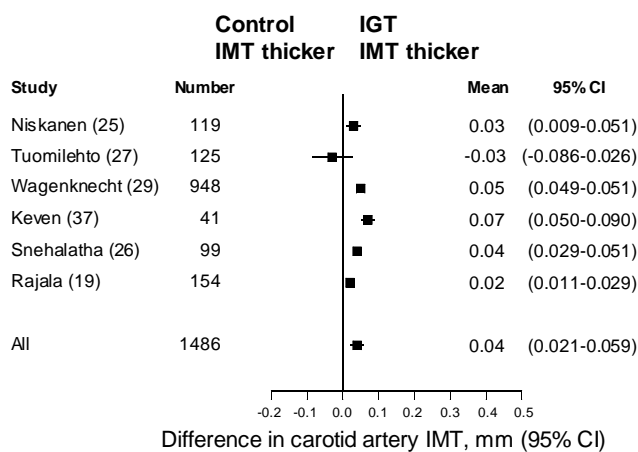| Study	Number		Mean	95% CI
Niskanen (25)	119		0.03	(0.009-0.051)
Tuomilehto (27)	125		-0.03	(-0.086-0.026)
Wagenknecht (29)	948		0.05	(0.049-0.051)
Keven (37)	41		0.07	(0.050-0.090)
Snehalatha (26)	99		0.04	(0.029-0.051)
Rajala (19)	154		0.02	(0.011-0.029)
All	1486		0.04	(0.021-0.059)

Difference in carotid artery IMT, mm (95% CI)

Figure 7. Meta-analysis showing the difference in carotid artery intima media thickness (IMT) between type 2 diabetes and controls (upper panel), and between patients with impaired glucose tolerance (IGT) and controls (lower panel). Means and 95% confidence intervals for the differences in separate studies and the summary of all studies are given.

Test for bias

Heterogeneity was indicated as the homogeneity test rejected the null hypothesis for the diabetes-control groups (p<0.001). The funnel plot (figure 8) showed a skewed distribution with an abundance of smaller studies showing larger IMT differences for diabetic patients compared with control subjects. Of the 14 studies with fewer than 500 subjects seven[*Bonora* 1997, *el-Barghouti* 1997, *Geroulakos* 1994, *Niskanen* 1996, *Pujia* 1994, *Visona* 1995, *Yamasaki* 1994] included only patients with well established diabetes. On the other hand just one of the 7 studies with 500 or more subjects dealt exclusively with patient with established diabetes[*Wei* 1996]. In the present meta-analysis five studies[*Ishizaka* 2003, *Keven* 1999, *Sigurdardottir* 2004, *Temelkova-Kurktschiev* 1999, *Wagenknecht* 1998] included only, or reported separately, newly detected diabetes patients and in those IMT was 0.11 (95% CI: 0.095 – 0.121) mm thicker than in control subjects compared to a corresponding difference of 0.13 (95% CI: 0.129 – 0.139) mm in the ten studies that included patients with known diabetes and control subjects The approach to measure IMT in the segment with the largest thickness was used by nearly 50% of the authors in the studies with less than 500 subjects [*el-Barghouti* 1997, *Geroulakos* 1994, *Mohan* 2000, *Niskanen* 1996, *Tuomilehto* 1998, *Yamasaki* 1994] and by one of the larger studies[*Taniwaki* 1999]. The studies performing the IMT measurements at the thickest point showed a larger IMT in diabetes patients (mean difference between diabetes patients and controls 0.21 [95% CI 0.206-0.219] mm). The corresponding mean difference in IMT, in studies measuring the thickness in predefined segments, was 0.09 [95% CI 0.091-0.097] mm. The differences between t2D and controls were similar in both sexes and in different ethnic groups.
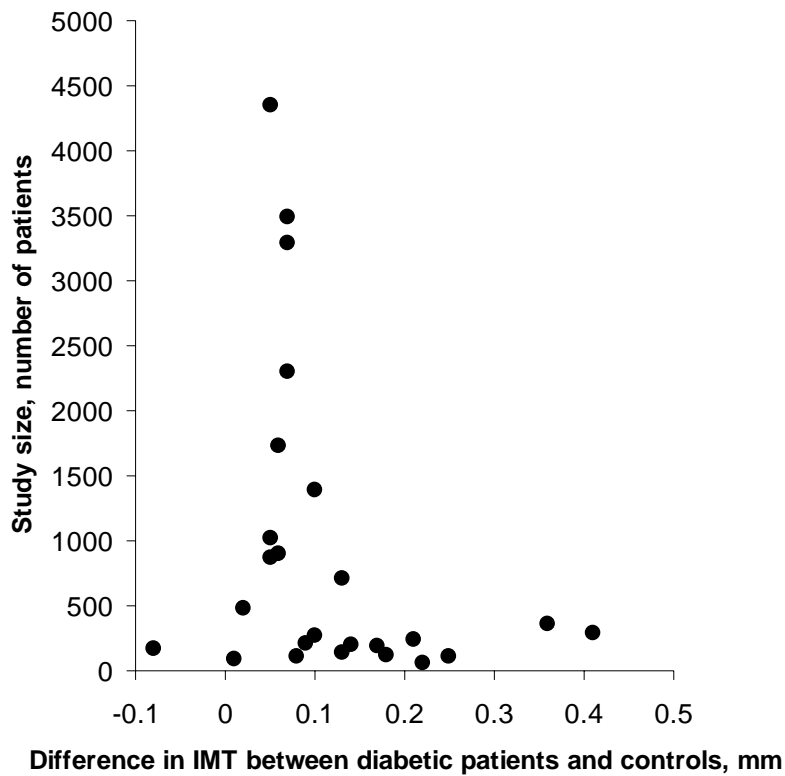
Figure 8.Funnel plot presenting difference in common carotid artery IMT between diabetic patients and control subjects by study size.

IMT in relation to age and cardivascular risk

In a large Swedish study [*Hedblad* 2000] the age-related change of carotid artery IMT can be estimated at 1% per year. The difference in IMT between individuals with type 2 diabetes patients and control subjects found in the present meta-analysis corresponds to an expected age difference of more than 10 years, using these Swedish population data.

Bots et al. [*Bots* 1997] used the Rotterdam population to estimate the gradient of risk of myocardial infarction and stroke per 1 standard deviation (0.163 mm) increase of IMT. They found the gradients 1.43 for myocardial infarction and 1.41 for stroke. In the previously mentioned Swedish study, a correlation

coefficient of 0.77 was observed between measurements of two observers. That yields a coefficient of $(0.77)^{1/2}$ between one observer and the true value. The gradient of risk adjusted for measurement error can be determined as $\exp(\log(1.43)/r) = 1.50$ for MI. The corresponding gradient for stroke was 1.48. The risk ratio of myocardial infarction when comparing the diabetes group versus controls is $\exp(\log(1.50)\cdot 0.12 / 0.163) = 1.38$, and for stroke the ratio is 1.37. Thus, the difference of IMT between diabetes patients and control subjects in the present meta- analysis is associated with increases in relative risks of 38% and 37% for myocardial infarction and stroke, respectively.

Paper II

(*Aim: To describe the prevalence of diabetes mellitus and IGT in a cohort of 64-year-old women in Gothenburg and to examine the variability and practical use of the OGTT in the screening for IGT and diabetes.*)

The outcome of the screening process is shown in figure 9.

Diabetes

The prevalence of diabetes in this cohort of 64-year-old women was 9.5%. New diabetes mellitus, detect by screening, was found in 4.8% and known diabetes in 4.7% of the women. Just performing fasting blood glucose measurements would have resulted in 44 fewer cases, i.e., 37% of new diabetes subjects who were identified by repeated OGTTs.

IGT

IGT, as defined by two OGTT, was found in 14.4% of the women. At the first examination, 22% of the women had IGT. At the re-examination of 367 women with IGT at the first examination, 175 (48%) turned out to have NGT.

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Screening procedure
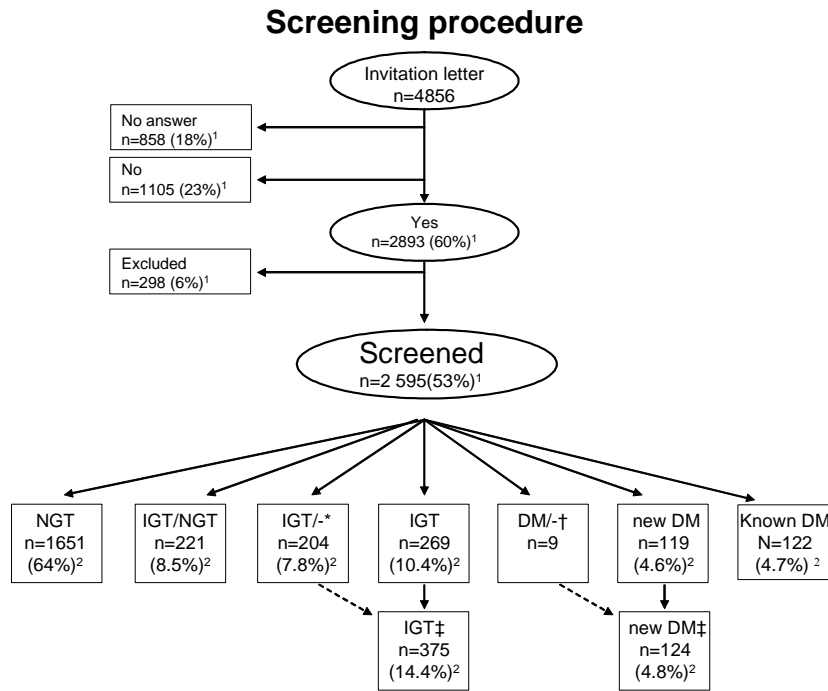
¹) % of invited n=4856

²) % of screened n=2595

*) IGT at the first test, did not participate in a second test.

†) DM value at the first test, refused to participate in the re-examination.

‡) IGT and new DM adjusted prevalence values.

Figure 9. The screening procedure.

Characteristics by glucose tolerance status

Table 1 shows that with worsening glucose tolerance there was a gradual increase in BMI, waist, waist-hip-ratio (WHR) and blood pressure, with the exception that known and treated diabetic women had lower blood pressure. The proportion of women with a family history of diabetes was doubled in the diabetic group in comparison with the NGT group. A closer analysis showed that women with IGT/IGT, in contrast to those with IGT/NGT had higher mean BMI, waist girth, WHR, diastolic blood pressure, heart rate and contained more subjects with family history of diabetes than the group with NGT women.

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Table 1 Characteristics of 64-year-old women by glucose tolerance after two OGTT.

	NGT	IGT/NGT	IGT	New diabetes	Known diabetes
Number	1651	221	269	119	122
Weight, kg	70.3±11.3	70.6±11.3	74.8±14.0*	77.5±12.7	80.0±15.2†
Height, cm	164.5±6.0	164.3±5.6	163.6±5.8*	163.7±6.1	163±5.5†
BMI	26.0±4.0	26.1±4.1	27.9±5.0*	28.9±4.4	30.1±5.5†
Waist, cm	86.6±10.7	87.9±10.9	92.4±12.0*	96.4±11.1	101±13.5†
Hip, cm	102.8±8.5	102.8±8.4	105.7±10.3*	107.1±9.6	109.3±11.8†
WHR	0.841±0.068	0.854±0.068*	0.874±0.076*	0.900±0.068	0.924±0.072†
Arm, cm	29.8±3.2	29.5±3.1	30.9±4.0*	31.2±3.8	32.4±4.3†
Neck, cm	34.7±4.0	35.0±2.3	36.1±2.8*	36.7±2.5	36.9±12.8†
Blood pressure Systolic, mm Hg	146 ±20.2	149 ±18.9*	154 ±20.2*	158 ±20.7	148±21.3†
Blood pressure Diastolic, mm Hg	83 ±9.0	84.0 ±9.3	86 ±9.2*	89 ±9	78±10†
Heart rate, beats/min	70±9	71±10	72 ±10*	73 ±11	69±11†
Family history DM	25 % (379/1514)	26 % (50/195)	34 % (84/249) *	48 % (53/111)	52% (44/84)†

Data are mean ± SD unless otherwise indicated. * p<0.05 in comparison with NGT;
† p < 0.001 for trend. NA – not applicable.

Impaired fasting glucose (IFG) as screening test

A fasting blood glucose FBG ≥ 5.6mmol/l (IFG) had a sensitivity of 0.87 and a specificity of 0.84 to identify women with diabetes. Out of 2475 women without known DM, 472 had FBG ≥ 5.6 mmol/l. Performing repeated OGTT in just this group (472 instead of 2475) would have resulted in identification of 103 new DM cases. Sixteen women with new DM were not identified with such an approach.

Non-participants

To explore the potential differences between participants and non-participants, attempts were made to contact the first 191 women who did not answer the two invitation letters. It was possible to interview 89 women by telephone contact.

Three of these women had known diabetes (3.4%) corresponding to 3.3% among the screened patients

Paper III

(*Aim; To investigate the occurrence of echolucent plaques in diabetic patients; to examine if carotid artery remodelling in women is present already at the diagnosis of new t2D, and if it is similar to that in known t2D and to investigate if non-atherosclerotic remodelling may occur in t2D.*)

In total 624 women underwent the ultrasound measurements. Out of the 122 known diabetic women; 15 had type-1 diabetes (t1D) according to the GAD-criteria; it was not possible to examine eight women; and 99 underwent the ultrasound examination. For the 119 screening identified women with diabetes, five had t1D and eight were not examined, and 106 were included. General characteristics of the study group are presented in Table 2. With the exception of smoking and LDL cholesterol, there was a continuously worsening of the cardiovascular risk factor profile in parallel with impairment of glucose tolerance.

Table 2. Characteristics of a population-based cohort of 64-year-old women by glucose tolerance group

	Normal glucose tolerance (n=99)	Type 2 diabetes	
		New (n=106)	Known (n=99)
BMI kg/m²	24.9±3.4	28.2±4.4	30.4±5.0**
Height, m	1.65±0.06	1.64±0.06	1.63±0.05*
Waist circumference, cm	85.2±9.1	95.0±11.3	101.9±12.3**
Smoking current, %(n)	15(15)	25(26)	24(23)
Systolic blood pressure, mmHg	131±17	143±18	144±21**
Diastolic blood pressure, mmHg	75±8	79±9	77±10*
HbA1c,% §	4.46±0.26	5.04±0.97	6.28±1.43**
HDL mmol/L	1.83±0.42	1.57±0.42	1.40±0.36**
LDL mmol/L	3.70±0.97	3.45±1.0	2.95±0.99**
Triglycerides, mmol/L §	1.12±0.59	1.35±0.73	1.84±1.1**
Previous myocardial infarction, %(n)	0	3(3)	10(10)†
Previous stroke, %(n)	0	2(2)	4(4)
Hypertension known,%(n)	0	51(54)	62(61)†
Statin therapy, %(n)	0	16(17)	39(39)†
Fibrate therapy, %(n)	0	1(1)	5(5)
Insulin therapy, %(n)	0	0	20(20)
Oral anti-diabetic therapy, %(n)	0	0	57(56)
Glitazone therapy, %(n)	0	0	10(10)

Data are mean±SD, § geometric mean±SD. Linear trend NGT, t2Dnew, t2Dknown
*p=0.002; **p<0.001 †No statistic test was performed as the NGT group by selection was free of cardiovascular diseases.

It was not possible to obtain complete ultrasound examinations in 195 women. As shown in Table 3, these women were more obese and showed a worse cardiovascular risk factor profile than the women with complete examinations. Mean CCA IMT values were significantly higher in the women in whom incomplete ultrasound examinations were performed compared with the women

with complete examinations (incomplete: 0.89±0.20 mm, n=195; complete: 0.84±0.16 mm; n=429; P<0.01).

Table 3. Comparison between the women with complete ultrasound and incomplete ultrasound examinations.

	Ultrasound examinations	
	Complete (n=429)	Incomplete (n=195)
Diabetes mellitus, %(n)	34(148)	39(76)
Impaired glucose tolerance, %(n)	31(131)	42(81)*
BMI	26.5±4.6	28.8±4.5†
Waist circumference, cm	89.8±11.7	96.9±12.0†
Systolic blood pressure, mm Hg	137±20	141±19†
HDL cholesterol, mmol/L	1.64±0.44	1.44±0.40†
Triglycerides, mmol/L	1.25±0.75	1.6±0.86†

Geometric mean ±SD *p<0.05 †p<0.01

IMT

In comparison to the NGT group, women with known t2D had larger mean composite IMT, mean bulb IMT, and mean CCA IMT, whereas women with new t2D had a larger mean CCA IMT (Table 4). The mean CCA IMT was increased in both the known and new t2D groups, also after adjustment for the covariates (Table 5).

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Table 4. Morphology of the carotid arteries. CCA- common carotid artery, IMT-intima media thickness. Composite IMT is the mean of IMT in CCA and carotid artery bulb.

	Normal glucose tolerance (n=99)	Type 2 diabetes	
		New (n=106)	Known (n=99)
Mean CCA IMT, mm	0.82±0.18	0.87±0.20**	0.91±0.18***
Mean bulb IMT, mm	1.00±0.62	1.02±0.32	1.11±0.35**
Mean composite IMT, mm	0.92±0.34	0.95±0.25	1.02±0.23***
Mean lumen CCA, mm	5.80±0.60	6.08±0.58***	6.21±0.67***
Women with any plaque,%(n)	47(46)	46(48)	58(57)
Women with any echolucent plaque %(n)	40(39)	41(42)	57(56)*
Mean total plaque area per subject mm² (mean, SD)	11.6±19.5	15.6±26.0	20.4±25.9†
Mean total plaque area for echolucent plaques per subject, mm², (mean, SD)	10.4±18.5	14.8±25.6	18.2±23.9†

Data are geometric mean±SD, unless stated. T-test *p<0.05, **p≤0.015; ***p<0.001 compared to NGT group. † p<0.05 for trend

Atherosclerotic plaques

The proportion of women with any echolucent plaque, mean echolucent plaque area, and mean total plaque area increased gradually in women with new and known t2D compared to the NGT group (Table 4). The ratio between the numbers of echolucent and total plaques did not differ between the groups (data not shown).
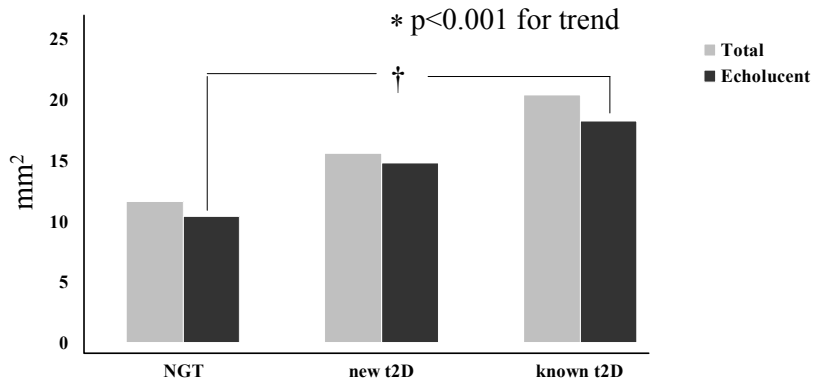
Figure 10. Total and echolucent plaquearea.

In a stepwise multiple regression analysis, known t2D was associated with increased occurrence of echolucent plaques after adjustment for BMI, height, systolic blood pressure, smoking, and LDL cholesterol (0.20 [0.01 to 0.40 95% CI]) in comparison to the NGT group. For echolucent plaque area and total plaque area the corresponding finding was (8.4 [2.1 to 14.7 95% CI] mm^2) and (7.4 [1.1 to 13.7 95% CI] mm²) respectively (Figure 10).

Table 5. Common carotid artery remodelling. Known (t2Dk, n=99) and newly detected (t2Dn, n=106) type 2 diabetes in comparison to healthy control women with normal glucose tolerance (n=99). Results are given as mean differences (95% CI) between t2D groups and control group**.**

Adjustments	Lumen diameter, mm		Mean CCA IMT, mm	
	t2Dk	t2Dn	t2Dk	t2Dn
Unadjusted	0.42 (0.24 to 0.60)	0.28 (0.12 to 0.44)	0.09 (0.04 to 0.14)	0.06 (0.008 to 0.12)
Log BMI, height	0.28 (0.06 to 0.49)	0.22 (0.04 to 0.40)	0.07 (0.004 to 0.13)	0.07 (0.005 to 0.12)
+ diastolic blood pressure,	0.31 (0.09 to 0.52)	0.20 (0.02 to 0.39)	0.07 (0.005 to 0.13)	0.07 (0.006 to 0.13)
+ serum LDL cholesterol	0.17 (-0.06 to 0.41)	0.18 (-0.01 to 0.36)	0.09 (0.02 to 0.16)	0.07 (0.01 to 0.13)
+ smoking	0.15 (-0.08 to 0.37)	0.14 (-0.03 to 0.31)	0.09 (0.02 to 0.16)	0.07 (0.01 to 0.14)

Vascular remodelling

As shown in Table 4 and 5, both known and new t2D were associated with increases in mean lumen diameter in comparison to the NGT group, also after adjustment for BMI, height and diastolic blood pressure.

Table 6. Lumen and IMT in common carotid artery by tertiles of plaque area, in relation to subject characteristics.

	Tertiles of plaque area		
	1	**2**	**3**
	n=78	**n=42**	**n=58**
Plaque surface, range, mm²	No plaque	4.7 – 20.5	20.9 – 145.7
Lumen diameter (LD), mm	6.1±0.7	6.2±0.6	6.2±0.6
Mean CCA IMT, mm	0.83±0.140	0.92±0.16	1.01±0.23**
Body mass index, kg/ m²	29.9±5.0	28.3±4.2	28.0±4.8*
Systolic blood pressure, mm Hg	141±18	143±21	144±21
Diastolic blood pressure, mm Hg	79±9	78±8	76±11**

*$p<0.05$; **$p \leq 0.001$ linear trend

With an attempt to separate the impact of atherosclerotic plaques all t2D women were divided by tertiles of plaque area. As shown in Table 6, tertile 3 contains the women with the largest plaque area and tertile 1 the women without any plaques. Table 6 also shows that women in tertile 2 and 3 had larger mean CCA IMT in comparison to tertile 1. Figure 11 demonstrates that, among women in tertile 3 the lumen diameter was significantly larger compared to tertile 1 also after adjustment for BMI and diastolic blood pressure. Similar findings were obtained if echolucent plaque area was used in instead of total plaque area in dividing the sample into tertiles (data not shown). In women without plaques, t2D had larger lumen diameter than NGT but the difference was not significant after adjustment for BMI and height (0.16 [-0.10 to 0.42, 95% CI] mm).
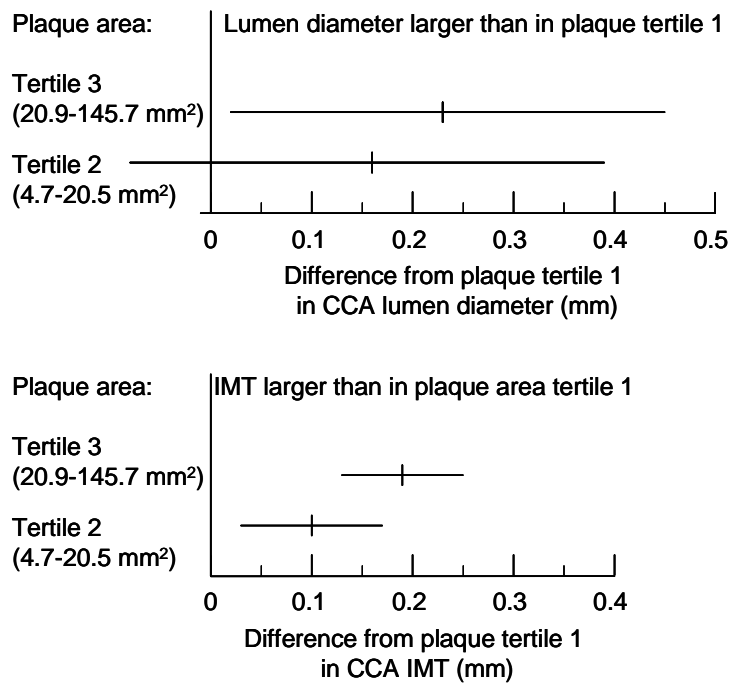
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Figure 11. Differences in lumen diameter and intima-media thickness (IMT) in the common carotid arteries (CCA) by tertiles of carotid plaque area in 64-year-old women. The figure shows the differences between plaque area tertile 2, and 3, versus tertile 1 in which there were no plaques (plaque area=0). Means and 95% confidence intervals are given after adjustment for BMI and diastolic blood pressure.

Paper IV

(*Aim: To examine if IGT, as diagnosed by doubled OGTT, is associated with subclinical carotid atherosclerosis assessed as IMT and occurrence of echolucent plaques. A post-hoc aim was to clarify if carotid IMT is thicker in groups of IGT compared to groups of NGT by performing an up-dated meta-analysis adjusted for differences in age.*)

As a consequence of the study design just healthy women, free of hypertension and cardiovascular diseases, were recruited to the NGT groups. These healthy NGT women were divided into two groups; one randomly selected and the other matched to the IGT group for BMI and WHR. As seen in Table 7 women in the IGT group showed significantly higher systolic blood pressure and

disadvantageous changes in serum concentration of HDL-cholesterol and triglycerides.

Table 7. Characteristics of the women by glucose tolerance group. (IGT-impaired glucose tolerance, NGT-normal glucose tolerance).

	IGT (n=205)	NGTm (matched to IGT group)* (n=96)	NGTr (randomly selected) (n=99)
Height, cm	163±5.9	164±6.4	165±5.7†
BMI, kg/m²	27.3±4.9	27.3±3.1	24.9±3.4‡
Waist circumference, cm	91.6±12.0	91.3±8.3	85.0±9.1‡
Smoking current, %(n)	18(37)	21(20)	15(15)
Systolic blood pressure, mmHg	142±19	134±18†	131±17‡
Diastolic blood pressure, mmHg	79±9	77±9	75±8‡
HDL cholesterol, mmol/L	1.62±0.42	1.73±0.40¶	1.83±0.42‡
LDL cholesterol, mmol/L	3.66±0.89	3.81±0.89	3.70±0.97
Serum triglycerides, mmol/L§	1.35±0.74	1.20±0.52¶	1.12±0.59†
Hypertension known, %(n)	26(53)	0	0
Statin therapy, %(n)	10(22)	0	0
Fibrate therapy, %(n)	0	0	0
HRT terapi %(n)	23(48)	16(15)	30(30)
Previous myocardial infarction %(n)	1(2)	0	0
Previous stroke %(n)	3(6)	0	0

*Matched to IGT for BMI and waist-hip ratio. Data are mean±SD, unless stated. §Geometric mean±SD. †$p<0.005$, ‡$p<0.0005$, ¶$p<0.05$ in comparison to IGT group.

Out of the 269 women with IGT after repeated OGTTs the 212 first detected were invited to ultrasound measurements. It was not possible to examine seven women and 205 were included. In 74 women it was not possible to obtain complete examinations from all ultrasound locations. As shown in table 8 these women were more obese and showed a worse cardiovascular risk factor profile. They also had thicker CCA intima-media.

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Table 8. Women with impaired glucose tolerance (IGT), with and without all ultrasound measurements.

	IGT not possible to assess measurements from all locals. (n=74)	**IGT** with measurements from all locals. (n=131)
Weight kg	77.1±16.2	70.8±9.8*
Waist cm	96.4±13.0	88.9±10.5*
BMI	29.2±5.2	26.3±4.4*
Bp syst	145±19	140±18
Bp dias	81±9	77±9 †
HDL	1.52±0.41	1.67±0.42 †
LDL	3.81±1.02	3.58±0.81
TG	1.73±0.69	1.37±0.73*
Mean CCA IMT	0.90±0.19	0.82±0.13*
Max CCA IMT	1.02±0.22	0.91±0.14*
Heredity for DM	25%	30%

Data are mean ± SD, t-test *p< 0.005, †p<0.05

IMT and plaques

Mean CCA IMT, mean bulb IMT and mean composite IMT did not differ between the groups (Table 9). The NGTm and NGTr groups were combined as there was no difference between the groups in IMT. There was still no difference in mean CCA IMT between the IGT and the combined NGT-group [0.84 (95% CI -0.03 to 0.03) vs 0.84 (95% CI -0.03 - 0.03) mm]. No differences in the results were found when statin treatment or medication for hypertension in the IGT group was taken into account. In the combined IGT, and NGT group CCA IMT correlated to systolic blood pressure (0.23, p<0.001), previous smoking (0.13, p=0.020), and negatively to HDL cholesterol, but not to LDL cholesterol, current smoking, or HbA1c (data not shown). The proportions of subjects with a family history of diabetes in any of the parents (FH+) were 29 % (n=59), and 17% (n=32), in the IGT and NGT groups, respectively (p=0.006). This difference in proportions was statistically significant for a family history of maternal (19% [n=38] versus 11% [n=20], p=0.027), but not for paternal diabetes. In the combined IGT and NGT groups FH+ was associated with 0.03 (96% CI -0.007 to 0.07) mm larger CCA IMT in comparison to those with no family history of

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diabetes (FH-). Women with a family history of maternal diabetes were associated with a larger CCA IMT than in those with FH- (mean difference 0.05 mm [95% CI 0.005 to 0.10 mm]). In a covariance analysis log CCA IMT was associated with maternal diabetes (partial correlation coefficient=0.12, p=0.027), independently of glucose tolerance group (IGT/NGT), systolic blood pressure, HDL cholesterol, and smoking.

Atherosclerotic plaques

As shown in Table 9 the proportion of women with any plaque, mean number of plaque per subject, and mean total plaque area per subject did not differ between the groups. The corresponding findings concerning echolucent plaques did not differ between the groups. The ratio between the numbers of echolucent and total plaques was similar between the groups (Table 9).

Table 9. Morphology of the carotid arteries.
(IGT-impaired glucose tolerance, NGT normal glucose tolerance, CCA common carotid artery). Composite IMT is the mean of IMT in CCA and carotid artery bulb.

	IGT (n=205)	NGT matched to IGT group[*] (n=96)	NGT randomly selected (n=99)
Mean CCA IMT, mm (n)	0.83±0.16 (205)	0.84±0.15 (96)	0.82±0.18 (99)
Mean bulb IMT, mm (n)	0.99±0.30 (181)	0.96±0.24 (90)	1.00±0.62 (98)
Mean composite IMT, mm (n)	0.91±0.19 (180)	0.91±0.16 (89)	0.92±0.34 (98)
Women with any plaque, %(n)	40(82)	38(37)	46(46)
Mean total plaque area per subject mm^2 (mean, SD)	12.0±25.4	10.4±17.7	11.9±19.6
Women with any echolucent plaque, %(n)	34(67)	34(33)	40(40)
Mean total area for echolucent paques per subject, (mean SD)	10.6±24.8	9.0±16.6	10.2±18.5

Geometric mean ±SD, unless stated
[*]Matched to IGT group for BMI and waist-hip-ratio

Meta-analysis

Three new studies including our own were found as an addition to the 9 previously reviewed studies. Three out of twelve studies showed that carotid IMT was thicker in the IGT group compared to the normal control group after adjustment for age (Figure 12). The observed variability in study results was greater than expected to occur by chance. The estimated mean difference in IMT, calculated by a random effect model, was 0.03 (95% CI 0.012 to 0.048) mm (Figure 12).
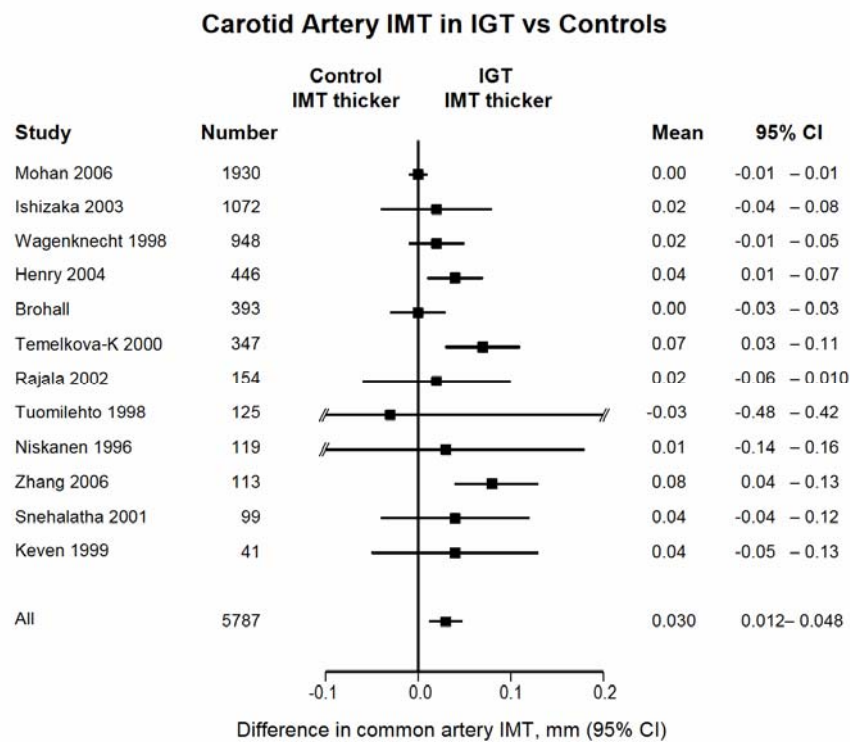

Carotid Artery IMT in IGT vs Controls

Study	Number		Mean	95% CI
Mohan 2006	1930		0.00	-0.01 – 0.01
Ishizaka 2003	1072		0.02	-0.04 – 0.08
Wagenknecht 1998	948		0.02	-0.01 – 0.05
Henry 2004	446		0.04	0.01 – 0.07
Brohall	393		0.00	-0.03 – 0.03
Temelkova-K 2000	347		0.07	0.03 – 0.11
Rajala 2002	154		0.02	-0.06 – 0.010
Tuomilehto 1998	125		-0.03	-0.48 – 0.42
Niskanen 1996	119		0.01	-0.14 – 0.16
Zhang 2006	113		0.08	0.04 – 0.13
Snehalatha 2001	99		0.04	-0.04 – 0.12
Keven 1999	41		0.04	-0.05 – 0.13
All	5787		0.030	0.012 – 0.048

Difference in common artery IMT, mm (95% CI)

Figure 12. Meta-analysis. Studies comparing intima-media thickness (IMT) between groups with impaired (IGT) and normal (NGT) glucose tolerance.

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Discussion

The major cause of death in type 2 diabetes is macrovascular, atherosclerotic disease. From a clinical standpoint it is of great importance to identify individuals at risk for, or suffering from diabetes as correctly and as early as possible, and to clarify when, and how atherosclerosis starts to develop. These issues are addressed in this thesis in the format of a meta-analysis of the current literature followed by a population based cross-sectional study. In order to identify and quantify atherosclerosis we have used measures obtained by B-mode ultrasound examinations of the carotid artery. The IMT of the carotid arteries is since many years an established surrogate measure of atherosclerotic disease, that is related to future cardiovascular diseases independently of traditional risk factors [*Bots* 1999, *O'Leary* 1999, *Salonen* 1991]. In addition, we have also measured the occurrence and characteristics of atherosclerotic plaques and studied the vascular remodelling that may occur as a consequence of atherosclerosis. Our working hypothesis was that there is a gradual enlargement of IMT and an increased occurrence of plaques, especially echolucent plaques, with worsening glucose tolerance, accompanied by a remodelling that might be visible already in subjects with IGT.

Paper I

Carotid artery IMT in type 2 diabetes and IGT a systematic review

The search in the data bases for studies evaluating the difference between type-2 diabetes, IGT and controls resulted in 23 studies. All studies following the inclusion criteria were included. The results obtained in the systematic review showed that type-2 diabetic patients had clearly significant larger carotid artery IMT than control subjects, on average 0.13 mm thicker. Similar results were obtained in men and women, and no obvious difference between ethnicities was found, although Caucasians were in great majority. The only study indicating that

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controls had larger IMT than t2D was Finnish [*Tuomilehto* 1998], however statistically non-significant, and with a wide CI. This study was performed at the 30th year of follow-up of a cohort consisting of men with very high mortality from coronary heart disease. The author speculated that the unexpected result could be due to selective survival of the subjects. A quality scoring between the included studies was not used and the different studies were not weighted differently. Even if the main interest was to summarise the current literature regarding the difference in IMT between diabetes, IGT and controls, the systematic review is also important as a study of heterogeneity and differences between studies.

Heterogeneity

The statistical homogeneity test indicated heterogeneity between the studies examining the difference in IMT between diabetic patients and controls. At a closer analysis it was found that the included studies differed in a number of aspects.

1. *Publication bias?* The 14 smaller studies, with less than 500 subjects, more often showed larger IMT-differences for diabetic patients compared with control subjects than did the larger studies. This may be explained by publication bias, favouring positive studies, but there are also other explanations.

2. *Selection of study groups*. One possibly source of discrepancy between the studies is the selection of study groups. Six out of seven of the larger studies [*Folsom* 1994, *Hedblad* 2000, *Henry* 2004, *Ishizaka* 2003, *Wagenknecht* 1998, *Wei* 1996] included population-based samples, whereas this was the case in only five of fourteen smaller studies [*Mohan* 2000, *Rajala* 2002, *Sigurdardottir* 2004, *Temelkova-Kurktschiev* 1999, *Tuomilehto* 1998]. Fifty percent of the smaller studies included only patients with well-established diabetes compared to 14% of the larger studies. Among newly detected t2D IMT was 0.11 (95% CI; 0.095-0.121) mm thicker than controls, compared to

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0.13 (95%CI; 0.129-0.139) mm in the known t2D group. However, this obvious statistically significant difference between the groups may not only be related to different durations of t2D but also to different methods to measure IMT by ultrasound.

3. *Ultrasound method*. Measurements of IMT made in segments of the thickest walls yielded larger differences in IMT between diabetes patients and controls [0.21 (95% CI; 0.206-0.219) mm] than assessing mean thickness in predefined segments [0.09 (95% CI; 0.091-0.097) mm]. In addition, measurement of IMT in the thickest wall segments was performed more often in smaller studies with a higher proportion of known diabetes than in larger studies.

4. *Age*. In eight studies the DM group was older than controls (ranging 1-7 years) but the opposite was seen in three studies (range 2-8 years). Age is a dominant determinant of IMT and this disparity might partly reduce the difference of IMT between t2D and controls. Any adjustment on behalf of differences in age between the groups was however not done in the meta-analysis.

Thus, any firm conclusions of the difference in IMT between known and newly detected t2D versus subjects with NGT cannot bee drawn from this review, showing a considerable variability in studied groups and used methods. There is today no consensus how to measure carotid IMT[*Lorenz* 2007]. We do not know which approach is superior in predicting future cardiovascular disease in diabetic patients, although attempts have been made to clarify this[*Iglesias del Sol* 2002]. Too few studies were available for reviewing differences in plaque occurrence and differences in subjects with impaired fasting glucose in relation to controls.

Clinical relevance

We have tried to estimate the clinical relevance of the difference in carotid artery IMT between diabetic patients and controls. We used the well-known facts that

carotid IMT is associated with increasing age and with future cardiovascular disease in order to perform such estimations [*Bots* 1997, *Chambless* 2002, *Hedblad* 2000, *O'Leary* 1992, *O'Leary* 1999]. The results showed that the observed difference in IMT can be interpreted as if the diabetes patients are more than 10 years older than the control groups, and that the relative risks of myocardial infarction and stroke are increased by 38%, and 37%, respectively.

Impaired glucose tolerance (IGT)

IGT is generally considered as a pre-diabetic state. The review showed that in 3/9 identifed studies IMT was thicken in those with IGT compared with NGT controls. The summary meta-analysis showed that IGT patients had a carotid IMT that was overall 0.04 (95% CI; 0.01 to 0.07) mm thicker than in the control subjects. However, in six out of nine studies the IGT group was older than the control group indicating that the difference in IMT between IGT and controls might be smaller than calculated [*Howard* 1993]. This issue will be discussed later (see Page 64).

Paper II

Prevalens of diabetes mellitus and impaired glucose tolerance 64-year-old Swedish women. Experiences of using repeated orol glucose tolerance tests.

In order to study the association between glucose tolerance and subclinical atherosclerosis a population based cohort was examined. We chose 64-year-old women as we wanted to keep the age-, sex- and ethnicity factors constant. Another reason for this age group is the rise in diabetes incidence among women after the age of 60 [*Rathmann* 2003, *Ryden* 2007]. The occurrence of subclinical atherosclerosis is also rising [*Joakimsen* 1999] and there is a high relative risk of cardiovascular disease in this age category of diabetic women [*Barrett-Connor* 1998, *Niskanen* 1998]. According to the calculation of study size a sample of 600

women with 200 in each group of of t2D, IGT and NGT were required, indicating a number of screened subjects of 2000-2500.

In the present study, 2595 women were screened with OGTTs. The response rate was initially > 80%, but > 20% refused in the end to participate. The inclusion rate was similar to that in a Danish and a German study [*Glumer* 2003, *Rathmann* 2003]. It is generally observed that the non-participants in studies are sicker than the participating study subjects. However, in the telephone interviews of a number of non-participating women we could not obtain any evidence that the non-participating women had higher prevalence of known diabetes. From ethical points of view the health status could not be examined among women refusing to participate.

OGTT

The results showed that repeated OGTTs seem to be needed in order to identify all women with diabetes according to the current WHO-definition in a middle-aged population. Otherwise, more than one third (37%) would have remained unidentified if only fasting blood glucose measurements had been used. Furthermore, the results of the OGTT showed considerable variability as more than 40% of the women with a diabetic OGTT at the first examination did not fulfil this criterion at the second examination. In addition, more than 20% had IGT at the first examination, but in almost 50% of the women there was normalization at the second examination. This high variability, observed in the present study, is a well known problem with the OGTT [*McDonald* 1965, *Rathmann* 2005]. As regard the variability of the OGT test, the coefficient of variation was 12% in the present study. It may also be questioned if the information to the patient of an abnormal result at a first test will not lead to drastic changes in life style, improving the results at the second test. However, it is clearly shown in our study that if subjects fulfil criteria for IGT or diabetes at one OGTT, and then show normal glucose tolerance at a repeated test, such subjects differ from healthy subjects with normal glucose tolerance in several

characteristics typical of the metabolic syndrome, i.e. high BMI, increased waist girth, and blood pressure. Thus, already a lone abnormal OGTT is a clear indication of increased risk, although this cross-sectional study provides no prospective information. The variability of the OGTT is related to factors such as duration of fasting, degree of physical activity, smoking, and inter-current diseases. We tried to control for these factors and obtained no indication that the duration of fasting played any major role in the study. Taken together, repeated OGTT is of great value when diagnosing diabetes and IGT, and the results do not indicate that the difference between the first and second OGTT results is primarily explained by changes in lifestyle habits.

Prevalence of diabetes and IGT

To the best of our knowledge there is no previously published study of the prevalence of diabetes and IGT in a cohort of middle-aged women, based on repeated OGTTs and the current definitions of these conditions. Using this approach, we observed a 9.5% prevalence of diabetes in 64-year-old women, whereas the prevalence of IGT was 14.4%. More than 40% of the women showed some impairment of glucose metabolism if impaired fasting glucose was also included. Similar to previous studies, we found that about half of the women with diabetes were newly diagnosed [*Glumer* 2003, *Rathmann* 2003]. Taken together these studies emphasise the need for better strategies to identify subjects with diabetes.

Screening method

It has been difficult to find simple and generally applicable methods for screening to identify diabetes [*Rathmann* 2005]. The results from the present study in combination with experiences from previous studies indicate a screening strategy using FBG $\geq$ 5.6 mmol/l as a first identification of cases targeted for further examinations with OGTT [*Anand* 2003, *de Vegt* 2001].

Paper III

Atherosclerotic plaques and carotid arterial remodelling in women with known and newly diagnosed diabetes.

In this cross-sectional study based on the cohort of 64-year-old women with t2D, IGT, and NGT ultrasound examinations of both carotid arteries were performed. The results of such examinations are dependent on both sonographer skill and patient characteristics.

Missing data

Of the, at screening identified diabetic patients it was not possible to examine 16 women of whom 8 had known diabetes. The non-participating diabetic women were more obese with higher weight, BMI, waist circumference but lower systolic blood pressure (data not shown). In total 626 women were examined with ultrasound, and in 195 women the examination was not complete. These women, with incomplete measurements, were more obese than those with complete examination, demonstrating the well-known fact that obesity is a factor that complicates the examination. In addition, women with incomplete ultrasound data had a more unfavourable risk factor profile and also a thicker mean CCA IMT. This has to be considered in the interpretation of data.

Ultrasound method

In the present study IMT was measured bilaterally in CCA, and the carotid bulb. Measurement of IMT in ICA was avoided due to the difficulty to obtain representative data. A well-validated approach was used, where the measurements of IMT in pre-defined segments from both CCA and the bulb were analyzed in an automated computerized system. This technique has been shown to have a low interobserver variability and a good reproducibility.[*Schmidt* 2003, *Schmidt* 1999]. Only the far wall is possible to examine, as previously shown by our group [*Wendelhag* 1991]. We report data on CCA and carotid bulb IMT and

also give the results of a composite endpoint that is the mean of CCA IMT mean and the bulb mean IMT. The rational is that the carotid bulb is much more a predilection site for atherslcerosis with an annual IMT progression of 0.02 mm compared to 0.01 or less for the CCA IMT [*Howard* 1993, *Mackinnon* 2004]. Previous studies have not succeded to show that CCA IMT is superior or inferior to any other IMT measure to predict future CVD[*Chambless* 1997, *Iglesias del Sol* 2002, *O'Leary* 1999]. The advantage of CCA IMT is that it is the easiest measure to obtain in most subjects.

Assessment of plaque occurrence and size was done in the entire CCA and bifurcation with good reproducibility. The assessment of plaque echogenicity was based on Gray-Weale et al [*Gray-Weale* 1988] but modified to two classes; 1) dominantly or substantially echolucent plaques, or 2) dominantly or uniformly echogenic. This classification was originally developed for large carotid plaques, but has also been shown to provide valuable information on plaque characteristics in smaller plaques [*Schmidt* 2005, *Schmidt* 2003].

Carotid plaques

The occurrence and increasing size of plaques are definite measures of atherosclerosis. Almost 60% of the women with known t2D had plaques in their carotid arteries compared to 40% in those in the NGT group. Almost all plaques were echolucent and there was no obvious increase in the proportion of echolucent plaques in parallel with worsening glucose tolerans. There was a gradual increase in echolucent plaque area that was lowest in NGT women, intermediate in new t2D and highest in known t2D. Our finding, that t2D is associated with increased occurrence of echolucent plaques, is in line with both accumulated data showing that echolucent plaques are associated with increases risk of cardiovascular disease [*Grogan* 2005, *Honda* 2004], and the association between diabetes and atherosclerotic disease [*Kannel* 1979]. However, the absolute majority of all plaques were echolucent. It may be assumed that small and early plaques contain less of fibrous tissue and calcium causing high echo-

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density. In the Tromsö study, increasing serum HbA1c concentrations across the cohort were also associated with increased risk of carotid plaques [*Jorgensen* 2004], but the association was only apparent with echogenic and not echolucent plaques. However, in that study all patients with diabetes were excluded, and there were also other methodological differences, such as only including plaques protruding into the lumen. In the present study a multivariate analysis showed that known t2D was associated with increased echolucent plaque area after adjustment for BMI, height, systolic blood pressure, smoking and LDL cholesterol in comparison to the NGT group. This result supports the concept that t2D causes atherosclerosis also by other mechanisms than those mediated by traditional risk factors.

Vascular remodelling

In comparison between groups, vascular features such as IMT and lumen diameter may differ by other causes than atherosclerosis. IMT is steadily increasing by age, and may also increase because of structural changes related to adaptation to wall stress and other causes [*Bots* 2005]. Lumen diameter is related to body size measured as height, body weight, BMI, and to blood pressure [*Bonithon-Kopp* 1996, *Henry* 2004].

Post-mortem studies performed by Glagov et al showed that human coronary arteries enlarge in relation to plaque area during the early phase of atherosclerosis, and that the lumen area is preserved or even overcompensated [*Glagov* 1987]. A few cross-sectional and one prospective studies have demonstrated that plaque occurrence or diffuse atherosclerosis in the carotid arteries is associated with enlarged vessel diameters [*Bonithon-Kopp* 1996, *Kiechl* 1999, *Montalcini* 2007, *Steinke* 1994]. The same seems to hold true for patients with known diabetes [*Henry* 2004].

In the present study clear proofs were found of vascular remodelling that had already occurred in women with screening detected t2D. Hence, CCA IMT, assessed as circumferential area was enlarged in combination with preserved or

even enlarged lumen diameter, remaining after adjustment for confounders such as height, obesity or differences in blood pressure. Further evidence that this was associated with the atherosclerotic burden in the carotid artery was obtained in the t2D women in whom lumen diameter increased by plaque burden after adjustment for differences in BMI and blood pressure. However in the subgroup of women without atherosclerotic plaques we found no differences in carotid artery diameters after adjustment for body size compared to the normal control group, indicating that remodelling is not related to diabetes per se. An intriguing fact is that we related the plaque area, measured in plaques that usually occur in the carotid bulb, to the lumen diameter in the CCA. However, other studies have shown that atherosclerosis in one part of the artery tree could affect another part of the artery [*Montalcini* 2007].

All used measures of IMT in the carotid arteries showed that both known and new t2D were enlarged in comparison to women with NGT. It should however, be kept in mind that as a consequence of the study design the women in the NGT group were free of cardiovascular disease and used no medication against hypertension or hyperlipidemi. Women on statin treatment had increased IMT in the different sections of the carotid arteries compared to t2D without medication, thus including them in the measurements did not reduce the differences seen between t2D and NGTr.

In the present study the difference between known t2D and controls was 0.09 mm (95% CI, 0.04 - 0.14) compared to 0.13 mm (95% CI, 0.129– 0.139) in the meta-analysis. The corresponding results for new t2D were 0.06 mm (95% CI, 0.01 – 0.11) and 0.11 (95% CI, 0.095 – 0.12). The confidence-intervals were, however overlapping between this study and the meta-analysis. The mean difference in reviewed studies measuring the IMT in predefined segments was 0.09 mm (95% CI, 0.091-0.097) even though 20% of these studies were including just new t2D. This difference is in harmony with our result. A former study in our group

between men with known t2D and controls showed a difference of 0.10mm [*Sigurdardottir* 2004].

Interpretation

Type 2 diabetes diagnosed by best available methods in a population sample is associated with carotid plaques seemingly increasing by diabetes duration. Apart from confirming results from the Horn study that t2D is associated with preserved carotid lumen diameter at increased IMT [*Henry* 2004] the present study also provides the new information that this has occurred already in subjects with very early t2D, detected by screening, and that this is related to the atherosclerotic burden. Complete ultrasound examinations were difficult to obtain in some women, who were associated with an increased occurrence of cardiovascular risk factors and indications of thicker IMT. It is reasonable to assume, that the exclusion of these women reduced the differences in atherosclerotic measures between women with and without diabetes.

Paper IV

IGT and subclinical atherosclerosis in the carotid arteries

IGT is generally regarded as a transient metabolic state occurring during the disease process leading to overt diabetes [*Alberti* 1996]. IGT is as such also a risk factor for future CVD [*Coutinho* 1999]. By defining IGT, as we did, by using a doubled OGTT, IGT would be a more selected group with an even higher risk factor for CVD. We included two healthy NGT groups; one randomly selected and one matched to the IGT group for body fat and body fat distribution. The assessment of subclinical atherosclerosis was based on measurements of IMT in CCA, the carotid bulb and the means of these measures. The carotid bulb is a predilection site for atherosclerotic process and shows a larger annual increase in IMT than the CCA [*Howard* 1993]. We also assessed the occurrence of plaques,

especially echolucent plaques which are known to be associated with increased risk of CVD in prospective studies [*Gronholdt* 2002, *Honda* 2004].

Missing data

In the screening process 269 women with IGT were identified after a doubled OGTT. The 212 first selected were further examined with ultrasound measurements. In seven women it was not possible to obtain readable ultrasound images, resulting in 205 women completing the study. The IGT women with incomplete ultrasound data (n=74 out of 205) were, as expected, more obese and had a thicker mean CCA intima-media.

Carotid IMT and plaque

In our population sample, none of the ultrasound measures indicated atherosclerotic disease associated with IGT. Hence, there was no difference in IMT measured in the CCA or carotid bulbs between the IGT group and the NGT groups. There was neither any indication of difference in the occurrence or size of carotid plaques. This was an unexpected finding given the fact that the IGT women were characterised by a more unfavourable cardiovascular risk profile with higher blood pressure, higher serum triglyceride levels and HDL cholesterol concentrations compared to the NGT group (Table 7). Potential confounders were concomitant treatment with statins, antihypertensive treatment and hormone replacement therapy. Adjusting for these variables or excluding women on such treatments did not change the results. A post-hoc analyse indicated that family history of maternal diabetes might be a confounder, as it was associated with an increase in common carotid artery IMT independently of the degree of glucose tolerance, and traditional cardiovascular risk factors. This observation is supported by results from previous studies showing that family history of diabetes is associated with CCA IMT enlargement in healthy subjects with normal glucose tolerance[*Kao* 2005, *Pannacciulli* 2003].

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Given the original power-calculation of the entire study and the results regarding IMT in the IGT and NGT groups, it is obvious that the study had an insufficient size to identify very small differences. In order to summarise the current data a new meta-analysis was undertaken including the present study, recently, and previously published reports.

Meta-analysis of studies measuring difference in intima-media thickness between IGT and normal glucose tolerance.

Our former meta-analysis was completed with the present study and two recently published studies [*Mohan* 2006, *Zhang* 2006]. In 2/3 of previously published studies the IGT group was older than the control group, the difference ranging from 1-6 years. We adjusted for age, as the annual increase in CCA IMT is calculated to 0.01mm [*Hedblad* 2000, *Howard* 1993, *Le Gal* 2003]. After adjustment for this difference, we found that in 3 out of 12 studies IGT was associated with a significant increased IMT compared to NGT [*Henry* 2004, *Temelkova-Kurktschiev* 2000, *Zhang* 2006]. In the meta-analysis IMT was overall 0.030 (95% CI 0.012 to 0.048) mm larger in the IGT group as if there was a difference of three years of age. Although heterogeneity was found, no obvious patterns of differences in IMT between IGT and NGT groups related to the different ultrasound methods could be discerned. Many of the studies were not performed in population-based samples, but represented varying selection criteria [*Ishizaka* 2003, *Keven* 1999, *Snehalatha* 2001, *Temelkova-Kurktschiev* 2000, *Zhang* 2006]. The possibility of publication bias of positive results must also be considered.

Interpretation

Our conclusion is that the present study was underpowered to identify small differences in IMT between the IGT and NGT groups. However, combining the present results with data from previous studies, a meta-analysis clearly shows that common carotid artery is slightly larger in IGT groups than that in subjects

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with NGT. However the atherosclerotic process does not seem to be pronounced, as large studies are needed to identify a minor increase in IMT. A family history of maternal diabetes seemed to be associated with increases in CCA IMT, independently of current glucose tolerance, given support to previous studies showing similar findings. Hence, it can be assumed that the increased risk of future cardiovascular disease associated with IGT is related to early sub-clinical atherosclerosis, and to impaired glucose tolerance as a marker of subsequent metabolic aberrations, including diabetes, hypertension, and dyslipidemia as powerful risk factors.

Limitations

The limitations of the present study are that only 64-year-old women were examined, that representativity may be questioned, and that exclusion criteria were used. The criteria used in the inclusion of women in the study excluded subjects with severe or inflammatory diseases. Hence, the results cannot be generalised to women with such diseases. The present study is further a cross-sectional study and no conclusion can be drawn regarding causality. Another limitation is that a larger sample size seems to be needed in order to identify sub-clinical atherosclerosis in women with IGT.

Complete ultrasound examinations were not possible in some subjects with increased risk of atherosclerosis. As a result the measured differences between the groups with glucose tolerance impairment and controls were probably less than the real differences if all measurements had been complete. The ultrasound technique does not allow a complete characterisation of the carotid artery geometry. As regards vessel geometry only lumen diameter and the far wall IMT measurements are representative of the underlying histology, necessitating calculations assuming symmetric wall changes in the common carotid arteries [*Wendelhag* 1991]. This seems to be the case as it has been shown that the increase in intimal thickness is uniform around the circumferences in the

common carotid artery, in which there is an axial, symmetric blood flow [*Zarins* 1983]. Another limitation is that only the carotid artery has been examined but measurements from this artery segment is an established surrogate measure of atherosclerotic disease [*Salonen* 1991].

Clinical implications

The results from this thesis indicate a screening strategy for diabetes using FBG ≥ 5.6 mmol/l (Fasting plasma glucose ≥6.1 mmol/l) as a first identification of cases targeted for further examinations with OGTT. A family history of diabetes among parents is important information as regards future diabetes and cardiovascular risk. The fact that IGT does not seem to be associated with very accelerated atherosclerosis, in combination with previous knowledge that life-style modifications reduces the risk for transition to diabetes, strongly indicate that IGT should be identified in order to start preventive measures. Our observation that already screening detected t2D has established atherosclerotic disease underlines the importance of early detection of diabetes and the need to start optimal risk factor reducing treatment.

Future perspectives

This study is a solid platform for a prospective study in order to follow the development of atherosclerotic disease in relation to characteristics at baseline and treatment efforts during follow-up.

Conclusions

Paper I. A systematic review of published studies using ultrasound to measure intima media thickness (IMT) in the common carotid artery (CCA) in type 2 diabetes and controls was performed, showing that in 20 out of 21 studies diabetic patients had greater carotid artery IMT than the subjects in the control groups. The meta-analysis demonstrated that type 2 diabetes was associated with an increase in common carotid artery IMT, which was 0.13 mm larger in diabetes groups than in control groups. This difference corresponds to a 10-year increase in age compared with age-matched controls and is associated with a nearly 40% increase in cardiovascular risk. The systematic review revealed heterogeneity between the different studies due to differences in the selection of study groups and also to methodological differences in assessing ultrasound measurements. The method to measure carotid IMT should be better standardised.

Paper II. The cohort of 64-year-old women in Göteborg was screened, and the prevalence's of known and new diabetes and impaired glucose tolerance (IGT) at both oral glucose tolerance tests (OGTT)s, were 4.7, 4.8 and 14.4 % respectively (n=2595). Half of the women with diabetes were previously undiagnosed and 37% of the diagnoses were based on a repeated OGTT. The degree of glucose tolerance impairment and the number of abnormal OGTTs were directly associated with occurrence of components of the metabolic syndrome. In future screening, impaired fasting glucose i.e. plasma-glucose ≥6.1 – 6.9 mmol/l might be useful in selecting subjects for further examinations with OGTT.

Paper III. In this cohort of 64 years old Swedish women with known (n=99) and new type-2 diabetes (n=106) and healthy subjects with normal glucose tolerance (n=99), ultrasound examinations showed that both new and known type 2 diabetes were characterised by enlarged IMT CCA compared to the women with normal glucose tolerance. Atherosclerotic plaques, which in general were echolucent, increased gradually in occurrence and size from controls, to new, and known type 2 diabetes. This difference remained after adjustment for

cardiovascular risk factors. Carotid artery plaque areas were associated with an increase in lumen diameter independently of blood pressure and BMI. This carotid vascular remodelling with enlargements in IMT and lumen diameter had occurred already in the women with new type 2 diabetes. The remodelling process occurred only in women with atherosclerotic plaques and was not associated to type 2 diabetes per se.

Paper IV. In the cohort of 64-year old women, IGT, as defined by repeated OGTT, was not associated with increased occurrence of sub-clinical atherosclerosis measured as IMT or plaque occurrence or size compared to healthy control women with normal glucose tolerance. As the study was under-powered to identify small differences in IMT a meta-analysis of 11 previously published studies, using only one OGTT for diagnosing IMT, and this study was performed. In three out of 12 reviewed studies IMT was larger in IGT groups than in control groups. A meta-analysis of the studies indicated a difference in IMT of 0.03 mm between IGT and controls and heterogeneity was shown. The 95% confidence interval of the present study (-0.03 to 0.03 mm) was within the confidence interval of the meta-analysis (0.012-0.048 mm), indicating that IGT is associated with a small increase in IMT in the CCA.

Populärvetenskaplig sammanfattning.

Det moderna västerländska levnadssättet har medfört att människan rör sig allt mindre och konsumerar allt energirikare mat. Över hela världen sker därför en påtaglig ökning av övervikt och fetma med åtföljande vällevnadssjukdomar som hjärtkärlsjukdom och diabetes. FN har alldeles nyligen klassificerat diabetes som en epidemi dvs. en sjukdom som ökar mkt kraftigt och med stora hälsorisker till följd. Diabetes har också visat sig vara en stor riskfaktor för hjärtkärlsjukdom. Normalt brukar kvinnor insjukna senare än män i kärlsjukdomar men när det gäller diabetes utjämnas könsskillnaden och kvinnor har nästan lika stor risk som män att avlida i hjärt- kärlsjukdom.

Diabetes kan diagnostiseras på två sätt, antingen som ett förhöjt fasteblodglukos eller som ett förhöjt värde vid ett belastningstest "oralt glukos tolerans test" (OGTT). I båda fallen krävs förhöjda värden vid två olika tillfällen. De personer som har ett förhöjt belastningsvärde men inte når upp till diabetesgränsen benämns ha "nedsatt glucos tolerans" (IGT) vilket i sig indikerar en ökad risk att på sikt utveckla diabetes. Vid ett OGTT får personen dricka en sockerlösning och efter två timmar skall blodsockret ha kommit ned till ett visst värde för att betecknas som normal glukostolerans.

Hjärtkärlsjukdom beror nästan alltid på åderförkalkning (ateroskleros) som i allmänhet utvecklas under lång tid utan några symptom. Ateroskleros uppträder oftast i vissa pulsådror, t.ex. i hjärtats kranskärl eller i halspulsådern. Med hjälp av ultraljudet kan man mäta kärlväggens tjocklek och även se eventuell förtjockning i kärlväggen "plack". Det har visat sig att dessa mätningar kan förutsäga risken för framtida kärlsjukdomar som stroke och hjärtinfarkt. Man kan också på detta sätt få en bild över vilka riskfaktorer som bidragit till aterosklerosutvecklingen.

Denna avhandling handlar om riskerna med diabetes och förstadier till diabetes för utvecklandet av ateroskleros.

I) I det första delarbetet identifieras och sammanfattas tidigare studier som jämfört väggtjockleken i halsartären (IMT) mellan typ 2 diabetiker, IGT och kontrollpersoner. Av 21 funna studier visade alla utom fyra att diabetiker hade tjockare kärlvägg än icke-diabetiker och den genomsnittliga skillnaden i meta-analysen uppmättes till 0,13 mm. Vad gäller förstadiet till typ 2 diabetes (IGT) var skillnaden inte så tydlig. I enbart tre av nio funna studier såg man en skillnad som i genomsnitt uppmättes till 0,04 mm. Översätter man skillnaden som uppmättes mellan diabetiker och icke-diabetiker till risker för framtida kärlsjukdomar så betyder detta att diabetikerna hade 38 % ökad risk för hjärtinfarkt och 37 % ökad risk för hjärninfarkt. Då väggtjockleken ökar normalt med åldern kan skillnaden sägas motsvara 10 års ökad ålder till diabetikernas nackdel. Vi fann dock att det förelåg en större skillnad i resultaten mellan studierna än vad som kunde förväntas vara en slumpmässig skillnad. Denna skillnad mellan studierna kunde bl.a. förklaras av olika mätmetoder vid ultraljudsundersökningarna, olika sätt att samla in studiedeltagare och kanske också av att det är lättare att få ett positivt resultat publicerat.

II) I det andra delarbetet redovisas erfarenheterna från insamlandet av den egna studiegruppen. I syfte att få tag på tillräckligt antal diabetiker undersöktes 2595 kvinnor, som alla var 64 år gamla, med ett upprepat oralt glukos tolerans test (OGTT). Som resultat av undersökningen fann vi 10 % diabetiker varav hälften var nyupptäckta. Vidare hade 15 % av kvinnorna IGT efter två upprepade OGTT. Vi fann att de kvinnor som hade ett förhöjt glukosvärde vid enbart ett test var mer överviktiga med ett större bukomfång och ett högre blodtryck jämfört med dem som hade normala glukosvärden. Hade vi enbart mätt fasteblodglukos och inte använt ett OGTT hade 37 % av de nyupptäckta diabetikerna missats. OGTT är resurskrävande och ett sätt att välja ut de som skulle genomgå testet vore att enbart testa de kvinnor som i fasteblodglukos låg strax under diabetesgränsvärdet dvs. mellan 5.6 och 6.0 mmol/l. Hade vi tillämpat denna strategi och testat enbart dessa 472 kvinnor i stället för 2475 hade vi missat 16 nya diabetesfall men sluppit över 2000 tester.

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III) I det tredje delarbetet mätte vi väggtjocklek, plackförekomst och diameter i halspulsådror hos diabetiker och friska kontroller i vårt eget material. Vi fann att kända diabetiker hade tjockast vägg; i genomsnitt 0.09 mm tjockare än kontroller. De nyupptäckta diabetikerna hamnade ungefär på halva skillnaden i väggtjocklek gentemot kontroller. Omkring 60 % av kända diabetiker hade plaque mot 40 % av kontrollerna. Vi såg också att plaqueytan var större hos de kända diabetikerna. Kärldiametern var dock bevarad eller kompensatoriskt förstorad hos diabetikerna trots att kärlväggen var tjockare och att där förelåg plaque. Denna kompensatoriska kärlombyggnad var tydlig även när vi tog hänsyn till andra faktorer som övervikt, längd och blodtryck. Kärlombyggnaden var tydligast hos de diabetiker som hade plaque och förklaras därigenom av aterosklerosutvecklingen och inte av diabetessjukdomen i sig. Diabetes bidrar till ateroskleros men först när aterosklerosplack har uppkommit, ser vi den kompensatoriska kärlombyggnaden.

IV) I det fjärde delarbetet undersöktes IGT i jämförelse med de friska kvinnorna. Här sågs igen skillnad vare sig i väggtjocklek eller i plaqueförekomst. En uppdaterad sammanställning av alla gjorda publicerade studier med tillägg av vår egen genomfördes, en s.k. metaanalys. Den förnyade utökade metaanalysen som omfattade 12 studier och 5787 individer visade en skillnad på 0,03 mm mellan IGT och kontroller. Vid beräkningar framkom att vår studie hamnade inom konfidensintervallet (sannolikhetsintervallet) för meta-analysen.

Sammanfattningsvis framkommer det att redan vid nyupptäckt diabetes ses ateroskleros i form av förtjockad kärlvägg, ökad mängd plack och därmed sammanhängande kärlombyggnad. Det är därför viktigt att upptäcka förstadierna till diabetes för att försöka förhindra uppkomsten av sjukdomen. Det är också viktigt att vid nyupptäckt diabetes tidigt och aktivt bekämpa höga blodsockernivåer, blodfetter och högt blodtryck.

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