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FACTORS PREDICTING MORTALITY IN TYPE 2 DIABETES

With special reference to physical exercise, blood pressure, proteinuria, inflammation and P wave duration

by

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To my family

ABSTRACT

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Factors predicting mortality in type 2 diabetes. With special reference to physical exercise, blood pressure, proteinuria, inflammation and P wave duration

From the Department of Medicine, University of Turku, Turku, Finland and the Department of Endocrinology, Division of Medicine, Turku University Hospital, Turku, Finland

Background: Type 2 diabetes patients have a 2-4 fold risk of cardiovascular disease (CVD) compared to the general population. In type 2 diabetes, several CVD risk factors have been identified, including obesity, hypertension, hyperglycemia, proteinuria, sedentary lifestyle and dyslipidemia. Although much of the excess CVD risk can be attributed to these risk factors, a significant proportion is still unknown.

Aims: To assess in middle-aged type 2 diabetic subjects the joint relations of several conventional and non-conventional CVD risk factors with respect to cardiovascular and total mortality.

Subjects and methods: This thesis is part of a large prospective, population based East-West type 2 diabetes study that was launched in 1982-1984. It includes 1,059 middle-aged (45-64 years old) participants. At baseline, a thorough clinical examination and laboratory measurements were performed and an ECG was recorded. The latest follow-up study was performed 18 years later in January 2001 (when the subjects were 63-81 years old). The study endpoints were total mortality and mortality due to CVD, coronary heart disease (CHD) and stroke.

Results: Physically more active patients had significantly reduced total, CVD and CHD mortality independent of high-sensitivity C-reactive protein (hs-CRP) levels unless proteinuria was present. Among physically active patients with a hs-CRP level >3 mg/L, the prognosis of CVD mortality was similar to patients with hs-CRP levels ≤3 mg/L. The worst prognosis was among physically inactive patients with hs-CRP levels >3 mg/L. Physically active patients with proteinuria had significantly increased total and CVD mortality by multivariate analyses. After adjustment for confounding factors, patients with proteinuria and a systolic BP <130 mmHg had a significant increase in total and CVD mortality compared to those with a systolic BP between 130 and 160 mmHg. The prognosis was similar in patients with a systolic BP <130 mmHg and ≥160 mmHg. Among patients without proteinuria, a systolic BP <130 mmHg was associated with a non-significant reduction in mortality. A P wave duration ≥114 ms was associated with a 2.5-fold increase in stroke mortality among patients with prevalent CHD or claudication. This finding persisted in multivariable analyses. Among patients with no comorbidities, there was no relationship between P wave duration and stroke mortality.

Conclusions: Physical activity reduces total and CVD mortality in patients with type 2 diabetes without proteinuria or with elevated levels of hs-CRP, suggesting that the anti-inflammatory effect of physical activity can counteract increased CVD morbidity and mortality associated with a high CRP level. In patients with proteinuria the protective effect was not, however, present. Among patients with proteinuria, systolic BP <130 mmHg may increase mortality due to CVD. These results demonstrate the importance of early intervention to prevent CVD and to control all-cause mortality among patients with type 2 diabetes. The presence of proteinuria should be taken into account when defining the target systolic BP level for prevention of CVD deaths. A prolongation of the duration of the P wave was associated with increased stroke mortality among high-risk patients with type 2 diabetes. P wave duration is easy to measure and merits further examination to evaluate its importance for estimation of the risk of stroke among patients with type 2 diabetes.

Key words: Type 2 diabetes, cardiovascular disease, mortality, physical activity, proteinuria, blood pressure, hs-CRP, ECG, P-wave duration

TIIVISTELMÄ

Teemu Vepsäläinen

Tekijät, jotka ennustavat kuolleisuutta tyypin 2 diabeetikoilla. Erityisesti fyysinen aktiivisuus, verenpaine, proteinuria, inflammaatio, ja P aallon kesto.
Lääketieteellinen tiedekunta, Turun yliopisto, Turku, Suomi ja Endokrinologian yksikkö, Turun yliopistollinen keskussairaala, Turku, Suomi

Tausta: Tyypin 2 diabeetikoilla on 2-4 kertaa niin suuri riski sairastua sydän- ja verisuonisairauksiin (CVD) kuin ei-diabeetikoilla. Useita CVD riskitekijöitä on tunnistettu, kuten lihavuus, hypertensio, hyperglykemia, proteinuria, liikkumaton elämäntyyli, ja rasva-aineenvaihdunnan häiriöt. Vaikka suurentunut CVD riski selittyykin osittain näillä riskitekijöillä, merkittävä osa on edelleen tuntemattomien tekijöiden aiheuttamaa.

Tavoite: Tutkia perinteisten ja ei-perinteisten CVD riskitekijöiden keskinäistä yhteyttä sydän- ja verisuonitauti sekä kokonaiskuolleisuusriskiin

Menetelmät: Tämä väitöskirjatutkimus toteutettiin osana laajaa, pitkittäistä väestöön perustuvaa Itä-Länsi tyypin 2 diabetes – tutkimusta, missä kattava kliininen alkututkimus, laboratoriokokeet ja EKG suoritettiin vuosina 1982–1984. Tutkimus käsittää 1,059 keski-ikäistä (45-64 vuotiaita) tyypin 2 diabeetikkoa. Seuranta-aika kesti 18 vuotta, joka loppui tammikuussa 2001 (tutkittavat tuolloin olivat iältään 63-81 vuotiaita). Päätetapahtumat olivat kokonais- sepelvaltimotauti- (CHD), CVD ja aivohalvauskuolleisuus.

Tulokset: Fyysisesti aktiivisilla potilailla oli merkitsevästi vähentynyt kokonais-, CVD tai CHD kuolleisuus riippumatta hs-CRP tasoista. Proteinuriassa liikunnan suojavaikutusta ei todettu. Fyysisesti aktiivisilla potilailla, joilla hs-CRP oli yli 3 mg/L, ennuste oli vastaava kuin potilailla, joilla hs-CRP oli ≤3 mg/L. Huonoin ennuste CVD kuolleisuuden suhteen oli potilailla, jotka olivat liikkumattomia ja joilla hs-CRP oli yli 3 mg/L. Lisäksi, proteinurisilla potilailla fyysinen aktiivisuus oli yhteydessä lisääntyneeseen kuolleisuuteen monimuuttuja-analyysissa ja systolinen verenpaine <130 mmHg verrattuna muihin systolisen verenpaineen luokkiin välillä 130-160 mmHg, oli yhteydessä merkittävästi lisääntyneeseen CVD sekä kokonaiskuolleisuuteen. Ennuste oli samankaltainen kuin potilailla, joilla systolinen verenpaine oli ≥160 mmHg. Potilailla, joilla ei ollut proteinuriaa, matala systolinen verenpaine <130 mmHg oli yhteydessä vähentyneeseen kuolleisuuteen. Kuitenkaan tämä yhteys ei saavuttanut tilastollista merkitsevyyttä. Potilailla, joilla oli oireinen sepelvaltimotauti tai klaudikaatio sekä P aallon kesto EKG:stä mitattuna ≥114 ms, oli 2.5 kertainen riski aivohalvauskuolleisuuteen, Tämä löydös oli merkitsevä myös monimuuttuja-analyysissä. Vastaavaa ei havaittu potilailla, joilla ei ollut oireista sepelvaltimotautia tai klaudikaatiota.

Johtopäätökset: Fyysinen aktiivisuus vähentää kokonais-, CVD ja CHD kuolleisuutta tyypin 2 diabeetikoilla, joilla ei ole proteinuriaa tai potilailla, joilla on kohonneet hs-CRP arvot. Osatekijänä voi olla liikunnan tulehdusta rauhoittava vaikutus. Proteinurisilla potilailla, liikunnan suojaavaa vaikutusta ei havaittu. Lisäksi proteinurisilla potilailla systolinen verenpaine <130 mmHg saattaa olla yhteydessä kohonneeseen CVD riskiin. Nämä tulokset viittaavat aikaiseen sydän- ja verisuonitaudin ehkäisyn tärkeyteen ennen merkittävien riskitekijöiden ilmaantumista. Proteinurian olemassaolo tulisi ottaa huomioon verenpaineen tavoitetasoja määritettäessä ehkäistäessä sydän- ja verisuonitautitapahtumia. Pidentynyt P aalto oli yhteydessä kohonneeseen aivohalvausriskiin korkean riskin potilailla. Helposti mitattavana riskitekijänä P aallon asemaa aivohalvauskuolleisuutta ennustavana tekijänä tulisi tutkia lisää.

Avainsanat: Tyypin 2 diabetes, sydän- ja verisuonitauti, kuolleisuus, fyysinen aktiivisuus, proteinuria, verenpaine, hs-CRP, EKG

TABLE OF CONTENTS

ABBREVIATIONS

ACCORD	Action to Control Cardiovascular Risk in Diabetes trial
ACEI	Angiotensin converting enzyme inhibitors
ADA	American Diabetes Association
AF	Atrial fibrillation
AGE	Advanced glycosylation end products
AHEAD trial	Action for Health in Diabetes trial
AHA	American Heart Association
apoB	Apolipoprotein B
ARB	Angiotensin receptor blockers
BMI	Body mass index
BP	Blood pressure
CAD	Coronary artery disease
CAN	Cardiac autonomic neuropathy
CHD	Coronary heart disease
CI	Confidence interval
CVD	Cardiovascular disease
DAN	Diabetic autonomic neuropathy
DN	Diabetic nephropathy
DPS	Diabetes Prevention Study
DR	Diabetic retinopathy
ECG	Electrocardiogram
ESRD	End-stage renal disease
FFA	Free fatty acid
GAD	Glutamic acid decarboxylase
GBM	Glomerular basement membrane
HbA_1	Hemoglobin A_1
HbA_{1c}	Hemoglobin A_{1c}
HDL	High density lipoprotein
hs-CRP	High-sensitivity C-reactive protein
HR	Hazard ratio or Heart rate
IAB	Interatrial block
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IL-6	Interleukin-6
INVEST	The International Verapamil SR-Trandolapril Study
LDL	Low density lipoprotein
LVH	Left ventricular hypertrophy

MA	Microalbuminuria
MAPK	Mitogen-activated protein kinase
MET	Metabolic equivalent task
MetS	Metabolic syndrome
MESA	Multi-Ethnic Study of Atherosclerosis
MI	Myocardial infarction
OGTT	Oral glucose tolerance test
OxLDL	Oxidized low-density lipoprotein
PAI-1	Plasminogen activator-1
PNMMVD	Prevalent non-major macrovascular disease
ROS	Reactive oxygen species
SD	Standard deviation
sdLDL	Small-dense low density lipoprotein
T2DM	Type 2 diabetes mellitus
UKPDS	United Kingdom Prospective Diabetes Study
VLDL	Very low density lipoprotein
vWF	von Willebrand factor
WHO	World Health Organization

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by the following roman numerals, and on some unpublished results.

I. Vepsäläinen T, Soinio M, Lehto S, Juutilainen A, Laakso M, Rönnemaa T. Proteinuria modifies the effects of physical activity on total and cardiovascular disease mortality rates in patients with type 2 diabetes. Diabetologia. 2010;53:1886-1889.

II. Vepsäläinen T, Soinio M, Marniemi J, Lehto S, Juutilainen A, Laakso M, Rönnemaa T. Physical activity, high-sensitivity C-reactive protein, and total and cardiovascular disease mortality in type 2 diabetes. Diabetes Care. 2011;34:1492-1496.

III. Vepsäläinen T, Laakso M, Kantola I, Lehto S, Juutilainen A, Rönnemaa T. Proteinuria modifies the effect of systolic blood pressure on total and cardiovascular disease mortality in patients with type 2 diabetes. J Intern Med. 2012;272:611-619.

IV. Vepsäläinen T, Laakso M, Lehto S, Juutilainen A, Airaksinen J, Rönnemaa T. P wave duration predicts stroke mortality among type 2 diabetic patients with prevalent coronary heart disease or claudication. Submitted.

1. INTRODUCTION

The incidence of type 2 diabetes mellitus (T2DM) in Finland is constantly rising. In patients with type 2 diabetes, atherosclerotic diseases are more common and this adds to the burden of illness to society. Mortality is higher than among non-diabetics after a cardiovascular disease (CVD) event: acute MI, stroke, or peripheral vascular disease (1-3). Of adult diabetic patients, >75% die from these complications (4). In addition to CVD, diabetes is often associated with microvascular complications, i.e., diabetic retinopathy (DR), nephropathy (DN) and neuropathy. Microalbuminuria (MA) and proteinuria, which are markers of incipient nephropathy, are strong and independent predictors of CVD and total mortality among patients with type 2 diabetes (5-7). These complications are a major burden to the patient, his relatives and the health care system. Hence, it is very important to identify high-risk patients before diabetic complications develop. It is important to tailor effective treatments for these patients with the aim to reduce CVD mortality.

Type 2 diabetes is associated with several CVD risk factors, e.g., obesity, high levels of circulating free fatty acids (FFA), low-grade inflammation, hyperglycemia, insulin resistance, hypertension, sedentary lifestyle and serum lipid and lipoprotein abnormalities, characterized mainly by high serum triglycerides and low high-density lipoprotein (HDL) cholesterol levels (8-10). Although much of the excess CVD risk can be attributed to these risk factors, a significant proportion of these risks are still unknown. There is also some evidence speaking in favor of a joint association of these risk factors with respect to major macrovascular disease events, especially among high-risk patients who carry serious comorbidities, e.g., microvascular complications or non-major coronary artery disease (CAD).

Due to the high prevalence of type 2 diabetes and CVD in Finland, a prospective, population based East-West type 2 diabetes study was launched in 1982-1984 in Kuopio and in Turku, to assess the CVD risk factor levels and their determinants in middle-aged patients with type 2 diabetes and their relation to macrovascular events, including total mortality and mortality due to CVD, coronary heart disease (CHD) and stroke. Altogether 1,059 patients participated at baseline. The most recent assessment of mortality was concluded in January 2001, 18-years after the baseline study.

In the present thesis the main objectives were to study 1) the association between physical activity in diabetic patients (stratified by urinary protein excretion and elevated inflammation markers) and mortality, 2) the association between blood pressure and proteinuria with respect to mortality and 3) the association between atrial functioning (measured by the duration of the P wave in electrocardiograms (ECG)) and prevalent non-major macrovascular disease with respect to mortality.

2. REVIEW OF THE LITERATURE

2.1 GENERAL ASPECTS OF TYPE 2 DIABETES

In 2010, diabetes mellitus affected some 285 to 350 million persons worldwide, 90% of these being type 2 diabetes patients (11-15). These estimates originate from health examination surveys and epidemiological studies. The varying prevalence of diabetes between the different studies is probably due to variations in inclusion and exclusion criteria and the number of studies used. In Finland, the prevalence of type 2 diabetes is growing into epidemic portions. In 2006, 16% of adult males and 11% of adult females had type 2 diabetes and 42% of all males and 33% of all females had disturbed glucose metabolism (16). In patients with type 2 diabetes, atherosclerosis is more common and mortality is higher after CVD events than among non-diabetic persons (1-3). The incidence of type 2 diabetes will probably rise as a consequence of lifestyle patterns contributing to obesity (16, 17); this will raise the prevalence of diabetic populations with CVD complications.

2.1.1 Pathophysiology of type 2 diabetes

Type 2 diabetes is characterized by insulin resistance, hepatic overproduction of glucose (gluconeogenesis) and β-cell dysfunction (18). Usually, T2DM becomes manifest in middle or old age. However, among children and young adults the incidence of T2DM has been increasing in recent years (19). T2DM is still underdiagnosed and almost half of the patients are not aware of the disease (20). It is associated with a strong familial and genetic predisposition (21-24).

Most patients with type 2 diabetes are obese, and obesity itself may cause or aggravate insulin resistance (18). Abdominal obesity and clustering of CVD risk factors usually precedes the onset of T2DM (25-28). An excess of visceral and subcutaneous abdominal fat leads to increased efflux of FFAs from the adipose tissue and influx into the liver. This leads to dyslipidemia and accumulation of triglycerides in the liver and skeletal muscle (28-31). In the prediabetic state, insulin secretion is increased to compensate for the reduced insulin action in the liver and peripheral tissues. Due to an impaired antilipolytic effect of insulin, FFA concentrations increase, and this further aggravates insulin resistance. This prediabetic state also includes low-grade inflammation, increased thrombogenesis and endothelial dysfunction.

Although insulin resistance is an important pathogenic factor for the development of T2DM, ultimately, β-cell failure is responsible for the progression of IGT and IFG to T2DM.

As long as β-cells are able to secrete sufficient amounts of insulin to offset the severity of insulin resistance, glucose tolerance remains normal. The first defects in insulin secretion are changes in the oscillatory patterns and progressive loss of the first phase

insulin release in response to food intake; this causes postprandial hyperglycemia. With time, also the second phase of insulin secretion becomes gradually reduced in some individuals. Overt diabetes is associated with a progressive decline in insulin secretion with little further change in insulin resistance (Figure 1) (28, 32, 33).

Type 2 diabetes does not usually lead to ketoacidosis. Hyperglycemia and type 2 diabetes are treated with lifestyle interventions, oral medications and finally, over time, because glycemic control tends to worsen, insulin (34) or nowadays also glucagon-like peptide-1 analogues.

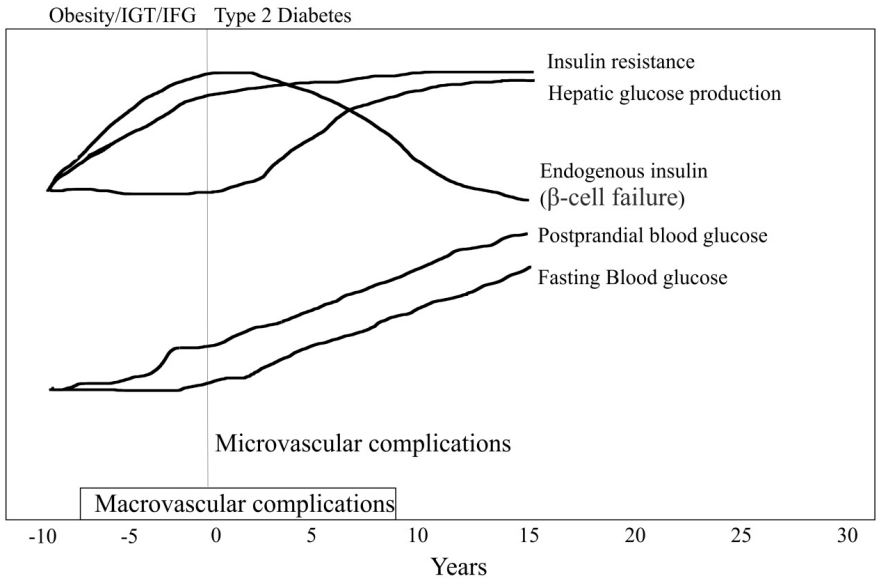


Figure 1. Natural history of type 2 diabetes (28).

2.1.2 Diagnostic criteria of type 2 diabetes

The first diagnostic criteria of diabetes were published by the World Health Organization (WHO) Study Group on Diabetes Mellitus in 1979, they were endorsed in 1980 and redefined in 1985. In 1985, the fasting plasma glucose criterion (≥7.8 mmol/L) and the criteria of impaired glucose tolerance (IGT) were presented (35-37). In 1997, the American Diabetes Association (ADA) lowered the diagnostic cut-off point of fasting plasma glucose to ≥7.0 mmol/L, because retinopathy evolved when fasting plasma glucose exceeded 7.0 mmol/L. A new category was introduced: impaired fasting glucose (IFG, 6.1–6.9 mmol/L) to supplement impaired glucose tolerance diagnosed by the oral glucose tolerance test (OGTT) (12, 38). In 1999, IFG was added to the WHO diagnostic criteria and at the same time, the terms "type 1" and "type 2" diabetes were adopted to replace "insulin dependent" and "non-insulin dependent" diabetes (12). Later, in 2005, ADA lowered the IFG category from ≥ 6.1 mmol/L to ≥5.6 mmol/L (39). The cut-point for diabetes is >11.0 mmol/L in the OGTT and ≥7.8 mmol/L for IGT. The diagnosis of

diabetes should preferable be based on venous plasma samples, not finger-stick glucose determinations. Glucose concentration measured from whole blood is approximately 11% times lower than the concentration in the plasma, and the difference is dependent on the patient's hematocrit. In human blood, glucose is evenly distributed between erythrocytes and plasma, like water. The molality of glucose is the same throughout the sample, but the concentration in the plasma is higher, because the concentration of water is higher in the plasma than in the erythrocytes (40).

Type 1 diabetes (T1DM) is characterized by β-cell destruction, which is usually due to autoimmune mechanisms. T1DM usually becomes manifest in young people and its diagnosis is based on symptoms of insulin deficiency: hyperglycemia, ketoacidosis and weight loss. Antibodies against insulin, islet cells and/or glutamic acid decarboxylase (GAD) antibodies are often detected (41). Complete insulin deficiency usually develops within five years of the diagnosis (41). Latent autoimmune diabetes in adults (LADA), characterized by islet-cell and/or GAD antibodies, usually becomes manifest at an older age (>30 years). It progresses rapidly and destroys β-cells, as in T1DM, but LADA patients are usually insulin independent for at least 6 months after the diagnosis (42, 43)

Monogenic forms of diabetes include maternally inherited mitochondrial diabetes (44) and six subtypes of genetically characterized maturity onset diabetes of the young (MODY) (45, 46). Other diabetes entities include gestational diabetes, a diabetic state that emerges during pregnancy and resolves at childbirth. It is associated with an increased maternal risk of T2DM in later life. Multiple endocrinopathy, diseases of the exocrine pancreas and some drug- and toxin-induced forms of diabetes are also known.

The diagnostic criteria are identical regardless of the etiology of the diabetes. Today, the diagnosis of diabetes is based on high levels of fasting glucose, on high post-challenge glucose levels, or on a glycosylated hemoglobin A_{1c} (HbA_{1c}) level of 6.5% or more. Symptoms (polyuria, polydipsia, weight loss) together with an increased random plasma glucose level ≥11.1 mmol/L fulfil also the criteria of diabetes (Table 1).

Table 1. Diagnostic criteria of diabetes and other hyperglycemic states.

Plasma glucose sample	Normal	IGT*	IFG†	Prediabetes‡	Diabetes
Fasting (mmol/l)	≤6.0 (WHO) ≤5.5 (ADA)		6.1–6.9 (WHO) 5.6–6.9 (ADA)	6.1-6.9	≥7.0
2-h-post challenge (mmol/l)	<7.8	7.8–11.0		7.8-11.0	>11.0
Random sample (symptomatic patient) (mmol/l)					>11.0
HbA_{1c} (%)				5.7-6.4	≥6.5 (48 mmol/mol)

Table modified from the Finnish national guidelines of diabetes treatment and from the WHO diagnostic criteria (12, 47, 122). The oral glucose load for adults is 75 g.

* IGT impaired glucose tolerance, † IFG impaired fasting glucose, ‡ Prediabetes is defined as IGT+IFG

2.2 CARDIOVASCULAR DISEASE AND TYPE 2 DIABETES

Type 2 diabetes increases the risk of CVD two- to four-fold compared with the general population. The risk seems to be even higher in women (9, 10, 48-51). The sex difference might be explained by a more unfavorable cardiovascular risk profile in diabetic women than in men. Diabetic women seem to have higher blood pressure and lipid levels than women without diabetes or men with diabetes, suggesting that the sex difference in CHD risk is mediated largely by differences in the levels of cardiovascular risk factors. Another explanation may be treatment bias favoring men. Recent studies found that men with diabetes or established cardiovascular disease are more likely to receive aspirin, statins or antihypertensive drugs than are women (50-55).

Patients with type 2 diabetes and a history of myocardial infarction (MI) or microvascular complications, e.g., proteinuria, are at a particularly high-risk for new manifestations of CVD. Previously, Haffner et al. observed that diabetic patients without prior MI have as high a risk of CHD death as do non-diabetic subjects with previous MI (48). Similar results were obtained from the same study cohort after prolonged follow-up for 18 years and also from a large epidemiological study which included all 3.3 million Danish residents on glucose-lowering therapy and aged at least 30 years (56, 57). These findings indicate that diabetes is a CVD equivalent.

There are, however, also results contradicting the concept that diabetes may be a CVD equivalent. In 2008, Bulugahapitiya et al. published a systematic review and meta-analysis of 13 studies, involving 45,108 patients aged 25-84 years. Follow-up varied from 5 to 25 years. In many of the studies the diagnosis of diabetes was self-reported and it was unclear if the subjects had type 1 or type 2 diabetes. Data on end points in most studies was collected by self-reporting. The authors reported that the risk of CHD was significantly lower (43%) in diabetic subjects without prior MI than in non-diabetic subjects with a prior MI, with a summary odds ratio of 0.56 (95% CI, 0.53-0.60), except in two of the studies (58). Other studies have reported similar results, especially concerning male subjects (59). The differences in these study results may be explained by different study designs and variable criteria for diagnosing diabetes, different criteria for CVD and different ways of verifying CVD at baseline and during follow-up.

Ethnicity, age and gender affect the CVD risk. For example, most of the studies not showing CVD equivalence for diabetes have been performed in US and UK populations (58). Furthermore, accumulation of CVD risk factors, hyperglycemia burden, characterized by the duration and severity of diabetes and the diabetes medication used by the patients affect the relative risk of CVD of an individual patient. Metabolic disturbances, including hypoglycemia induced with intensive glucose lowering medication and microvascular complications may further increase the risk of CVD in diabetic subjects (60).

In conclusion, diabetes increases the risk of CVD events substantially, and very often the risk may be as high as for individuals with prior CVD.

In the following chapter, the CVD risk factors related to type 2 diabetes are reviewed with focus on high-risk diabetic patients, treatment of T2DM and the pathophysiology of diabetic vascular disease.

2.2.1 Pathophysiology of diabetic vascular disease

Type 2 diabetes accelerates the progression of atherosclerosis (9, 10). It augments pathological atherosclerotic processes, endothelial dysfunction and the hallmark of early atherosclerotic lesions: localized accumulation of foam cells that herald fatty streaks. Diabetes also alters vascular smooth muscle cells in such a way that promotes the formation of atherosclerotic lesions, plaque instability and, ultimately, clinical events. Diabetes modifies also the coagulability of blood and increases platelet adhesion and aggregation. These changes may augment the progression of atherosclerosis and the consequences of plaque rupture resulting in thrombotic occlusion. Some of the crucial abnormalities related to vascular phenomena described above include hyperglycemia, insulin resistance and dyslipidemia (61). The atherosclerotic process begins well before type 2 diabetes is present. The prediabetic state, which is closely associated with the metabolic syndrome (MetS), is characterized by a number of adverse changes in CVD risk factors which in concert increase the risk for CVD (60-62). Once the patient has developed frank diabetes, the abnormal metabolic state of diabetes further augments the conventional CVD risk factors and the MetS, resulting in accelerated atherosclerosis (9, 28, 60).

2.2.1.1 Endothelium function

The healthy endothelium reacts to regulation of inflammation, platelet activation, leukocyte adhesion, blood vessel tone and thrombogenesis by vasodilation and by launching anti-atherogenic and anti-inflammatory responses (63). When these mechanisms fail, i.e., if the endothelium is not healthy, atherosclerosis ensues and progresses. Endothelial dysfunction is characterized by increased vascular tone and by vascular smooth muscle cell growth and migration, increased inflammatory gene expression and thrombogenesis. These adverse events are due to decreased nitric oxide bioavailability, increased endothelin-1 and angiotensin II concentrations, activation of the transcription factors nuclear factor kB (NF-kB) and activator protein 1, increased productions of tissue factor and plasmin activator inhibitor 1 (PAI-1) and decreased prostacyclin concentrations (61). Endothelial dysfunction may be considered as an early phase in the pathogenesis of CVD and the development of microvascular complications (64, 65). Hyperglycemia, and the constellation of risk factors associated with insulin resistance and the metabolic syndrome, contributes to endothelial dysfunction in type 2 diabetes (64-66). However, the molecular mechanisms overlap and make it difficult to identify specific mechanisms. Diabetic subjects express complex mechanisms that lead to atherosclerosis and microvascular complications (Figure 2). Among the pathogenic features, oxidative stress and inflammatory responses may be the first abnormalities which trigger several other mechanisms in diabetes-associated atherosclerosis (67, 68).

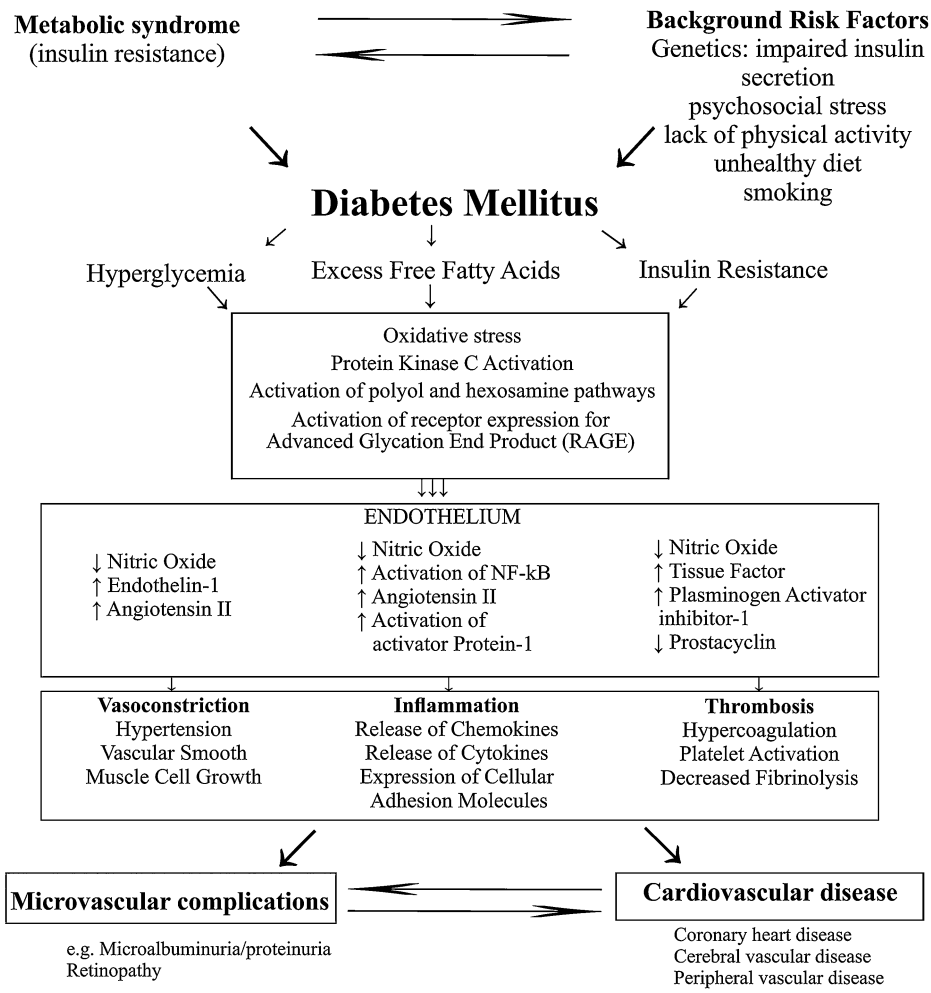


Figure 2. Endothelial dysfunction, risk factors and co-morbidities in type 2 diabetes mellitus [Modified from Beckman et al. (61)].

2.2.2 Diabetes-specific CVD risk factors

2.2.2.1 Hyperglycemia

Hyperglycemia, measured by the plasma glucose or by the HbA_{1c} level, induces overproduction of superoxide by the mitochondrial electron transport chain. Among the cells ill affected by hyperglycemia are the capillary endothelial cells of the retina, the mesangial cells of the renal glomerulus and the neurons and Schwann cells of peripheral nerves (64). These cells are at particularly high risk for damage because they are lack the capacity to regulate glucose uptake during hyperglycemia (69, 70). The mitochondrial electron transport chain activates four detrimental pathways: the polyol pathway, the hexosamine pathway, the protein kinase C (PKC) pathway and the advanced glycation end (AGE) products formation pathway (64).

The polyol pathway increases the susceptibility of tissues to intracellular oxidative stress and it seems to affect nerve conduction velocities, at least in diabetic animal studies (64, 71). The AGE product formation leads to both microvascular and macrovascular complications, including retinopathy, nephropathy and accelerated atherosclerosis. The PKC pathway affects the expression of a variety of genes and has been linked to early retinal and renal changes among patients with diabetes. The hexosamine pathway leads to expression of pathologic genes which are linked to abnormal glomerular cell gene expression and cardiomyocyte dysfunction (64).

These pathways are key in the process of diabetic microvascular complications. For macrovascular complications, also factors related to insulin resistance and conventional risk factors are involved (28, 72). Hyperglycemia induces platelet adhesion and aggregation, vascular smooth muscle cell dysfunction and accelerates oxidative stress and LDL oxidation (61, 73).

Previous studies have reported that, after adjustments for other risk factors, an increase of 1% in the HbA_{1c} level is associated with a 12-14% increase in mortality, 18% increase in CVD events and a 37% increase in renal failure or retinopathy (74-76).

The causal relationship between HbA_{1c} and CVD events suggests that an intensive therapeutic strategy to lower HbA_{1c} might reduce the occurrence of these outcomes. However, recent trials have published controversial results on the treatment of glycemia. The United Kingdom Prospective Diabetes Study (UKPDS) authors reported that, over a follow-up of 10 years, newly diagnosed type 2 diabetes patients treated intensively (HbA_{1c} 7.0%) vs. those treated by standard means (HbA_{1c} 7.9%) did have a 12% reduction in diabetes-related endpoints, but most of the risk reduction (25%) was due to a reduction in microvascular complications. T2DM-related mortality or all-cause mortality did not differ between the groups (77). After an additional 10 years of post-intervention follow-up, UKPDS authors observed a statistically significant reduction in the rate of MI (among non-obese 15% and among obese subjects 33%) and in total mortality (among non-obese 13% and among obese subjects 29%). These findings were significant despite the fact that there no longer were any differences in mean HbA_{1c} after the first year (mean HbA_{1c} in both groups 8.1%). The authors concluded that the intervention phase of the trial had a similar "legacy effect" on macrovascular complications as was observed in the DCCT/EDIC study involving patients with type 1 diabetes (78). In 2008-2009 three studies (ACCORD, ADVANCE, VADT) showed that intensive treatment of hyperglycemia, targeting near normoglycemia, of patients with longstanding type 2 diabetes does not reduce CVD mortality. A recent review (79) on the management of hyperglycemia among middle-aged patients with diabetes and chronic kidney disease concluded: "Intensive control (mean achieved HbA1c level of 6.6%) did not improve clinical outcomes, with the exception of nonfatal MI, compared to conventional glycemic control (mean HbA1c, 7.4%) through a mean follow-up of 6.7 years. Intensive glycemic control also resulted in increased severe hypoglycemia." On the other hand,

microalbuminuria and new-onset macroalbuminuria were reduced among intensively treated patients, but since there is a lack of long-term benefits from this and since intensive glycemic control is associated with adverse effects, the ultimate clinical role of intensive glycemic control is not settled (79).

These results suggest that intensive treatment of hyperglycemia should be initiated as soon as diabetes is diagnosed to reduce micro- and macrovascular complications. Intensive glycemic control appears to have a prolonged legacy effect on diabetes related complications, i.e., there is a sustained benefit with respect to cardiovascular disease outcomes still long after the trial. On the other hand, caution is in order when treating older patients with longstanding diabetes, patients with a poor unawareness of hypoglycemia and patients with existing CVD because of the adverse effects of intensive treatment. Nevertheless, the most effective prevention and treatment of microvascular and macrovascular disease must always involve a multifactorial approach including pharmacological and lifestyle intervention (80-84).

2.2.2.2 Insulin resistance and hyperinsulinemia

Insulin resistance is a major contributor to the pathogenesis of type 2 diabetes and metabolic syndrome (MetS). It plays a key role in associated metabolic abnormalities, such as dyslipidemia and hypertension (85, 86). Insulin resistance may be defined as reduced sensitivity to the action of insulin of the liver, skeletal muscle and adipose tissue (85). Insulin resistance is characterized by increased hepatic gluconeogenesis, reduced glucose uptake in the muscle, endothelial dysfunction and increased release of FFA from the adipose tissue (87). Insulin resistance has been associated with a 2.5-fold risk of CVD mortality after adjustments for other classical CVD risk factors (88). Compensatory hyperinsulinemia ensues when beta-cells attempt to overcome the underlying defect of insulin resistance by increasing insulin secretion (85). However, hyperinsulinemia causes also insulin resistance through negative feedback in the insulin signaling pathway (89).

Insulin resistance, although genetically influenced, is closely associated with obesity and ectopic fat accumulation, and may be mediated by increased FFAs (85, 90, 91). Ectopic fat accumulation, particularly in the abdomen, could be associated with reduced peripheral and hepatic insulin sensitivity. Both obese non-diabetic and obese type 2 diabetic patients exhibit hepatic insulin resistance, which apparently is a dominant component in the pathogenesis of fasting hyperglycemia in type 2 diabetes (92).

In obese patients, increased triglyceride levels in the skeletal muscle reduce insulin action. Elevated levels of circulating FFAs inhibit insulin signaling pathways. Visceral fat plays a key role in this effect, because lipolysis from visceral fat is more pronounced than from other depots, e.g., subcutaneous fat, and visceral fat cells are more resistant to suppression of lipolysis by insulin (93). FFAs derived from visceral fat lipolysis are released into the portal vein and drain into the liver. This not only promotes hepatic fat

accumulation but can also cause hepatic insulin resistance and gluconeogenesis (93-96).

Increased FFA flux to endothelial cells results in increased FFA oxidation and finally to mitochondrial overproduction of ROS that leads to activation of the four damaging pathways, as was discussed previously (64). These mechanisms have been proposed to inhibit insulin secretion and also associate insulin resistance with CVD mortality. Compensatory hyperinsulinemia stimulates the intact mitogen-activated protein kinase (MAPK) pathway that contributes to pro-atherogenic events by increasing smooth muscle cell proliferation, collagen formation, production of growth factors and inflammatory cytokines (97). In addition, hyperinsulinemia causes exaggerated responses in tissues that still are insulin sensitive, e.g., to stimulation of the sympathetic nervous system that could contribute to hypertension (98, 99).

Inflammation plays also a major role in the development of insulin resistance. Adipocytes especially from the visceral fat, synthesize and secrete bioactive molecules, collectively known as adipocytokines. These molecules include tumor necrosis factor alpha, resistin, adiponectin, leptin, IL-6 and IL-1. These substances are associated with inhibition of insulin signaling pathways in adipocytes and hepatocytes (100).

Insulin resistance also underlies the development of the metabolic syndrome (MetS) (101), which is a combination of several cardiometabolic risk factors including obesity, impaired glucose metabolism, hypertension and dyslipidemia. It is also a precursor for type 2 diabetes (102, 103). Depending of ethnicity, the risk of type 2 diabetes among patients with MetS is 3.5-5.2 higher than among people without MetS (104). There is controversy regarding the pathophysiological basis as well as the clinical impact of the MetS, and especially the dichotomization of its components (105). Risk assessment is a progressive function and using dichotomous variables may result in loss of crucial information concerning the magnitude of risk factors. Nevertheless, the MetS is associated with an increased risk of cardiovascular disease among patients with type 2 diabetes as well as other patients (106). In a 22 year follow-up study, Pyörälä et al. observed that the MetS predicts significantly increased CVD and stroke mortality among Finnish men (107). A large meta-analysis involving over 170,000 subjects reported that individuals with the MetS had nearly a double risk of CVD mortality and a 1.6-fold risk of total mortality compared to patients without MetS (108). Similar results have been provided by Mottillo et al. in a meta-analysis which included almost 950,000 patients (109). The prevalence of MetS, as of diabetes, will increase due to increases in the aging population, obesity and sedentary lifestyle. Today, almost one-quarter of the Finnish population has MetS (110).

2.2.2.3 Microalbuminuria and proteinuria

Approximately 180 liters (20%) of plasma flow is filtered by the glomeruli daily. The glomerulus filters blood and retains larger proteins, including most of the serum albumin

(molecular weight >67 kd). Usually, proteins with a molecular weight below 60 kd pass freely through the glomerular basement membrane (GBM). These proteins, molecules and the small amount of albumin that pass freely through GBM are actively reabsorbed within the tubular system. In normal urine, only 40-80 mg of protein is detected per day and of this 10-15 mg (30-40%) is albumin and the rest is epithelial cells originating from the loop of Henle, called Tamm-Horsfall protein. The urine contains also small quantities of low-molecular-weight proteins, such as β2-microglobulin, α1-microglobulin and retinol-binding protein (111). Transient increases in proteinuria may occur with exercise, fever, upright posture, pregnancy and hypertension.

Urinary protein excretion may be quantified in terms of excreted albumin or excreted total protein. Previously, a daily excretion of >150mg protein was generally considered abnormal proteinuria and a sign of kidney disease. However, it has since been found that albuminuria reflects even more accurately incipient renal damage, especially in diabetes related kidney disease. Glomerular filtration of proteins depends on a 3-layer structure called the glomerular filtration barrier: the endothelium with a glycocalyx, the GBM and the glomerular epithelial cells called podocytes. Circumstances related to diabetes and MetS, such as pro-inflammatory cytokines and adipokines (e.g. TNF-α, IL-6, leptin and adiponectin) and hyperglycemia, damage the glomerular filtration barrier, which leads to thickening of the GBM and mesangial expansion, podocyte injury and endothelial dysfunction. As a result of these changes, proteinuria increases which allows glomerular passage of proteins with a molecular mass larger than albumin; such proteins are normally retained in the circulation. Concomitant tubulointerstitial injury may also contribute to albuminuria/proteinuria by impairing proximal tubular protein reabsorption. Finally, proteinuria itself, along with other factors, may lead to progressive glomerulosclerosis and tubulointerstitial fibrosis, with a subsequent decline in GFR (111-115).

MA is defined as a persistent, increased urinary excretion of albumin less than macroalbuminuria. Table 2 shows the detailed diagnostic thresholds for MA and macroalbuminuria (116, 117). Of all patients with hypertension 11-40% have MA (117-120). Patients with MA have also an increased prevalence of left ventricular hypertrophy (LVH) and retinal microvascular lesions (117, 118, 121). Other risk factors for the development of MA include smoking, a sedentary lifestyle and genetic factors (117, 118, 121). MA is usually the first manifestation of diabetic nephropathy (DN), i.e., albuminuria followed by a decline in glomerular filtration rate, which in time, often over 10-20 years, progresses to overt proteinuria and eventually to renal failure. The natural history of DN is described in Figure 3. Data on the natural history of DN is largely based on studies among type 1 diabetes patients, the high CVD mortality among type 2 diabetes patients is often an obstacle to sufficient follow-up (116). DN is the leading cause of end-stage renal disease (ESRD) (122). Among type 2 diabetes patients approximately 25-28% have MA or more advanced stage of DN. This has been observed to worsen at a rate of 2-3% per year (117).

Table 2. Diagnostic thresholds for MA and proteinuria (table modified from the Finnish National Guidelines of Diabetes Treatment) (123).

	24-h urine albumin (mg/24 h)	Overnight urine albumin (µg/min)	Albumin/creatinine ratio (Spot urine)
Normal	<30	<20	<2.5 (male) and <3.5 (female)
Microalbuminuria	30 – 300	20 – 200	2.5 - 25 (male) and 3.5 – 35 (female)
Macroalbuminuria	>300	>200	>25 (male) and >35 (female)

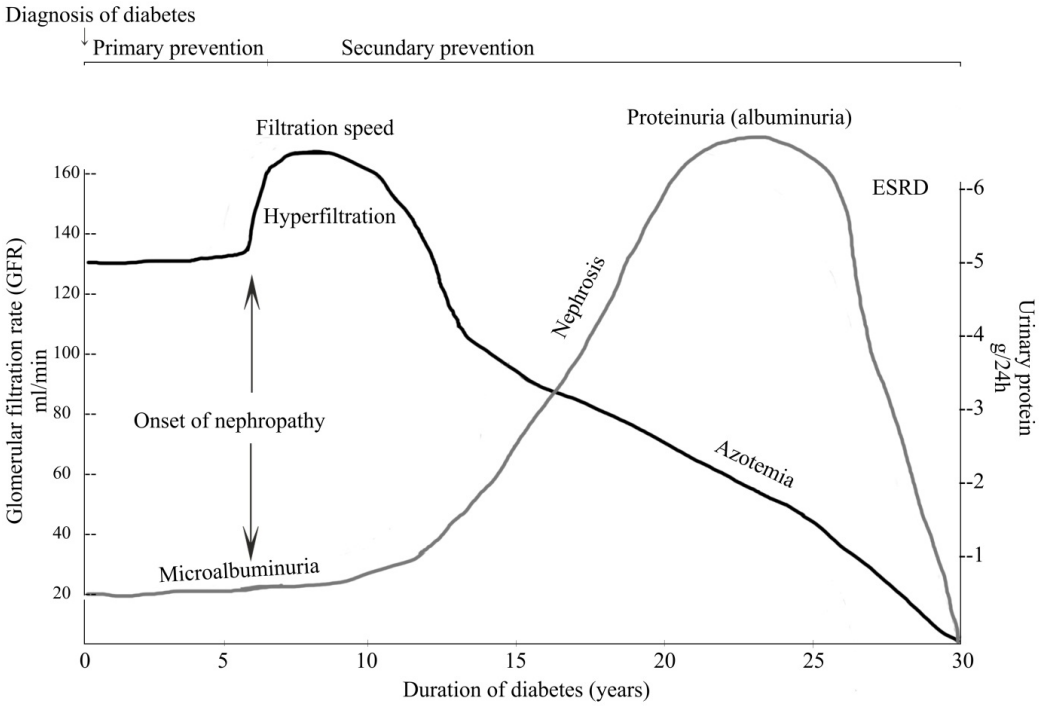


Figure 3. Natural history of diabetic nephropathy in type 1 diabetes (Figure adapted from Finnish National Guidelines of Diabetes Treatment) (123).

MA does not only precede DN, but it also predicts premature CVD mortality. Several studies have shown an association between, on the one hand, of MA and proteinuria and, on the other hand, increased total and CVD mortality among type 2 diabetes patients as well as among the general population (5-7, 124-127). This association is independent of the conventional CVD risk factors. The date of a prospective study among middle-aged individuals was adjusted for conventional risk factors and for the presence of diabetes, and it turned out that MA was associated with increased CVD mortality (RR 3.22; 95% CI 1.28 - 8.06) and all-cause mortality (RR 1.70; 95% CI 0.86 - 3.34), especially among hypertensive patients (RR 2.87; 95% CI 1.22 - 6.33) (128). Previously, Miettinen et al.

have reported that among patients with type 2 diabetes proteinuria, measured with a spot urine sample of total urinary protein, was associated with a more than double risk of CVD mortality when compared with patients with no proteinuria. Borderline proteinuria was associated with 1.3-fold risk of CVD mortality (Fig. 4). The link between microvascular and macrovascular complications may reside in endothelial dysfunction (64). Thus, MA may be a marker of generalized vascular damage.

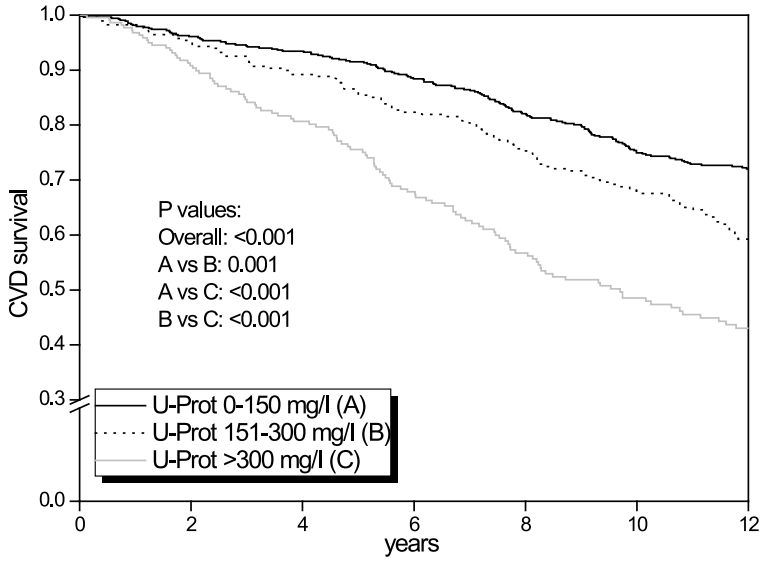


Figure 4. Mortality among diabetic patients stratified by baseline total proteinuria (adapted from Miettinen et al.[5]).

Key elements in treating MA are good glycemic control and good BP control (129). Tight glycemic control seems to be associated in a reduction of MA. On the other hand, new-onset macroalbuminuria among middle-aged diabetic patients with comorbidities (mean achieved HbA_{1c} level of 6.6%) resulted in a significant increase in adverse events when the HbA_{1c} was less than 7.0%; a typical adverse event was hypoglycemia (79).

Also hypertension increases the risk for development and progression of MA (128). Accumulating evidence suggests that reducing systolic BP to levels even lower than 120mmHg could reduce the incidence and progression of nephropathy, especially among patients treated with angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) (130, 131). However, the largest positive effect of ACE inhibitors or ARBs is probably seen among patients with a baseline systolic BP >145mmHg (132). When reducing systolic BP to less than 130 mmHg, the risk of adverse events and the mortality rises among patients with underlying CVD (131-135). Recent ADA guidelines suggest that, among patients without underlying comorbidities or patients with newly-onset diabetes, stricter BP targets, such as <130 mmHg or HbA_{1c} <6.5% may be appropriate, if achievable with a reasonable treatment burden (136).

2.2.2.4 Cardiac function and P wave duration

A resting 12-lead electrocardiograph (ECG) is a common procedure for evaluation of patients with CVD for regards to previous MI and signs of myocardial ischemia (137). Patients with type 2 diabetes may exhibit several abnormal changes in the ECG: sinus tachycardia, prolonged corrected QT interval, QT dispersion, changes in heart rate variability, ST-T changes and LVH. These ECG changes help to evaluate cardiac autonomic neuropathy (CAN) and detect signs of myocardial ischemia, also in asymptomatic patients (137, 138). Previous studies have associated changes in several of these variables with increased CVD and CHD mortality (138-143).

CAN results from hyperglycemia and other diabetes related risk factors that induce damage, by mechanisms previously discussed, to the autonomic nerve fibers that innervate the heart and blood vessels (144). These mechanisms lead to abnormalities in heart rate control and vascular dynamics. Reduced heart rate variation is the earliest indicator of CAN (145). Other symptoms of CAN include resting tachycardia, exercise intolerance, slow heart rate recovery after exercise, orthostasis, "silent" or painless MI and increased mortality (144-146). CAN has been associated with diabetic cardiomyopathy, which might predispose to diastolic and systolic filling abnormalities and ultimately to heart failure (144). Prolonged P wave duration, a marker of atrial conduction, is also associated with CAN (147). CAN is one of the manifestations of diabetic autonomic neuropathy (DAN).

Prolonged P wave duration has been suggested as a conveniently measurable risk factor for subclinical heart disease. Prolonged P wave duration has been associated with atrial fibrillation (AF) and also more recently with increased mortality due to any cause, CVD and stroke among the general population (147-150). A normal P wave has duration of less than approximately 110-120 ms (152). Prolonged P wave duration signifies a conduction delay between the right and left atrium due to impulse slowing or blockage, often but not exclusively in Bachmann's bundle (interatrial tract). On the ECG this conduction delay is thus referred to as an interatrial block (IAB) (153-155). IAB is one of the most common ECG abnormalities but surprisingly often overlooked and underdiagnosed (156-160). Atrial tissue sampling from patients with IAB shows consistently intercellular fibrotic changes and intracellular inclusions, particularly in the sarcomere and sarcoplasmic reticulum (161).

Diabetes, CAD and conventional CVD risk factors, such as hypertension, hypercholesterolemia, smoking, obesity, physical inactivity and high age have been associated with IAB. It is possible that these CVD risk factors increase the duration of the P wave via endothelial injury which generates an ischemia-induced interatrial conduction delay (162). The National Health and Nutrition Examination Survey (NHANES) reported that patients with diabetes have a significantly longer mean P wave duration compared to the general population (148). Interestingly, a subgroup analysis done in the same study showed that there is a significant interaction between the use of AV nodal agents (beta blockers or calcium channel blockers) and all-cause mortality. P wave duration was associated with increased all-cause mortality only among subjects who were taking AV

nodal agents. The authors speculate that the use of AV nodal agents may be a confounder which impacts the results through indication bias of uncertain nature (148). In support of this speculation, beta blockers and amiodarone have previously been found to influence the duration of the P wave favorably. Furthermore, beta-blockers have been associated with a lower rate of AF recurrence during exercise testing in the general population. (163). There is still much to be learned about the association between P wave duration and mortality among type 2 diabetes patients.

2.2.2.5 Other diabetes related CVD risk factors

Diabetic retinopathy

Diabetic retinopathy (DR) can affect the peripheral retina, the macula or both. It is a leading cause of visual disability and blindness among people with diabetes (164). The severity of DR varies from non-proliferative to proliferative DR characterized by neovascularization (164). The prevalence of DR increases in concert with the duration of diabetes (165). Other risk factors, besides a genetic predisposition and hyperglycemia, are hypertension, insulin resistance, high BMI, renal disease, dyslipidemia and smoking (65, 167-168). DR is independently associated with CVD mortality (169).

Diabetic neuropathy

Diabetic neuropathy includes somatic neuropathy and autonomic neuropathy (65). Somatic neuropathy affects usually peripheral nerves and is present among approximately half of the diabetic population (170). It predisposes to foot ulcerations and amputations. DAN may affect a wide range of organs and functions, including the cardiovascular system, the gastrointestinal tract, erectile function and hypoglycemia awareness (144). The prevalence of autonomic neuropathy ranges from 7.7% to 90%, depending on how autonomic neuropathy is defined in the respective patient cohorts (144). Diabetic neuropathy has been linked to increased CVD mortality (65).

Thrombogenesis

Increased concentrations and activities of plasma fibrinogen, von Willebrand factor, factor VII and plasminogen activator inhibitor 1 (PAI-1) have been associated with increased CVD events among patients with and without type 2 diabetes (171-180). These factors measure blood coagulation and endothelial cell function and, taken together, promote thrombosis in type 2 diabetes patients.

2.2.3 Conventional cvd risk factors

2.2.3.1 Hypertension

Hypertension is usually associated with the metabolic syndrome and related disturbances and occurs more often in patients with type 2 diabetes than in the general population

(181, 182). Hypertension is defined as systolic blood pressure (BP) >140 mmHg and/or diastolic BP >90 mmHg (182). Hypertension is a major risk factor for atherosclerosis and the leading global cause for mortality (181, 183, 184). It has been suggested that, in type 2 diabetes, some 35% - 75% of the CVD risk can be attributed to hypertension (185, 186). UKPDS reported that every 10 mmHg increase in systolic BP raises the risk of CAD by 15% (187). This finding is similar to what has been reported for the general population (188).The WHO Multinational Study reported a 5-fold risk of CVD morbidity and mortality among men compared to diabetic patients without hypertension and proteinuria; for women the risk was no less than 8-fold (189). In addition, elevated BP is associated with insulin resistance, compensatory hyperinsulinemia, dyslipidemia and endothelial dysfunction (183).

It is well documented that reducing BP under 150/90 mmHg reduces CVD and total mortality among diabetic patients and among the general population (186). UKPDS observed that, among patients randomly assigned to tight BP control, achieving mean BP of 144/82 mmHg, when the pretreatment level was 150/85 mmHg, reduced significantly the incidence of microvascular and macrovascular events over a follow-up time of 9 years (133). Another landmark trial, the HOT study, which assessed various diastolic BP goals, observed that patients assigned to a diastolic BP goal of 80 mmHg had less adverse outcomes that higher diastolic BP groups. It is noteworthy, however, that diabetic patients were only a subgroup in the HOT study and only 6% of the study population had CAD at entry. The mean achieved BP was actually 139.7/81.1 mmHg among the general study population (mean BP was not reported for the diabetes subgroup) (134).

The present guidelines recommend starting BP treatment for patients with type 2 diabetes when their BP is over 140/90 mmHg and a recommended goal of 130/80 mmHg or less if the patient has signs of microvascular complications. This recommendation is based largely on observational data, which poses an interpretation challenge, since sicker patients may have lower blood pressure at baseline while healthier or more adherent patients may achieve the target goals more easily. There is a paucity of evidence from randomized clinical trials to support these BP recommendations (190).

The Action to Control Cardiovascular Risk in Diabetes blood pressure trial (ACCORD BP) was undertaken among high-risk type 2 diabetic patients. It studied the effect on major cardiovascular events of a target systolic BP <120 mmHg. There were no significant differences between the study groups with respect to CVD and all-cause mortality, but the intensive treatment group had higher rates of serious adverse events with the exception of stroke deaths, which were less common among the intensively treated patients (135).

Similar results were observed in the International Verapamil SR-Trandolapril Study (INVEST). The study assigned 6,400 diabetic patients with CAD to tight systolic BP (<130 mmHg), usual control (130-140 mmHg) and uncontrolled (≥140 mmHg) groups.

There were no statistically significant differences between the tight or usual BP control groups. The all-cause mortality rate was 11.0% in the tight-control group and 10.2% in the usual-control group (P = 0.06). Furthermore, during an extended follow-up all-cause mortality was 22.8% in the tight control and 21.8% in the usual control group (P = 0.04) (191). Moreover, the number of trials that have studied the association between BP targets <130/80 mmHg and the progression rate of microvascular complications is small and the results vary (182, 190, 192). These results will be discussed in more detail (see section 6.5.3). Nor are there any long term follow-up studies on the associations between BP, microvascular complications and CVD mortality.

2.2.3.2 Dyslipidemia

The dyslipidemia of type 2 diabetes is characterized by high triglyceride levels, low HDL cholesterol levels and high levels of small dense low-density lipoprotein (sdLDL) cholesterol. Several studies have proposed that hepatic triglyceride-rich very-low-density lipoprotein (VLDL) overproduction could be the critical factor underlying dyslipidemia (193-195): insulin resistance raises the influx of FFA to the liver (29). In response, the liver increases triglyceride rich VLDL production and cholesterol ester synthesis which leads to hypertriglyceridemia (30, 31). Hypertriglyceridemia reduces HDL cholesterol synthesis and changes the composition of HDL so that it becomes less antiatherogenic (31). At the same time, the composition of LDL cholesterol is changed and sdLDL is predominantly produced, although average plasma LDL cholesterol levels may not be increased (196, 197). SdLDL particles are proatherogenic and are more prone to oxidation, forming oxidized LDL (ox-LDL). Oxidation of LDL increases their uptake by monocytes and vascular smooth muscle cells in the vessel walls (197, 198). Ox-LDL is associated with endothelial dysfunction, foam cell formation and thrombus formation and with apoptosis of vascular smooth muscle cells. These changes make the subject more prone to plaque rupture (199).

These lipid abnormalities, especially high LDL, have been associated with CVD morbidity and mortality, and they are particularly frequent among patients with renal complications or patients with poor glycemic control (192, 200-204). Consequently, better glycemic control has been suggested as a means to reduce lipid abnormalities (205). Several studies have shown the benefits of pharmacological (primarily statin) cholesterol lowering therapy in the primary and secondary prevention of CVD events (206, 207). A meta-analysis of 14 randomized statin trials among 18,686 diabetic patients followed for a mean of 4.3 years reported that, for each mmol/L reduction in LDL cholesterol, there was a 9% proportional reduction in all-cause mortality and a 13% reduction in vascular mortality (208). Therefore, ADA has recognized that LDL cholesterol is a primary target of lipid lowering therapy (136). In addition, lifestyle changes focusing on the dietary habits, weight loss and increased physical activity should be recommended to improve the lipid profile in patients with diabetes (136).

2.2.3.3 Chronic low-grade inflammation

Inflammation is present before T2DM and CVD develop. This suggests that there are common mechanisms underlying these disease processes (209). The Multi-Ethnic Study of Atherosclerosis (MESA) reported that high levels of the inflammatory markers C-reactive protein (CRP) and interleukin (IL)-6 were associated with an increased risk of T2DM (210). Diabetes, obesity and insulin resistance are associated with subclinical inflammation characterized by overexpression of cytokines produced by adipose tissue, activated macrophages, and other cells (211-214). Inflammatory mediators, such as CRP, cytokines (IL-1β, IL-6, and TNF-α), adiponectin and fibrinogen are involved in insulin signaling pathways and perpetuation of the inflammatory response (212). These changes lead to chronic inflammation of the vessel walls, promotion of lipid accumulation and atherosclerosis (212).Stress, diet-induced hyperglycemia and hypertiglyceridemia also stimulate inflammation (215-217).

Several cytokines and acute-phase reactants have been studied as predictors of atherosclerotic disease, but special focus has been put on the high-sensitivity C-reactive protein (hs-CRP) (218). CRP is produced primarily by the liver in response to inflammatory cytokines (e.g. IL-6) and it is also produced in adipose tissue and atherosclerotic plaques (219, 220). It is involved in atherogenesis by promoting endothelial cell activation, macrophage recruitment and foam cell generation within the arterial wall (220). CRP is also an acute phase protein; during acute infections, the level of CRP may increase over 10000-fold.

High sensitivity-CRP (hs-CRP) assays allow detection of even modest elevations of CRP (221). According to AHA guidelines, a subject is considered to have a low risk of CVD if CRP is <1.0 mg/L, intermediate risk when CRP is between 1.0 – 3.0 mg/L and high-risk when CRP is >3.0 mg/L (222). Two measurements should be performed, optimally two weeks apart. If the value is over 10 mg/L, an infection might be present and reassessment should be made within two weeks. When the hs-CRP value is within the reference limits, IL-6 is a crucial factor that mediates CRP synthesis from the hepatocytes (223).

hs-CRP levels rise in type 2 diabetes, especially among patients with MA or other microvascular complications (222, 224). Several epidemiological studies have observed strong independent associations between elevated hs-CRP values and CVD mortality (225, 226). Soinio et al. observed that patients with type 2 diabetes and hs-CRP levels >3.0 mg/L have an approximately 1.6-fold higher CHD mortality when compared with patients with hs-CRP ≤3.0 mg/L during 7-years of follow-up (227) (Fig. 5). It has, consequently, been contemplated whether anti-inflammatory treatments might reduce excess CVD mortality among type 2 diabetic patients.

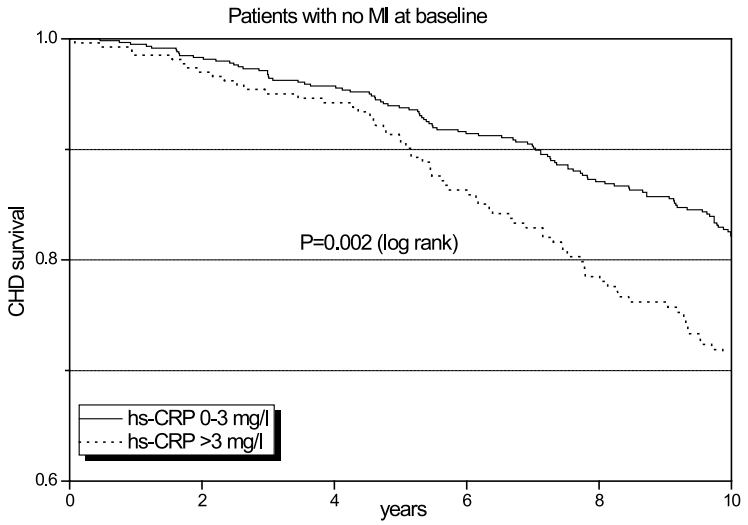


Figure 5. CHD mortality among patients with type 2 diabetes stratified by baseline hs-CRP levels (adapted from Soinio et al. [227]).

2.2.3.4 Smoking

Smoking is associated with an increased incidence of type 2 diabetes. Smoking is also a major risk factor for CVD among these patients (188, 228). Smoking has a multitude of effects on atherosclerosis and thrombogenesis, including increased inflammation and oxidative stress, damage to β-cell function and endothelial dysfunction (229-231). It is also associated with central obesity (232). Therefore, all patients with type 2 diabetes should be encouraged to stop smoking (136). In the East-West type 2 diabetes study approximately 16% of the diabetics and 19.5% of the controls smoked (184).

2.3 LIFESTYLE HABITS AND CVD IN PATIENTS WITH TYPE 2 DIABETES

Several studies have highlighted repeatedly and conclusively the importance of early lifestyle intervention in the management of patients with type 2 diabetes. The aims of the intervention are weight reduction, a healthy diet and increased physical activity to prevent or postpone type 2 diabetes among high-risk individuals (80-84). The Finnish Diabetes Prevention Study (DPS) was the first randomized, controlled clinical trial to show that a relative risk reduction of almost 60% can be achieved with intensive dietary and physical activity counseling (81). In a longer follow-up of the same study cohort, researchers observed that active lifestyle intervention of relatively short duration (a median of 4 years) postponed the development of diabetes for approximately 5 years. It was argued that this might be due to sustained lifestyle changes as well as to a legacy effect of previous improvements in glycemia (233). The Look AHEAD (Action for Health in Diabetes) trial is a multi-center randomized clinical trial comparing the effects

of intensive lifestyle intervention, diabetes support and education on the incidence of major CVD events among 5145 overweight or obese type 2 diabetes patients. The study showed that after four years of follow-up, patients in the intensive lifestyle intervention group had significantly greater improvements in weight, fitness, HbA_{1c}, systolic BP and HDL cholesterol levels (234). These improvements in the risk factor profile might also reduce CVD mortality. However, when the follow-up time of the Look AHEAD trial was extended to a median of 10 years, there was no significant reduction in the risk of CVD morbidity or mortality as compared with a control group. However, it should be noted that study patients who were recruited to the trial were motivated to lose weight and at the end of the follow-up, the differential weight loss between the trial groups was only 2.5%. In addition, weight loss was achieved through caloric restriction and increased physical activity. It is unclear whether intervention focused on changes in dietary composition (e.g., the Mediterranean diet) could have yielded different outcomes (235). Recently, it was reported that an energy-unrestricted Mediterranean diet, supplemented with extra-virgin olive oil or nuts, results in a substantial reduction in the risk of major cardiovascular events among high-risk persons (236). ADA guidelines on nutrition recommendations in diabetes highlight the importance of weight loss because of the effect of obesity on insulin resistance. Carbohydrate from fruits, vegetables, whole grains, legumes and low-fat milk are recommended as part of healthy diet (237).

2.3.1 Physical activity

Regular physical activity and exercise are associated with reductions in total and CVD mortality among patients with type 2 diabetes and in the general population (238, 239). There is also evidence that not only leisure time physical activity but also occupational activity and daily walking or cycling to and from work are important components of a healthy lifestyle among diabetic patients (239, 240). Regular physical activity could reduce CVD mortality through several mechanisms. Meta-analyses have reported that exercise leads to improvements in metabolic control, measured by HbA_{1c}, plasma glucose or insulin sensitivity. The overall beneficial effect of exercise on HbA_{1c} levels is modest, average HbA_{1c} reduction is approximately -0.8% (240). It has been found that 4 months of physical exercise training among patients with type 2 diabetes resulted in significant decrease in HbA_{1c} (9.6-8.6%) and the 2 hour plasma glucose concentration in OGTT decreased (19.7-16.5 mmol/L).

Exercise training induces a significant decrease in serum LDL-cholesterol and an increase in serum HDL-cholesterol (241, 242). Improvements are small, but they are clinically significant when incorporated in a summary variable of macrovascular, microvascular and nonvascular end points, and reportedly similar to what is gained from intensive pharmaceutical intervention. Exercise training has also been associated with reductions in the need for antihyperglycemic medication. Regular physical activity improves and maintains cardiorespiratory fitness, muscular strength, endurance and body composition. It has a favorable effect on CVD risk factors and inflammation. In particular, it has specific

beneficial effects by reducing hypertension, hyperlipidemia (even when combined with a rigorous calorie-restricted diet) and on obesity. Physical activity and exercise might also be beneficial with respect to endothelial function, vascular distensibility, myocardial functioning and diabetes related CAN (240).

Physical activity can be measured by several techniques which can be classified into methods related to some activity criterion or to some objective or subjective endpoint. These techniques vary with regard to e.g. cost, feasibility and accuracy. Some methods are more suitable for evaluating physical activity in laboratory conditions and others are more suitable for large population based studies. The greatest obstacle to evaluating physical activity of study subjects has been the lack of an adequate criterion with which objective and subjective techniques may be compared. The interrelations of various objective and subjective assessment methods may be of some value, but due to errors in all methods, it is difficult to determine validity of any one of them (243). For validation of physical activity methods, the double-labelled water method has become the gold standard which is based on the stable ^{18}O and ^{2}H isotopes of labelled water and their elimination rates. These elimination rates allow calculation of the production of carbon dioxide, and the subject's total energy expenditure (243, 244). From this, physical activity can be derived mathematically after assessment of the subject's basal energy expenditure (243, 244). Other reliable and validated criterion methods are include calorimetry and behavioral observation. These methods have important drawbacks, such as cost, invasiveness and usability only in laboratory conditions (243, 244). Objective methods include motion sensors, such as pedometers and accelerometers and heart rate monitoring. These techniques are often used, as they are relatively cheap, easy to use and provide valid data for most common physical activities. Accelerometers can also estimate energy expenditure and intensity related to movement. Activity questionnaires, including interviews and diaries, are the most common tools for assessment of physical activity. These are considered as subjective methods, because they rely only on the patient's or subject's own interpretation of physical activity. Subjective methods are relatively easy to use: the methodology is cheap and allows application in large populations. The major drawback is that the reliability and validity of the measurement is low and may well result in an underestimation or overestimation between physical activity and study outcome (243, 244). Questionnaires are helpful for to classifying subjects into distinct categories of physical activity (e.g., physical inactive or active) (243, 244).

In population based studies the metabolic equivalent task (MET) is often used to assess and classify physical activity. After the physical activity interview corresponding MET values are assigned based on the intensity of the activity. One MET is defined as the energy expenditure for sitting quietly. It is equivalent to 3.5 mL of oxygen uptake per kilogram of body weight per minute. As an example, 4 METs requires four times the person's metabolic energy expenditure of sitting quietly, and it is equivalent to brisk walking.

Physical activity and exercise are the cornerstones of treatment of patients with type 2 diabetes, along with dietary and pharmacological interventions (81, 136, 245). Current guidelines recommend that patients with type 2 diabetes follow the same guidelines of physical activity as the general population. Thus, the recommendation is that the subject performs at least 150 minutes per week of aerobic exercise of moderate intensity and resistance exercise at least twice a week. However, higher levels of exercise intensity yield greater benefit (136, 246). There is still relatively limited amount of data between physical activity and diabetes related risk factors on CVD and total mortality.

3. AIMS OF THE STUDY

The present thesis is based on the Finnish East-West type 2 diabetes study and specifically on the 18-year follow-up data. The purpose was to examine the association between certain risk factors and CVD and total mortality, and to bring novel data for identifying patients with an increased risk of CVD events in a large cohort of middle-aged Finnish patients with type 2 diabetes.

The specific aims were:

1. to examine the association between physical activity and well-known CVD risk factors (proteinuria and high hs-CRP) and mortality (I, II)

2. to examine the relation between two major CVD risk factors (proteinuria and blood pressure) with respect to mortality (III)

3. to examine the association between P wave duration and mortality (IV)

4. SUBJECTS AND METHODS

4.1 STUDY SUBJECTS

4.1.1 Subjects in the East-West type 2 diabetes study

1,059 patients aged 45-64 years with type 2 diabetes who were living in the Turku University Central Hospital district in West Finland or in the Kuopio University Hospital district in East Finland were identified on the basis of a national drug reimbursement register that is maintained by the Social Insurance Institution (184). Of these patients, 328 men and 221 women were from West Finland (participation rate 79%) and 253 men and 257 women were from East Finland (participation rate 83%). 147 were treated with diet only, 762 were treated with oral medication and 150 were treated with insulin. The mean age of the men with diabetes from West Finland was 57±0.3 years and from East Finland 56.8±0.3 years; the corresponding figures for women were 58.7±0.3 years and 58.9±0.3 years. Among the insulin treated patients, type 1 diabetes was excluded by C-peptide measurements. All diabetic subjects included in the final study population had a plasma C-peptide concentration of at least 0.20 nmol/L six minutes after 1 mg of intravenous glucagon. This cut-off point was chosen, because C-peptide values below these limits are associated with ketoacidosis (247). None of the study subjects had a history of ketoacidosis. The records of all patients, whose fasting plasma glucose in baseline was <8.0 mmol/L, were checked to confirm that the WHO diagnostic criteria for diabetes were fulfilled (36). The subjects whose diagnosis of diabetes could not be verified underwent the oral glucose tolerance test (OGTT).

The number of the study subjects varies in the four studies, because of different exclusion criteria and the availability of study variables (Fig. 6).

4.1.2 Studies I-II

A total of 452 subjects who had angina pectoris, possible or definite stroke, possible or definite MI, intermittent claudication or amputation at baseline were excluded from the statistical analyses, as were 30 subjects who died or had a severe CVD event (MI, stroke or lower limb amputation) during the first two years after the baseline examination. The exclusion criteria were based on the assumption that subjects very likely had changed their exercise habits due to severe disease at baseline. Thus, the final study population consisted of 577 patients with type 2 diabetes (314 men, 263 women). Due to missing data on proteinura, an additional 3 patients (1 man, 2 women) were excluded from all proteinuria analyses.

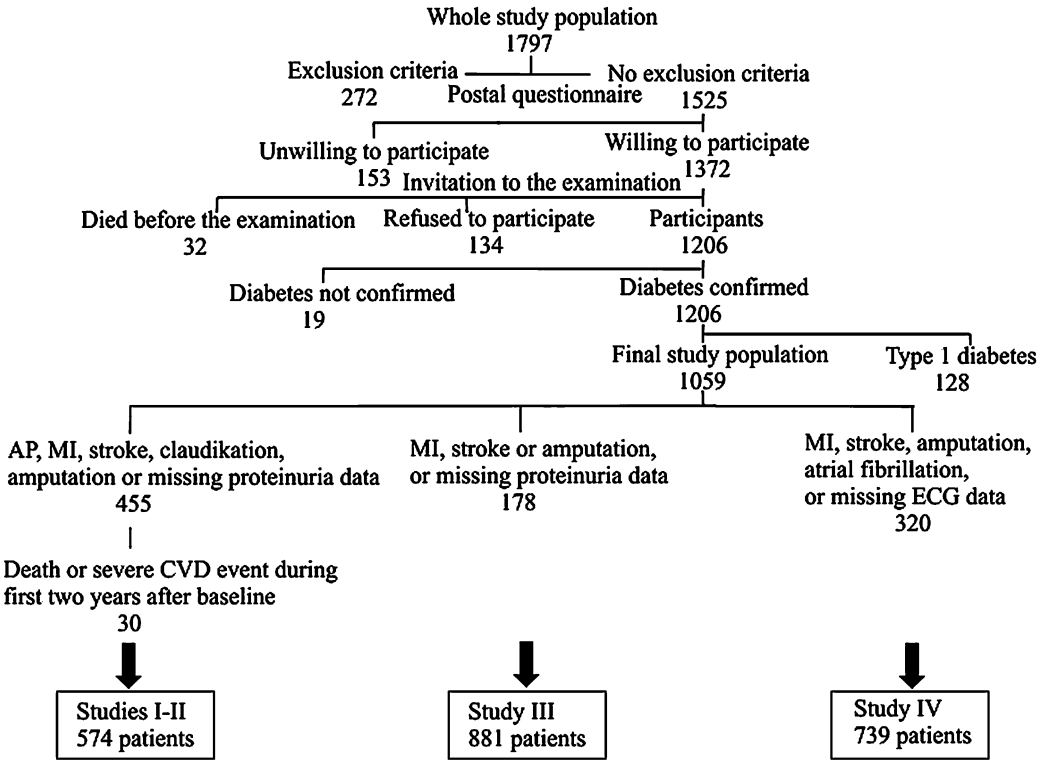


Figure 6. Study populations.

4.1.3 Study III

A total of 174 subjects who had possible or definite stroke, possible or definite myocardial infarction (MI) or lower-extremity amputation at baseline were excluded from the statistical analyses. Due to missing data on proteinuria 4 additional patients were excluded from all statistical analyses involving proteinuria. Thus, the final study population comprised 881 patients with type 2 diabetes (460 men, 421 women).

4.1.4 Study IV

A total of 320 subjects were excluded from all analyses in this study. The exclusion criteria were: major previous CVD event (possible or definite stroke, possible or definite MI or lower-extremity amputation) or missing three-dimensional computerized ECG data or known AF. Thus, the final study population comprised 739 patients with type 2 diabetes (381 men, 358 women).

4.2 METHODS

4.2.1 Baseline study

The baseline examination was carried out between 1982 and 1984 at one outpatient visit at the Clinical Research Unit of the University of Kuopio or the Rehabilitation Research Centre of the Social Insurance Institution in Turku. The examinations in Turku were performed by the one and the same physician Tapani Rönnemaa (T.R.) and in Kuopio by Markku Laakso (M.L.). The visit included an interview to determine history of smoking, alcohol use, physical activity and medication use. Chest pain symptoms and symptoms of intermittent claudication were recorded by specially trained nurses. The examinations and the methods have been described in detail (184). Smoking and alcohol in studies I-IV was included as a dichotomous variable (smoking: current smokers vs. non-smokers; alcohol use: user vs. non-user).

The Rose classification was used to evaluate the presence of typical angina pectoris and intermittent claudication by specially trained nurses (248). Whitehall changes according to Minnesota coding were used to identify ischemic changes on the ECG (249). The medical records of patients who reported that they had been admitted to hospital for chest pain were reviewed by two investigators (M.L. and T.R.) after careful standardization of the methodology. The WHO criteria were used to define a definite or possible previous MI (249) based on chest pain symptoms, ECG changes and determination of enzyme activities. Definite or possible stroke was also defined according to WHO criteria: a clinical syndrome of neurological deficits persisting for over 24 hours observed by a neurologist, without other diseases explaining the symptoms (250). Thromboembolic and hemorrhagic strokes, but not subarachnoidal hemorrhage, were included under the heading stroke. Also non-traumatic lower-extremity amputations were recorded.

The height of the subjects was read on a scale to the nearest 1 cm with the person standing without shoes. Body weight was measured with subject bare foot, dressed in light shorts, and was read on a scale to the nearest 1 kg. The body mass index (BMI) was calculated (kg/m^2).

BP was measured with the subject sitting, after a 5 min rest, from the right arm with a mercury sphygmomanometer (cuff size 12.5 x 40 cm) by one of the investigators (M.L.) in East Finland and by a trained laboratory nurse in West Finland. The BP was measured twice at an interval of 1.5 minutes, and the latter (I, II, IV) or the calculated mean (III) was used for statistical analyses. Systolic and diastolic BP was read to the nearest 2 mmHg. The disappearance of the Korotkoff sounds (5th phase) was recorded as diastolic BP. Three series of quality-control measurements of BP were arranged during the 2-year period of the study. There were no statistically significant differences between the BP values measured by investigators in East and West Finland (251). Hypertension was defined as systolic blood pressure $\geq$ 160 mmHg, diastolic pressure $\geq$ 95 mmHg or use of antihypertensive drugs treatment in studies I-II and IV.

Biochemical methods

All blood specimens were taken at 8.00 AM, after the subject had fasted for 12 hours. Samples were centrifuged within 1 hour and frozen immediately at -20 °C. The analyses were performed duplicate, except for hemoglobin A_1 (HbA_1). The HbA_1 (reference range in non-diabetic subjects 5.5-8.5%) level was determined by affinity chromatography (Isolab, Akron, OH, USA). Serum total cholesterol and triglycerides were determined enzymatically (Boehringer, Mannheim, Germany). Serum HDL cholesterol level was determined enzymatically after precipitation of low-density lipoproteins (LDL) and VLDL with dextran sulphate $MgCl_2$ (252). The day- to-day variation of the measurements was 2.2% for cholesterol and 4.4% for triglycerides, and the intra-assay variation was 1.6% for cholesterol and 2.6% for triglycerides. Plasma glucose was determined with the glucose oxidase method (Boehringer).

The Coomassie brilliant blue method was used to measure the total urinary protein concentration in a morning spot urine specimen (253). The interassay coefficient of variation was 7% at protein levels 100 and 250 mg/L, and 3% at 600 mg/L. The plasma creatinine level was determined by the Jaffe method. We used the Cockcroft-Gault (CG) equation to estimate the glomerular filtration rate (GFR) in study III: 1.23 x (140 – age (years)) x weight (kg) x 0.85 (if female) /plasma -creatinine (μmol/L). The body surface area (BSA) in m^2 was calculated as 0.007184 × height (cm)0.725 × weight (kg)0.425. The value was used to correct the CG equation for BSA: CG-determined estimated GFR × (1.73 m2/BSA) (254, 255).

Serum hs-CRP was analyzed from baseline samples with a latex turbidimetric immunoassay in 2001 (Wako Chemicals, Neuss, Germany). The analytical detection limit was 0.06 mg/L. The interassay coefficient of variation was 3.3% and 2.7% at mean hs-CRP levels of 1.52 (n = 116) and 2.51 (n = 168) mg/L, respectively.

Evaluation of physical activity

At the baseline examination occupational, commuting and leisure-time physical activity was assessed using a self-administered questionnaire. After the interview a corresponding MET value was assigned for each of the three categories according to the intensity of the activity (METs) (256). Occupational activity was divided into six classes: a) no work (1.5 METs), b) sitting office work (1.75 METs), e.g. car driving, secretarial, c) other sitting work (2.5 METs), e.g. salesman, repairer, d) light moving work (3.5 METs), e.g. store-assistant, active office work, e) moderate moving work (5 METs), e.g. light industrial work, f) active work (7.25 METs), e.g. heavy industrial work, farm work, g) very heavy manual work (10 METs), e.g. woodcutter, lifting > 40kg. The daily commuting journey was categorized into three classes summer and winter separately: a) using motorized transportation (1.5 METs), b) bicycling (5 METs), c) walking (3.5 METs). The leisure-time physical activity was classified separately for summer and for winter as a) little exercise (2 METs), e.g. reading, b) irregular exercise (3 METs), e.g. fishing, walking the dog, c) regular exercise (2.5-12.5 METs), where subjects stated their regular sport

activities and whether it involved sweating and/or breathlessness, e.g. swimming, skiing. In all statistical analysis the summer and winter levels were combined and the mean was used. The questionnaire on physical activity was adapted from studies undertaken in Gothenburg (257, 258) and the Mobile Clinic Health examination survey of the Social Insurance Institution in Finland. The questionnaire before use was repeated and validated after an interval of about 3 months on 1400 subjects. The reliability estimated by kappa coefficients was at least 0.6 (256).

There was a wide variation in the physical activity class (occupational, commuting and leisure-time physical activity) with highest MET. Therefore, the highest intensity of occupational, commuting or leisure-time activity was used to represent overall activity level of the subjects.

ECG measures

All participants underwent a standard 12-lead resting ECG at baseline at 8 - 9 o'clock AM. Before recording the subject had fasted for 12 hours. ECG abnormalities were classified according to the Minnesota code, including LVH (259). The coder of the ECGs was blinded to the glucose tolerance status and other information on the study subjects. The ischemic changes by ECG (the Whitehall criteria) included Minnesota codes 1.1-1.3, 4.1-4.3, 5.1-5.3, 7.1.

A three-dimensional computerized ECG recording was performed. Measurements for atrial conduction were based on the Dalhousie ECG Program v. 8.0 (260). The program was implemented on an SM-4 computer and adapted for use by the ECG terminals and data communication equipment (Kone 620, Kone Oy, Finland). P wave duration was measured as the time from the onset of the P wave to the end of P wave. The spatial magnitude curve of the X, Y, Z components was used after selective averaging. Computer-identified time points including the onset point of the P wave were displayed, visually verified and obvious measurement errors corrected.

4.2.2 Follow-up study

The 18-year follow-up period lasted until 1 January 2001. Information on the vital status of the participants and copies of death certificates of all deceased subjects who had died before 1 January 2001 were obtained from the Cause-of-Death Register (Statistics Finland). All death certificates of the participants were reviewed by two investigators (Seppo Lehto and Auni Juutilainen). For the final classification of causes of death, hospital records and autopsy records were also used, if available.

The study endpoints were total mortality, CVD mortality [International Classification of Diseases 9th revision (ICD-9) codes 390–459], CHD mortality (ICD-9 codes 410–414) and stroke mortality (ICD-9 codes 431-438).

4.2.3 Statistical methods

All statistical analyses were performed using PASW statistics (version 18.0; SPSS Inc., Chicago, IL, USA) and SAS (version 9.2; SAS Institute Inc., Cary, NC, USA). Data for continuous variables are expressed as mean ± SD or median (interquartile range) and categorical variables as percentage. Baseline characteristics were compared using analysis of variance for continuous variables or the Kruskal–Wallis test, when appropriate, and the χ^2-test for categorical variables. Because the distributions of serum triglycerides and proteinuria were skewed, statistical analysis followed only after logarithmic transformation of these data. The association between study variables and the mortality were analyzed by univariate and multivariate Cox regression analyses and the Kaplan-Meier procedure. Unadjusted and adjusted hazard ratios and their 95% confidence intervals were calculated. Adjustments used in all studies included age, sex, duration of diabetes, area of residence (East or West Finland), total cholesterol, use of alcohol (user vs. non-user), smoking (smoker vs. non-smoker), HDL cholesterol, total triacylglycerol (log), HbA_1, diabetes medication (diet alone, oral drugs, insulin) and BMI. Several other adjustments were used depending of the study. Physical activity (study III and IV), hypertension (no or yes, yes being defined as systolic blood pressure ≥ 160 mmHg, or diastolic pressure ≥ 95 mmHg, or drug treatment) (study I, II, IV), estimated GFR, diastolic BP, BP medication (study III), heart rate and presence of left ventricular hypertrophy (study IV). There were no interactions between sex and any of the outcome variables. Therefore, men and women were combined for all statistical analyses. $P < 0.05$ was considered to be statistically significant.

Study I

Participants were classified according to physical activity (0-4 MET and >4 MET) and proteinuria (≤ 300 mg/L or > 300 mg/L).

Study II

Participants were classified according to physical activity (0 - 4 MET and >4 MET) and hs-CRP (<1.0 mg/L, 1.0 - 3.0 mg/L or >3.0 mg/L). Hs-CRP cut off-points are based on the recommendations of American Heart Association for low risk (<1.0 mg/L), intermediate risk (1.0 – 3.0 mg/L) and high risk (>3.0 mg/L) (222). In the Kaplan-Meier analyses we combined low and average hs-CRP groups, because they did not differ with regard to CVD mortality in relation to physical activity.

Study III

Participants were categorized by systolic BP into four groups (<130 mmHg, 130–139 mmHg, 140–159 mmHg and ≥160 mmHg), as recommended by the European Society of Hypertension for hypertension management (182). Participants were also stratified according to the proteinuria: no proteinuria (≤150 mg/L) or borderline/clinical proteinuria (>150 mg/L). The cut-off level for proteinuria was based on the observation

that proteinuria >150 mg/L is a significant predictor of total and CVD mortality, and indicates patients at high risk of total and CVD mortality as well as incipient renal impairment (5).

Study IV

Participants were categorized according to P wave durations into two groups; normal or prolonged (<114ms or ≥114ms). Various cut off values have been suggested for prolonged P wave duration, such as 110ms or 120ms, where most of the recommendations are based on earlier textbooks and other publications that often did not involve original work (152). Therefore, analyses was made using several cut off values for P wave duration, 104 ms, 108 ms, 110 ms, 114 ms and 120 ms (corresponding to 50, 60, 70, 80 and 90 percentiles of the distribution) to find out a threshold of P wave duration associated with a steep increase in mortality. Death rates/1000 patient years according to 50, 60, 70, 80 and 90 percentiles of P wave duration were as follows; for total mortality: 58.9, 59.6, 60.6, 64.7, 75.4, and for stroke mortality: 6.7, 7.7, 8.1, 9.1 and 11.6. For lower percentiles mortality risk increases relatively smoothly but after 80th percentile the risk increased more rapidly. Therefore the 80th percentile cut off value to categorize participants according to P wave duration into two groups; normal or prolonged (<114 ms or ≥114 ms) was used. Furthermore, participants were stratified according to PNMMVD (yes or no). PNMMVD stratification was made because total and stroke mortality was significantly increased among patients with PNMMVD: 76.8% (322 out of 419) of patients with PNMMVD and 58.4 % (187 out of 320) of patients without PNMMVD died during follow-up (P <0.001 log rank). The corresponding figures for stroke mortality were 9.1% (38 out of 419) and 6.6% (21 out of 320), respectively (p=0.02 log rank).

4.2.4 Ethics

The Ethics Committees of the Turku University and Turku University Central Hospital, and the University of Kuopio approved the study. Informed written consent was obtained from all participants.

5. RESULTS

5.1 CLINICAL CHARACTERISTICS

5.1.1 Baseline general characteristics

The baseline characteristics and CVD risk factors of the original study population in relation to total mortality are presented in Table 3. Patients who died during the 18-year follow-up were significantly older, had worse cholesterol, urinary protein, estimated GFR, hs-CRP and HbA_1 levels. Their physical activity levels were lower and their BMI was higher. Moreover, deceased patients had more likely insulin treatment, retinopathy,

Table 3. Baseline characteristics of type 2 diabetes patients in relation to total mortality.

	Total mortality		
	No	Yes	P value
N	291	768	
Area of residence, Turku,%	55.3	50.5	0.162
Age, years	56.3 ± 5.39	58.8 ± 4.70	<0.001
Women,%	45.7	44.9	0.819
Duration of diabetes, years	8.0 ± 4.0	8.1 ± 4.1	0.846
HbA_1,%	9.3 ± 2.4	10.1 ± 2.2	<0.001
Diabetes treatment			<0.001
diet only,%	27.1	8.9	
oral drugs,%	63.9	75.0	
insulin therapy,%	8.9	16.1	
Total cholesterol, mmol/L	6.47 ± 1.29	6.84 ± 1.78	<0.001
HDL cholesterol, mmol/L	1.30 ± 0.359	1.18 ± 0.351	<0.001
Triglycerides, mmol/L	1.97 ± 1.34	2.85 ± 3.09	<0.001
BMI, kg/m^2	28.8 ± 4.93	29.4 ± 5.19	0.083
Current smokers,%	13.4	17.7	0.092
Alcohol users,%	41.6	35.9	0.090
Physical activity, MET	4.4 ± 1.9	3.7 ± 1.8	<0.001
Systolic BP	150 ± 22	154 ± 24	0.005
Diastolic BP	86 ± 11	86 ± 12	0.921
BP medication,%	39.9	52.7	<0.001
Hypertension / -medication,%	52.6	66.9	<0.001
Hs-CRP	2.38 ± 3.64	4.12 ± 6.58	<0.001
Urinary protein, mg/L	153 ± 264	378 ± 818	<0.001
Estimated GFR, ml min^{-1} $1.73m^{-2}$	92.5 ± 20.0	87.5 ±21.3	0.001
Retinopathy (mild/proliferative),%	21.4	30.0	0.005
Heart rate, bpm	71 ± 12	76 ± 15	<0.001
QTc, ms	418 ± 27	421 ±29	0.182
P wave duration, ms	103 ± 17	103 ± 23	0.995
LVH,%	10.0	16.5	0.007
CHD at baseline *,%	13.7	29.3	<0.001
PNMMVD †,%	48.1	72.1	<0.001

Data are expressed as the mean ± SD, unless otherwise indicated

* Baseline CHD by symptoms (typical angina pectoris) and ECG changes, † Prevalent non-major macrovascular disease: CHD (ischemic ECG changes and typical symptoms of angina pectoris), or claudication

CHD or claudication, LVH and their resting heart rate was higher. After 18-years of follow-up, 291 (27.5%) subjects of the original study population were alive.

5.1.2 Studies I-II

The general characteristics of the study population at baseline are presented in Table 4 by physical activity. Physically active type 2 diabetes patients were slightly younger, had lower triglyceride levels, BMI, and blood pressure, smoked less often, used insulin more often and had lower hs-CRP level than physically inactive patients. Physically active patients also tended to have a lower total cholesterol concentration.

Table 4. Baseline characteristics and number of subjects with various outcomes by physical activity among patients with type 2 diabetes

	Physical activity		
	0-4 MET	**> 4 MET**	**P value**
N	347	230	
Area of residence, Turku,%	62.0	64.8	0.491
Age, years	58.2 ± 5.1	56.5 ± 5.3	<0.001
Women,%	50.1	38.7	0.007
Duration of diabetes, years	7.9 ± 4.0	7.8 ± 4.0	0.689
HbA_1,%	9.7 ± 1.8	9.9 ± 3.0	0.222
Diabetes treatment			0.054
diet only,%	13.0	16.1	
oral drugs,%	76.9	68.3	
insulin therapy,%	10.1	15.7	
Total cholesterol, mmol/L	6.62 ± 1.68	6.39 ± 1.38	0.089
HDL cholesterol, mmol/L	1.24 ± 0.35	1.28 ± 0.36	0.195
Triglycerides, mmol/L	2.39 ± 2.58	1.89 ± 1.24	0.006
BMI, kg/m^2	29.9 ± 5.9	28.2 ± 4.4	<0.001
Current smokers,%	19.6	11.3	0.008
Alcohol users,%	37.5	42.6	0.216
Hypertension / -medication,%	61.7	52.2	0.024
hs-CRP, mg/L	3.1 ± 3.8	2.4 ± 3.7	0.044
High sensitivity CRP, N			0.080
< 1 mg/L	118	86	
1 – 3 mg/L	120	89	
> 3 mg/L	106	50	
Proteinuria, N			0.287
No	288	200	
Yes	56	30	
Subjects with end points, N			
Total mortality	234	122	
CVD mortality	143	74	
CHD mortality	98	51	

Data are expressed as the mean ± SD, unless otherwise indicated

Among the physically active group, 5.2% had proteinuria (>300 mg/L) and 94.8% had not; 8.7% had hs-CRP >3.0 mg/L and 91.3% had hs-CRP <3.0 mg/L. The corresponding

figures among the physically inactive were 9.8% had proteinuria (>300 mg/L) and 90.2% had not and 18.4% had hs-CRP >3.0 mg/L and 81.6% had hs-CRP <3.0 mg/L

5.1.3 Study III

The general characteristics of the study participants at baseline by the four systolic BP categories are shown in Table 5. Higher baseline systolic BP levels were significantly associated with female gender, increased age, diastolic BP, BP medication use, total cholesterol, triglycerides, BMI, CHD or claudication and proteinuria and a lower prevalence of smoking. Patients with systolic BP between 130–139 mmHg and ≥160 mmHg used least alcohol. Among patients with no proteinuria, 16.3% had systolic BP <130 mmHg,

Table 5. Baseline characteristics and number of subjects with endpoints by systolic blood pressure.

	Baseline systolic BP (mmHg)				
	<130	**130–139**	**140–159**	**≥160**	**P value**
N	129	112	327	317	
Area of residence, Turku,%	65.9	52.7	54.1	51.1	0.039
Age (years)	56.0±5.69	57.1±5.01	57.8±5.00	59.2±4.52	<0.001
Women,%	29.5	44.6	44.0	60.6	<0.001
Duration of diabetes, years	7.9±4.9	7.7±4.3	8.1±3.7	8.0±3.7	0.898
HbA_1,%	10.0±2.9	9.8±2.0	9.9±1.9	9.9±2.3	0.813
Diabetes treatment					0.851
Diet alone,%	15.5	10.7	14.7	14.5	
Oral drugs,%	71.3	72.3	72.5	73.2	
Insulin therapy,%	13.2	17.0	12.8	12.3	
Total cholesterol, mmol/L	6.34±1.49	6.55±1.38	6.70±1.78	6.87±1.76	0.019
HDL cholesterol, mmol/L	1.19±0.35	1.29±0.39	1.21±0.35	1.25±0.37	0.060
Triglycerides, mmol/L	1.89±0.99	1.96±1.16	2.64±3.14	2.83±3.27	0.001
BMI, kg/m^2	28.1±4.88	28.6±5.38	29.6±4.87	29.7±5.78	0.009
Current smokers,%	23.3	18.8	17.4	11.7	0.016
Alcohol users,%	51.9	29.5	41.6	29.7	<0.001
Physical activity, METs	4.3±2.2	4.2±2.0	3.9±1.7	3.9±1.7	0.091
Diastolic BP, mmHg	76.8±9.21	78.8±9.22	86.0±9.37	92.3±11.8	<0.001
BP medication,%	17.1	27.7	48.6	64.4	<0.001
Urinary protein, mg/L	130(80–230)	120(0–228)	140(90–230)	170(100–305)	<0.001
Proteinuria, *n*					
no (<150 mg/L)	77	72	180	143	
yes (≥150 mg/L)	52	40	143	174	
Estimated GFR, ml min^{-1} $1.73m^{-2}$	90.1±17.3	91.4±19.8	91.3±20.4	88.8±23.0	0.420
Retinopathy (mild/proliferative),%	21.9	27.3	25.9	29.4	0.42
CHD without MI at baseline*,%	14.7	14.3	15.6	21.1	0.155
PNMMVD†,%	51.9	52.7	59.0	64.7	0.033
Subjects with end points, N					
Total mortality	83	69	229	229	
CVD mortality	50	46	156	145	
CHD mortality	40	35	104	102	

Data are expressed as the mean ± SD or median (interquartile range), unless otherwise indicated. *P* value obtained by analysis of variance, the Kruskal–Wallis test or chi-square test. * Baseline CHD by symptoms (typical angina pectoris) and ECG changes without prior MI, † Prevalent non-major macrovascular disease: CHD (ischemic ECG changes and typical symptoms of angina pectoris), or claudication.

15.3% had 130–139 mmHg, 38.1% had 140–159 mmHg and 30.3% had ≥160 mmHg. The proportion of patients by BP with proteinuria was 12.7%, 9.8%, 35.0% and 42.5%, respectively.

5.1.4 Study IV

The general characteristics of the study population at baseline are presented in Table 6 by P wave duration. A prolonged duration of the P wave at baseline was associated with male gender, increased age, triglycerides, BMI and increased urinary protein. Prolonged P wave duration was also associated with a higher prevalence of hypertension and prevalent non-major macrovascular disease (PNMMVD). 16.1% of the subjects

Table 6. Baseline characteristics and number of subjects with various outcomes by P wave duration among patients with type 2 diabetes.

	P wave duration		
	<114 ms	**≥114 ms**	**P value**
N	647	92	
Area of residence, Turku, %	51.8	48.8	0.489
Age, years	57.5±5.29	59.0±4.21	0.001
Women, %	50.6	41.2	0.031
Duration of diabetes (years)	7.9±3.9	8.0±3.9	0.649
HbA_1, %	9.90±2.39	10.1±1.93	0.257
Diabetes treatment			0.823
diet only, %	13.5	14.7	
oral drugs, %	74.2	71.8	
insulin therapy, %	12.3	13.5	
Total cholesterol, mmol/L	6.68±1.66	6.81±1.98	0.381
HDL cholesterol, mmol/L	1.25±0.363	1.19±0.310	0.051
Triglycerides, mmol/L	2.38±2.58	3.10±4.08	0.006
BMI, kg/m^2	29.0±5.23	30.2±5.13	0.007
Current smokers, %	16.7	17.6	0.772
Alcohol users, %	35.9	43.5	0.0.70
Physical activity, MET	4.1±1.9	3.85±1.8	0.091
Hypertension / -medication, %	56.6	74.7	<0.001
Urinary protein, mg/L	220±445	396±879	0.001
Heart rate, bpm	74±13	75±15	0.368
LVH, %	12.3	16.5	0.160
PNMMVD*, %	52.7	70.0	<0.001
Death rates/1000-patient-years			
Total mortality	52.9	64.7	0.039
CVD mortality	33.7	43.5	0.070
CHD mortality	24.7	30.3	0.326
Stroke mortality	5.7	9.1	0.153

Data are expressed as the mean ± SD, unless otherwise indicated

* Prevalent non-major macrovascular disease: CHD (ischemic ECG changes and typical symptoms of angina pectoris), or claudication

had prolonged P wave duration (≥114 ms) and PNMMVD, 6.9% had prolonged P wave duration, but no PNMMVD. 40.6% had not prolonged P wave duration and PNMMVD, and 36.4% had not prolonged P wave duration and no PNMMVD. PNMMVD patients' median P wave duration was 106 ms (SD±14.4), minimum 62 ms and maximum 174 ms. Patients without PNMMVD had median P wave duration of 104 ms (SD±10.8), minimum 70 ms, maximum 135 ms.

5.2 PHYSICAL ACTIVITY, PROTEINURIA AND MORTALITY (I)

During 7,746 patient-years of follow-up, 354 (61.7%) patients died, 216 (37.6%) of them from CVD. In the physically active group 25 out of 30 participants with proteinuria died (16 from CVD) and 97 out of 200 participants without proteinuria died (58 from CVD). In the group of physically inactive participants 45 out of 56 participants with proteinuria died (26 from CVD) and 187 out of 288 participants without proteinuria died (116 from CVD). The event rates of total, CVD and CHD mortality per 1000 patient-years of follow-up among physically active (>4 MET) and inactive (0-4 MET) patients stratified by baseline proteinuria (0-300 mg/L and >300 mg/L) are shown in fig 7.

The physically more active group of participants with no proteinuria had a significantly reduced relative risk of total mortality, CVD mortality and CHD mortality by univariate and multivariate analyses (Table 7.). Univariate analyses showed that there were no significant differences in any of the outcome variables between physically active and inactive patients with proteinuria. However, by multivariate analysis the physically active group with proteinuria had a significantly increased risk of total and CVD mortality.

Table 7. Hazard ratios (physically active vs. inactive) for total mortality, CVD mortality and CHD mortality stratified by proteinuria. Variables used for multivariable adjustment: age, sex, diabetes duration, HbA1, total cholesterol, presence of hypertension, smoking, alcohol, BMI, area of residence, type of diabetes therapy, triglycerides(log), and HDL cholesterol

	Hazard ratio (95% CI)					
	No adjusted relative risk		Age-adjusted relative risk		Multivariate-adjusted relative risk	
Variables	Active vs. inactive	P	Active vs. inactive	P	Active vs. inactive	P
Total Mortality						
No proteinuria	0.61 (0.48-0.78)	<0.001	0.65 (0.51-0.84)	0.001	0.66 (0.51-0.86)	0.002
Proteinuria	1.11 (0.68-1.81)	0.688	1.21 (0.73-2.00)	0.455	1.83 (1.00-3.36)	0.049
CVD Mortality						
No proteinuria	0.59 (0.43-0.80)	0.001	0.63 (0.46-0.86)	0.004	0.63 (0.45-0.88)	0.007
Proteinuria	1.24 (0.66-2.31)	0.500	1.38 (0.73-2.61)	0.33	2.43 (1.09-5.40)	0.030
CHD Mortality						
No proteinuria	0.62 (0.42-0.90)	0.011	0.67 (0.46-0.97)	0.035	0.66 (0.44-0.97)	0.037
Proteinuria	1.09 (0.49-2.45)	0.833	1.14 (0.50-2.61)	0.75	1.49 (0.51-4.38)	0.472

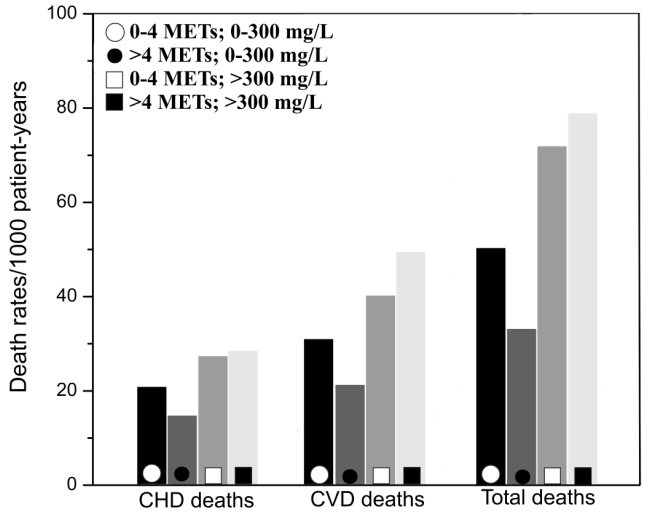


Figure 7. Total, CVD and CHD mortality among physically active (>4 MET) and inactive (0-4 MET) type 2 diabetic patients stratified by baseline proteinuria (0-300 mg/L and >300 mg/L). Event-rates are expressed per 1000 patient-years of follow-up

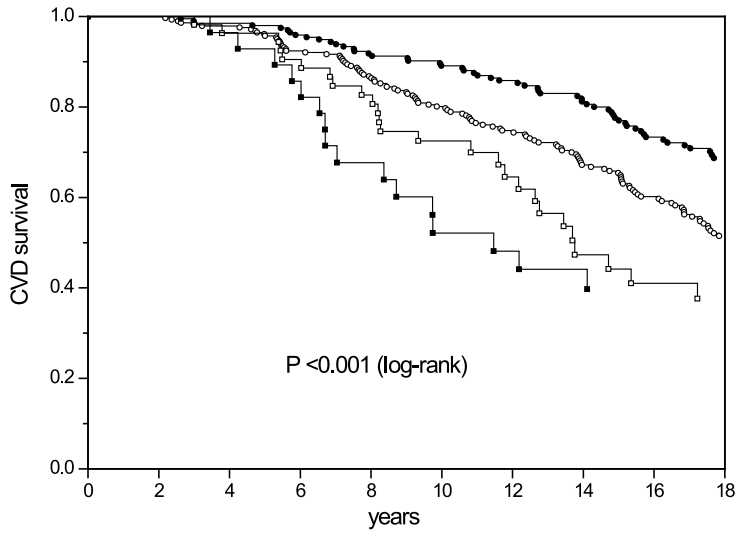


Figure 8. Kaplan-Meier survival curve for CVD mortality among physically active and inactive type 2 diabetic patients stratified by baseline urinary protein. MET represents the physical activity of the subjects. ○, physically inactive patients (0–4 METs) with urinary protein levels ≤300 mg/L; ●, physically active patients (>4 METs) with urinary protein levels ≤300 mg/L; □, physically inactive patients (0-4 METs) with urinary protein levels >300 mg/L; ■, physically active patients (>4 METs) urinary protein levels >300 mg/L. *P* value denotes the difference between the survival curves (log rank).

The Kaplan-Meier curves in Figure 8 show that the advantageous effect of physical activity with respect to CVD mortality in non-proteinuric patients became evident after 5 years of follow-up. The disadvantageous effect of physical activity in patients with proteinuria became evident after 4 years of follow-up, but was no longer observed after 15 years.

5.3 PHYSICAL ACTIVITY, HS-CRP AND MORTALITY (II)

During 7,797 patient-years of follow-up 356 (61.7%) patients died, including 217 (37.6%) from CVD. In the physically active group 30 out of 50 participants with hs-CRP levels >3.0 mg/L died (21 from CVD) and 87 out of 175 participants with hs-CRP levels 0-3.0 mg/L died (52 from CVD). In the physically inactive group 85 out of 106 participants with hs-CRP levels >3.0 mg/L died (57 from CVD) and 147 out of 238 participants with hs-levels 0-3.0 mg/L died (84 from CVD). The event rates of total, CVD and CHD mortality per 1000 patient-years of follow-up among physically active (>4 MET) and inactive (0-4 MET) patients stratified by baseline hs-CRP levels (0-3.0 mg/L and >3.0 mg/L) are shown in fig 9.

In age-adjusted or multivariate analyses among subjects with hs-CRP levels ≤3.0 mg/L there were no significant differences in any of the outcome variables between physically active and inactive patients. However, by both univariate and multivariate analyses among patients with hs-CRP >3.0 mg/L the physically more active group had significantly lower total mortality, CVD mortality and CHD mortality (Table 8).

Table 8. Hazard ratios (physically active vs. inactive) for total mortality, CVD mortality and CHD mortality stratified by serum high sensitive C-reactive protein concentration

	HR (95% CI)			
	Age adjusted relative risk		Multivariate-adjusted relative risk	
Variables	Active vs. inactive	P value	Active vs. inactive	P
Total mortality				
hs-CRP < 1.0 mg/L	0.79 (0.53-1.19)	0.266	0.96 (0.57-1.59)	0.863
hs-CRP 1.0 - 3.0 mg/L	0.82 (0.57-1.17)	0.263	1.10 (0.71-1.70)	0.667
hs-CRP > 3.0 mg/L	0.51 (0.33-0.77)	0.001	0.51 (0.30-0.85)	0.011
CVD mortality				
hs-CRP < 1.0 mg/L	0.68 (0.39-1.18)	0.167	0.74 (0.36-1.51)	0.408
hs-CRP 1.0 – 3.0 mg/L	0.97 (0.61-1.53)	0.889	1.59 (0.89-2.84)	0.119
hs-CRP > 3.0 mg/L	0.55 (0.33-0.92)	0.022	0.53 (0.28-0.99)	0.045
CHD mortality				
hs-CRP < 1.0 mg/L	0.63 (0.33-1.21)	0.163	0.70 (0.30-1.63)	0.405
hs-CRP 1.0 -3.0 mg/L	1.09 (0.63-1.87)	0.765	1.61 (0.82-3.17)	0.166
hs-CRP > 3.0 mg/L	0.49 (0.26-0.92)	0.027	0.36 (0.15-0.84)	0.019

Variables used for multivariable adjustment: age, sex, area of residence, diabetes duration, total cholesterol, HDL cholesterol, triglycerides(log), proteinuria(log), smoking, alcohol, HbA_1, presence of hypertension, BMI and type of diabetes therapy.

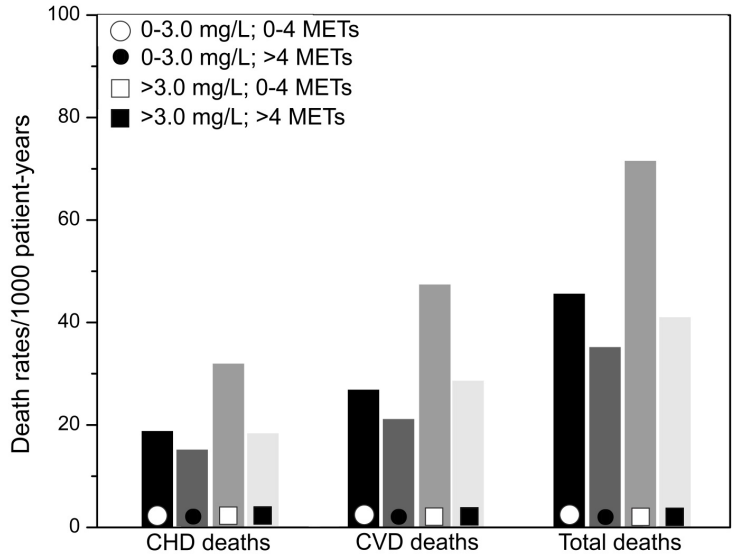


Figure 9. Total, CVD and CHD mortality among physically active (>4 MET) and inactive (0-4 MET) patients with type 2 diabetes stratified by baseline hs-CRP levels (0-3.0 mg/L and >3.0 mg/L). Event-rates are expressed per 1000 patient-years of follow-up.

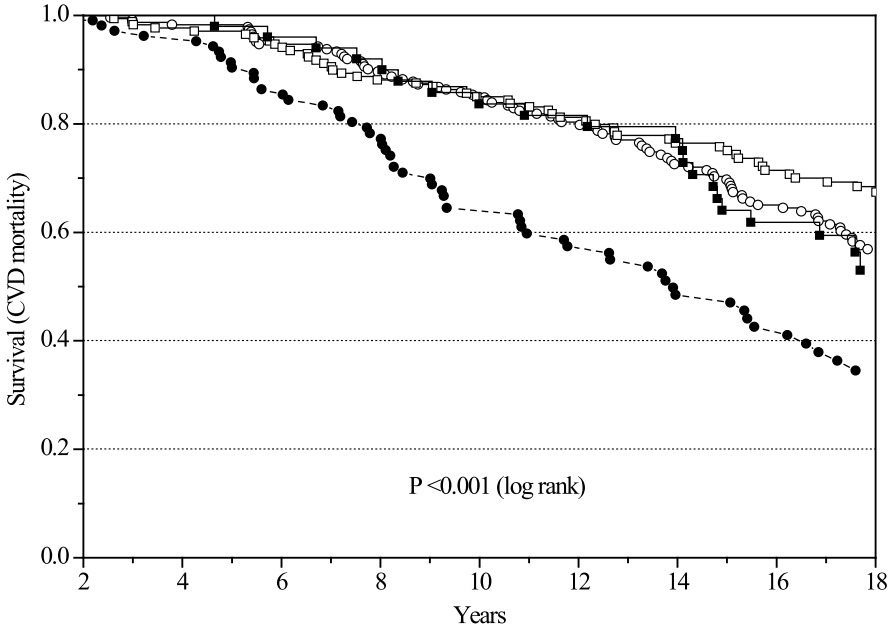


Figure 10. Kaplan-Meier survival curve describing CVD mortality among physically active and inactive type 2 diabetic patients stratified by baseline hs-CRP levels. MET represents the physical activity of the subjects. ○, physically inactive patients (0–4 METs) with hs-CRP levels ≤3.0 mg/L; ●, physically inactive patients (0–4 METs) with hs-CRP levels >3.0 mg/L; □, physically active patients (>4 METs) with hs-CRP levels ≤3.0 mg/L; ■, physically active patients (>4 METs) with hs-CRP levels >3.0 mg/L. *P* value denotes the difference between the survival curves (log rank).

The Kaplan-Meier curves in Figure 10 show that patients with hs-CRP levels >3.0 mg/L at baseline and who were physically inactive had a poorer prognosis than those who were physically active. This became evident after 4 years of follow-up. Physically active patients with a high hs-CRP had a similar prognosis with respect to CVD mortality as physically inactive patients with low or intermediate CRP levels.

5.4 SYSTOLIC BLOOD PRESSURE, PROTEINURIA AND MORTALITY (III)

During 10,784 patient-years of follow-up, 607 (68.9%) patients died, including 395 (44.8%) from CVD. The number of patients without proteinuria in the BP groups <130 mmHg, 130–139 mmHg, 140–159 mmHg and >160 mmHg were 77, 72, 180 and 143, respectively; the numbers of patients with proteinuria were 52, 40, 143 and 174, respectively. The event rates of total, CVD and CHD mortality per 1000 patient-years of follow-up among systolic blood pressure groups (<130, 130-139, 140-159, ≥160 mmHg) stratified by baseline proteinuria levels (0-150 mg/L and >150 mg/L) are shown in fig 11.

By Cox regression analyses, there was a statistically significant interaction between proteinuria and baseline systolic BP (P=0.01) for total mortality, and an interaction of borderline significance (P=0.05) for CVD mortality. For CHD mortality the P value for interaction was 0.07 in the univariate analysis and 0.11 in the multivariate analysis. Because of this interaction, the mortality rates were examined by systolic BP categories separately for subjects with and without proteinuria (Table 9).

After adjustment for confounding factors, patients with proteinuria and a systolic BP <130 mmHg had a significantly increased total and CVD mortality compared to those with a systolic BP between 130 and 139 mmHg (approximately 2-fold higher mortality) and to those with a systolic BP between 140 and 159 mmHg (1.7-fold higher mortality; P<0.05 for both). CVD mortality was 1.7-fold higher among patients with a systolic BP <130 mmHg compared to those with a systolic BP ≥160 mmHg. Total mortality did not significantly differ between patients with systolic BP <130 mmHg or ≥160 mmHg. Among patients without proteinuria, a systolic BP <130 mmHg tended to be associated with slightly reduced CVD mortality. There was no interaction between proteinuria and diastolic BP and therefore only the results for systolic BP are shown.

Table 9. Relative mortality of patients by systolic BP category stratified by the presence or absence of proteinuria, compared to patients with a systolic BP <130 mmHg.

	Hazard ratio (95% CI)				
	Systolic BP (mmHg)				
Variables	**<130**	**130–139**	**140–159**	**≥160**	**P for interaction**
Total mortality					
Age adjusted					
No proteinuria	1.00	1.06 (0.69–1.64)	1.33 (0.92−1.91)	1.14 (0.78−1.67)	
					0.01
Proteinuria	1.00	0.51 (0.31−0.82)*	0.57 (0.40−0.82)*	0.65 (0.46−0.91)*	
Multivariate adjusted†					
No proteinuria	1.00	1.04 (0.66−1.65)	1.29 (0.88−1.91)	1.10 (0.73−1.68)	
					0.02
Proteinuria	1.00	0.50 (0.30−0.83)*	0.62 (0.42−0.91)*	0.78 (0.53−1.16)	
CVD mortality					
Age adjusted					
No proteinuria	1.00	1.32 (0.77−2.27)	1.47 (0.92−2.34)	1.27 (0.78−2.07)	
					0.05
Proteinuria	1.00	0.46 (0.24−0.86)*	0.66 (0.43−1.03)	0.64 (0.42−1.0)*	
Multivariate adjusted†					
No proteinuria	1.00	1.30 (0.73−2.32)	1.33 (0.80−2.21)	1.08 (0.63−1.86)	
					0.05
Proteinuria	1.00	0.43 (0.22−0.84)*	0.61 (0.38−0.97)*	0.62 (0.38−1.02)	
CHD mortality					
Age adjusted					
No proteinuria	1.00	1.33 (0.73−2.45)	1.15 (0.67−1.99)	1.12 (0.64−1.96)	
					0.07
Proteinuria	1.00	0.38 (0.18−0.81)*	0.57 (0.35−0.94)*	0.55 (0.33−0.90)*	
Multivariate adjusted†					
No proteinuria	1.00	1.39 (0.72−2.67)	1.13 (0.63−2.05)	1.09 (0.58−2.04)	
					0.11
Proteinuria	1.00	0.40 (0.18−0.88)	0.61 (0.36−1.05)	0.63 (0.36−1.11)	

* $P<0.05$ for the difference between patients in various systolic BP groups and patients with systolic BP <130 mmHg; † variables included in multivariate adjustment: age, sex, diabetes duration, HbA_1, total cholesterol, smoking, alcohol, BMI, area of residence, physical activity, diastolic pressure, BP medication, type of diabetes therapy, triglycerides(log), estimated GFR, HDL cholesterol and presence of retinopathy and of CHD without MI.

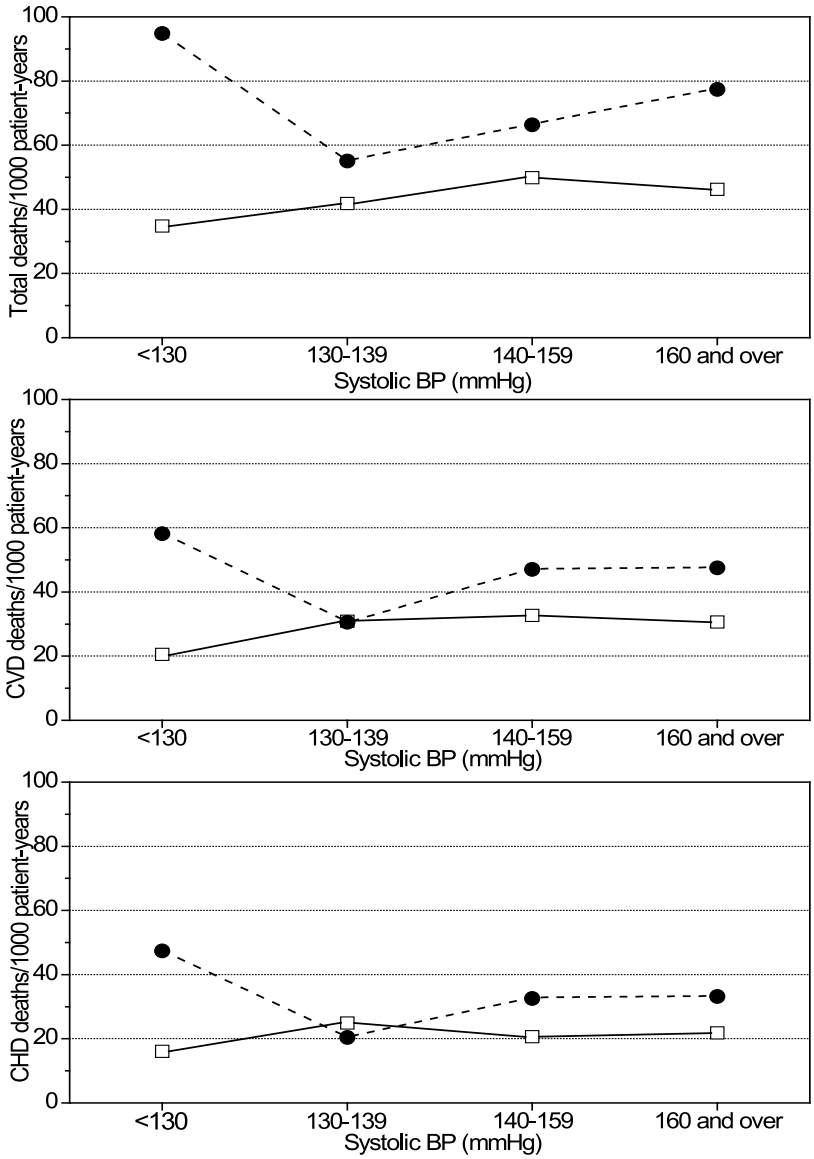


Figure 11. Total, CVD and CHD mortality in patients with no proteinuria (solid line and □,) and with proteinuria (dashed line and ●) by systolic BP categories. Event-rates are expressed per 1000 patient-years of follow-up.

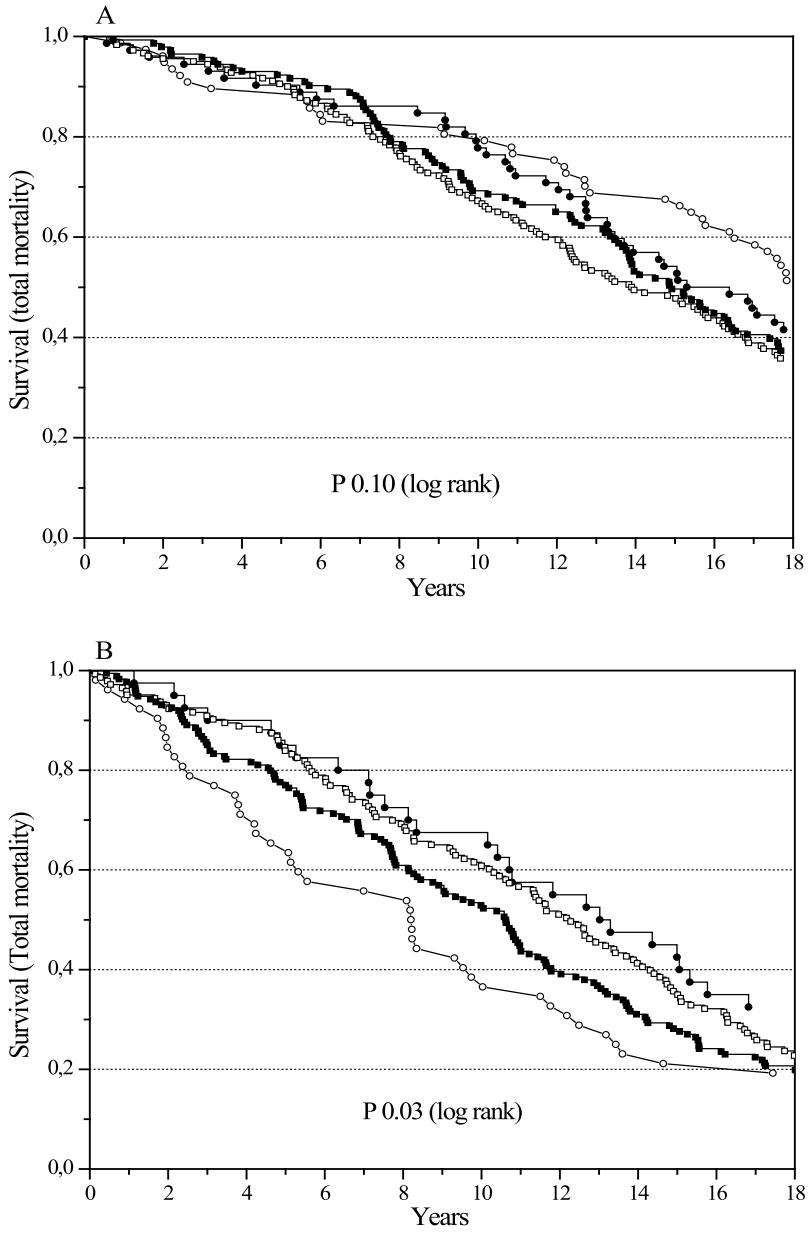


Figure 12. Kaplan–Meier total survival curves by systolic BP categories stratified by baseline proteinuria: urinary protein ≤150 mg/L (A) and >150 mg/L (B). ○, systolic BP <130 mmHg; ●, systolic BP 130–139 mmHg; □, systolic BP 140–159 mmHg; ■, systolic BP ≥160 mmHg. *P* value denotes the difference between the survival curves.

The Kaplan–Meier curves in Figure 12 show that patients with no proteinuria and a systolic BP <130 mmHg tended to have the lowest total mortality (upper panel, highest line). On the other hand patients with proteinuria and a systolic BP <130 mmHg had the highest total mortality (lower panel, lowest line). The mortality difference emerged already after 2 years of follow-up.

To study whether the high risk of CVD death in patients with proteinuria and a systolic BP <130 mmHg could be explained by differences in the prevalence of baseline CHD without prior MI, the prevalence of CHD by symptoms and ECG changes was scrutinized in the population stratified by proteinuria and systolic BP. Among patients with proteinuria, the prevalence of CHD was 28.8%, 20.0%, 15.4% and 22.4% by systolic BP <130, 130–139, 140–159 and ≥160 mmHg, respectively (P = 0.18). In patients with no proteinuria, the corresponding prevalence figures were 5.2%, 11.1%, 14.4% and 19.6% (P = 0.027).

5.5 P WAVE DURATION AND MORTALITY (IV)

During 9,185 patient-years of follow-up a total of 509 (68.9%) patients died, 59 (8.0%) died from stroke. Among the participants without prolongation of the P wave duration, 221 out of 300 with prevalent non-major macrovascular disease (PNMMVD) died (22 from stroke) and 160 out of 269 participants without PNMMVD died (19 from stroke). Among the participants with prolongation of the P wave duration, 101 out of 119 with PNMMVD died (16 from stroke) and 27 out of 51 participants without PNMMVD died (2 from stroke). The event rates of total and stroke mortality per 1000 patient-years of follow-up according to P wave duration in patients with or without PNMMVD are shown in fig 13.

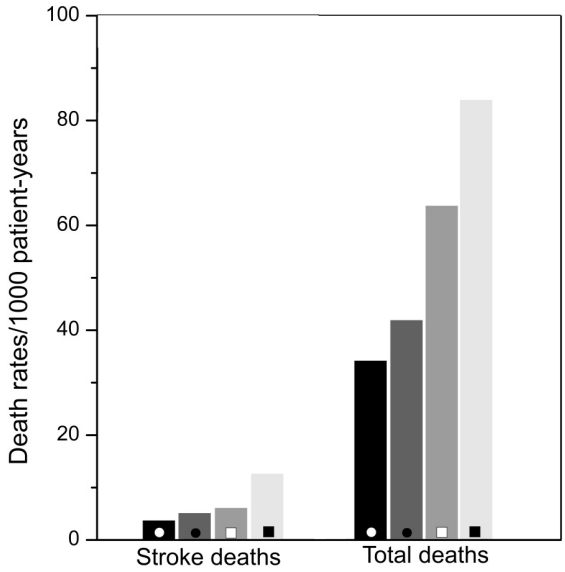
By Cox regression analyses, there was a statistically significant interaction between P wave duration 114 ms (80th percentile) and PNMMVD with respect to stroke mortality. This cut off value for P wave duration was used to calculate hazard ratios for mortality among patients with and without PNMMVD (Table 10).

Among the patients with PNMMVD those who had prolonged P wave duration had significantly an increased risk for total and stroke mortality in univariate analysis. This trend was also seen in multivariate analysis, but the association was significant only for stroke mortality. The relative risk for stroke mortality among PNMMVD patients with prolonged P wave duration was approximately 2.5 times higher in both univariate and multivariate analysis when compared to patients with normal P wave duration. Among patients without PNMMVD prolonged P wave duration was not associated with total or stroke mortality.

Table 10. Hazard ratios (P wave ≥114 ms vs. P wave <114 ms) for total mortality, CVD and stroke mortality stratified by prevalent non-major macrovascular disease (PNMMVD†)

	HR (95% CI)			
	Age-adjusted relative risk		Multivariate-adjusted relative risk	
Variables	P wave ≥114 vs. <114 ms	P for Interaction	P wave ≥114 vs. <114 ms	P for Interaction
Total mortality				
No PNMMVD†	0.75 (0.50-1.13)		0.70 (0.45-1.09)	
		0.020		0.047
PNMMVD	1.31 (1.03-1.66)*		0.118 (0.90-1.54)	
Stroke mortality				
No PNMMVD	0.47 (0.11-2.04)		0.36 (0.08-1.66)	
		0.057		0.089
PNMMVD	2.25 (1.18-4.31)*		2.45 (1.11-5.37)*	

* P<0.05 for the difference between P wave ≥114 vs. <114 ms. Variables in multivariate adjusted: age, sex, area of residence, diabetes duration, total cholesterol, HDL cholesterol, triglycerides(log), proteinuria(log), smoking, alcohol, HbA1, presence of hypertension, BMI, type of diabetes therapy, physical activity, heart rate and left ventricular hypertrophy. † Prevalent non-major macrovascular disease: CHD (ischaemic ECG changes and typical symptoms of angina pectoris), or claudication.



Total and stroke mortality according to P wave duration stratified by prevalent non-major macrovascular disease (PNMMVD): ○ No PNMMVD with P wave duration ≥114 ms; ● No PNMMVD with P wave duration <114; □ PNMMVD with P wave duration <114 ms; ■ PNMMVD with P wave duration ≥114 ms. Event-rates are expressed per 1000 patient- years of follow-up. Number of patients without PNMMVD in the two P wave duration groups (<114ms and ≥114ms) were 269 and 51, respectively; the respective number of patients with PNMMVD were 300 and 119.

Figure 13. Total and stroke mortality among patients with type 2 diabetes by P wave duration stratified by prevalent non-major macrovascular disease: CHD (ischemic ECG changes and typical symptoms of angina pectoris) or claudication (PNMMVD). Event-rates are expressed per 1000 patient-years of follow-up.

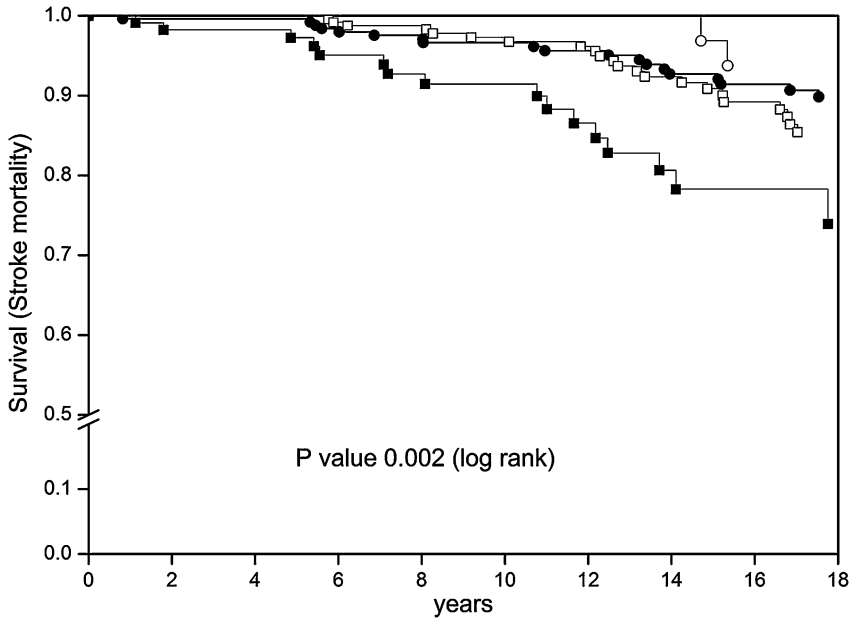


Figure 14. The Kaplan-Meier survival curve for stroke mortality according to P wave duration stratified by prevalent non-major macrovascular disease (PNMMVD): ○ No PNMMVD with P wave duration ≥114 ms (n= 51); ● No PNMMVD with P wave duration <114 (n=269); □ PNMMVD with P wave duration <114 ms (n=300); ■ PNMMVD with P wave duration ≥114 ms (n=119). *P* value denotes the difference between the survival curves (log rank).

The Kaplan-Meier curve (Fig.14) indicates the cumulative survival with respect to stroke mortality for patients with normal or prolonged P wave duration stratified by baseline PNMMVD. Patients with PNMMVD and P wave duration ≥114 ms had poorer prognosis than those with P wave duration <114 ms. This started to become evident after four years of follow-up. Among patients without PNMMVD there was no statistical difference between P wave duration curves in Kaplan-Meier analysis.

6. DISCUSSION

6.1 STUDY SUBJECTS

This thesis is based on the Finnish East-West type 2 diabetes study, which is a prospective follow-up study aiming to investigate conventional and non-conventional risk factors for fatal and non-fatal CVD events and to explore any East-West differences that impact the CV risk of patients with type 2 diabetes. The present thesis is focused on the 18-year follow-up study performed in 2001.

The original study population in 1982-1984 consisted of 1,059 patients with type 2 diabetes. They were 45-64 years old and lived either in the Turku University Central Hospital district in West Finland (participation rate 79%) or in the Kuopio University Hospital district in East Finland (participation rate 83%). The cohort was identified on the basis of a national drug reimbursement register maintained by the Social Insurance Institution of Finland. The study cohort represents fairly well patients with type 2 diabetes. The selection of study subjects was population based and the participation rate was high. In addition, OGTT was performed, if the diagnosis could not be verified on the basis of physicians' certificates or medical records, and type 1 diabetes patients were excluded from the study by the glucagon-stimulated C-peptide test. However, selection of the participants from the drug reimbursement registry leaves out many patients treated with diet alone, and it is likely that the proportion of diet-treated type 2 diabetic patients in this study is lower than in reality. Based on a large population based study carried out in 1979-1980 in Finland, the proportion of diet treated patients was 34% (262).

Type 2 diabetic patients in this study had poor glycemic control. The mean HbA_1 was approximately 9.7% among men and 10.2% among women. It is also noteworthy, that at the time of the baseline study, the WHO diagnostic criteria for type 2 diabetes differed from those of today (12). At the time of the baseline study, the fasting plasma glucose limit for diabetes was 7.8 mmol/L, now it is $\geq$7.0 mmol/L. This increased stringency excluded many subjects who currently would be considered as having diabetes. It is also noteworthy that study patients had been diagnosed with type 2 diabetes at least 8 years before the baseline examination. Therefore, study patients had already developed relatively severe CVD complications. This was considered in the analysis by excluding patients with severe CVD complications at baseline. Therefore, the study population probably represents type 2 diabetic patients with more severe disease.

6.2 METHODS

6.2.1 Physical activity

Physical activity was assessed with a self-administered questionnaire. A MET value was assigned for each of the categories (occupational, commuting or leisure-time activity) according to the intensity of the activity. Using a questionnaire for assessment of habitual physical activity is crude and imprecise (243, 244). It has, however, been suggested that questionnaires can be used to classify subjects to distinct physical activity classes (e.g., low vs. high physical activity) (243, 244). The questionnaire was adapted from studies undertaken in Gothenburg (257, 258) and the Mobile Clinic Health examination survey of the Social Insurance Institution in Finland. The questionnaire was validated to use after an interval of about 3 months on 1400 subjects. The reliability estimated by kappa coefficients was at least 0.6 (256).

The highest intensity of occupational, commuting or leisure-time activity was recorded to represent overall activity level of the subjects to reduce the wide variation in the physical activity classes. Similar questionnaires for assessment of physical activity have been used previously in population based studies (239, 245). These questionnaires, together with interviews and diaries, are the most common tools for the assessment of physical activity in population based studies, because the methodologies are relatively cheap and easy to carry out and can be used to classify patients into groups of low and high physical activity (243, 244). Previous studies have shown that self-reported physical activity is associated with decreased CVD mortality (239, 245). Thus, the present study may have yielded some random misclassification of self-reported physical activity, particularly over reporting of physical activity, and this could result in underestimation rather than overestimation of the impact of physical activity on outcome.

6.2.2 Laboratory methods

All blood specimens were drawn at baseline at 8.00 o'clock AM after a 12-hour fast, centrifuged within one hour and immediately frozen at -20°C. High sensitivity-CRP measurements were made in 2001 from the frozen blood samples. Juonala et al analyzed the stability of the stored CRP samples of 39 subjects of this study in 2006. The mean ± SD (median) CRP values in serial measurements were 2.10 ± 2.26 mg/L (1.7 mg/L) in 2001 and 1.90 ± 1.81 mg/L (1.6 mg/L) in 2006 (263). Thus, during some 20 years of storage, a reduction on the measured hs-CRP values may have occurred. Nevertheless, this decrease should be similar for all patients. The correlation between the values in samples analyzed at 5-year intervals was very high (r=0.997), and the coefficient of variation between the two measurements was only 6.5% (263). In this same study population hs-CRP values have previously been associated with an increase in CVD mortality (227). At the time of the baseline study, HbA_1 was routinely used instead of HbA_{1c}. HbA_1 includes different glycosylated derivatives, HbA_{1a1}, HbA_{1a2}, HbA_{1b},

HbA_{1c} and hence the reference range is higher than for HbA_{1c} alone. Measurements of HbA_1 are susceptible to higher interference by unstable intermediates than HbA_{1c} and standardization of different methods was difficult. However, the method used in this study was less sensitive to metabolic changes than other techniques (264).

At the baseline examination, the total urinary protein concentration was measured from spot urine samples with the Coomassie brilliant blue method, not with a specific assay for albumin which constitutes about one half of the total urinary protein (265-268). MA was not identified as a predictor of mortality until 1984 (269). In study I and III, cut-off points of 150mg/L and 300 mg/L were used for limits of urinary protein. In other studies, urinary protein was used in multivariate adjustments as a continuous variable. The cut-off point of 150 mg/L for urinary protein corresponds roughly to albuminuria of 60-140 µg/min, as MA by definition is a state of persistent, increased urinary excretion of albumin (20-200 µg/min) (116). The cut-off point of 300mg/L is close to the conventional cut-off point for urinary albumin excretion rate of 200-300 mg/24h, i.e., proteinuria, if the mean daily urine volume is approximately 1.5 L (267, 268). Urine albumin correlates strongly with total protein excretion and converges toward the line of unity as proteinuria increases, especially among the elderly. As a screening test for proteinuria, albuminuria performed well with a sensitivity of 91.7% (95% CI 87.7-94.5%), a specificity of 95.3% (95% CI 94.9-95.7%) and a negative predictive value of 99.8% (95% CI 99.7-99.9%) (270). The use of minor total proteinuria as a surrogate marker for albuminuria will apparently yield quite similar results as MA. The cut-off points in the present study were chosen to represent patients at high risk for CVD events. Both of these cut-off points are associated with increased CVD mortality (see section 2.2.2.3).

In the present study baseline urinary protein was measured only once and this probably increases random variation and decreases the strength of the association between proteinuria and study variables. If urinary albumin data, more specific to diabetic nephropathy, were available, the association between proteinuria and study outcomes would probably be even stronger.

6.2.3 ECG and blood pressure measurements

Blood pressure was measured at baseline after the patient had sat for 5 minutes. The measurement was made with the patient sitting from the right arm with a mercury sphygmomanometer. The baseline BP was used to examine the association between study variables and outcomes, especially in study III. In order to take into account random variation of the BP, the BP was measured twice with an interval of 1.5-min interval; the latter (I, II, IV) or the calculated mean (III) was used in the statistical analyses. Three series of quality-control measurements of BP were arranged during the 2-year period of the study and although there were no statistically significant differences between the BP values by investigators in East and West Finland (251), it is possible that BP values measured by an MD might be slightly higher than the BP values measured by a nurse. However, these inaccuracies of BP measurements probably underestimate

rather than overestimate the association between BP and study outcomes in study III. In the other studies, we used hypertension as a dichotomous variable (no hypertension or hypertension; hypertension being defined as systolic blood pressure ≥ 160 mmHg, diastolic pressure ≥ 95 mmHg or antihypertensive drug treatment).

ECG examinations were performed at baseline. First, a conventional 12-lead resting ECG was recorded and the Minnesota code was used for classification. The Minnesota coding is an established method in epidemiological studies to study the association between ECG variables and CVD events (259). The coding was performed by persons blinded to any information on the study subjects' glucose tolerance and other data.

A three-dimensional computerized ECG was performed. The duration of the P wave and its association with study outcomes was analyzed using computerized ECG data, which is probably a more objective method to measure P wave duration than manual assessments from ECG strips. The P wave duration was used as a marker of atrial conduction. More complete assessment of the P wave indices could provide a deeper insight into these variables with respect to their association with study outcomes.

6.3 CHANGES IN TREATMENT PRACTICES OF PATIENTS WITH TYPE 2 DIABETES AFTER BASELINE

Follow-up lasted for 18 years. The guidelines for the treatment of diabetes have changed since the times of the baseline study (136). The control of diabetes and treatment of hyperglycemia have become stricter and more effective, since new treatment options and information about type 2 diabetes and its complications is presented continuously. At baseline, only less than 10 subjects took lipid-lowering medication and none took statins, since statins became available only in the latter half of the 1990s. Today, at least 50% of all diabetic patients use statins, which prevent CHD events effectively (208). Therefore, statin treatment did not cause any notable bias in this cohort. This is important, since statin treatment decreases serum CRP concentrations and reduces CHD events also independently of their effects on serum lipids (271, 272). Nor was data on the use of ACE inhibitors available at the time of study start, since the first ACE inhibitor, captopril, was approved by the Food and Drug Administration only in 1981.

At the time of our baseline examination, risk assessments and interventions for CVD in type 2 diabetes patients were not routinely performed, in contrast to the situation in clinical practice today. Diabetes and conventional CVD risk factor control has become more favorable and also acute cardiological procedures have evolved and become readily available since the baseline study. Probably the prognosis of patients with diabetes and diabetic complications is better than it was some 20 years ago. Therefore, it is not possible to draw final conclusions on how strong the association between novel risk factors and mortality among patients with type 2 diabetes patients is today, nor can we determine their impact on a person's health and well-being.

6.4 END POINTS

In studies I-III the end points were total mortality, CVD mortality (ICD-9 codes 390–459) and CHD mortality (ICD-9 codes 410–414). In study IV the endpoints were total mortality and stroke mortality (ICD-9 codes 431-438). Mortality data was obtained from the Cause-of Death Register (Statistics, Finland). All the causes of death were reviewed by one of the authors of the Finnish East-West type 2 diabetes study (Seppo Lehto or Auni Juutilainen). To prevent misclassification, hospital records and autopsy records were used when available. The original study design made only total stroke mortality data available and it was not possible to separate between thromboembolic and hemorrhagic stroke mortality.

6.5 RESULTS

6.5.1 Physical activity, proteinuria and mortality (I)

Physical activity reduced significantly the risk of total, CVD and CHD mortality in type 2 diabetes patients with no proteinuria. The beneficial effect of physical activity in patients with no proteinuria on mortality was independent of conventional CVD risk factors, duration of diabetes, diabetes treatment and glycemic control. On the other hand, patients with proteinuria lost the protective effect of physical activity. In fact, increased physical activity was associated with higher total and CVD mortality, implying that physical activity may be harmful in these patients. This suggests that preventive measures to reduce CVD and total mortality in diabetic patients have to be initiated early, preferably at the time of diagnosis. . Trials on the treatment of hyperglycemia in patients with type 2 diabetes are in line with this conclusion. The UKPDS demonstrated a positive effect of strict glycemic control on CVD events in newly diagnosed diabetic patients after a 10-year follow-up (78), whereas other trials aiming to reach near normal HbA_{1c} level failed to demonstrate a reduction in total and CVD mortality in type 2 diabetes among patients who had had the disease for many years (273-275).

Several mechanisms could explain why high physical activity reduces CVD and total mortality. A review of 24 randomized trials reported that physical activity has favorable effects on insulin sensitivity, HbA1c, weight loss, hypertension, serum lipid profile and maximal exercise capacity in patients with type 2 diabetes (240). Physical activity also reduces inflammation and cytokine response, attenuates left ventricular diastolic dysfunction and improves endothelial vasodilatation. Regular physical activity may also reduce oxidative stress (276, 277).

In this study, patients with proteinuria did not benefit from physical activity. In fact, physically active patients with proteinuria had higher mortality. The number of patients with proteinuria was much smaller than that of patients with no proteinuria which hampers the statistical power of this study. Several mechanisms do, however, suggest that

these findings are likely to be valid. First, exercise causes acute oxidant stress, whereas longer term exercise may exert antioxidative properties (277). The antioxidative defense is crucial in combating superoxide formation by the electron transport chain. Diabetic patients with long-term complications have significantly lower activities of antioxidant enzymes, superoxide dismutase, glutathione peroxidase and glutathione reductase and their total antioxidant status is lower than among patients without complications (278). Maybe patients with proteinuria have enhanced superoxide production by exercise, and since the levels of antioxidant enzymes are low at the outset, the result would be accelerated atherogenesis and CVD events. It is also known that there is an exaggerated increase of blood pressure in response to exercise in subjects with albuminuria and sympathetic over-activation; this fact may also contribute to the increased mortality (279). Finally, in the Look AHEAD trial, which was carried out among overweight and obese type 2 diabetes patients, 22.5% had abnormal exercise stress results by ECG, angina pectoris, heart rate recovery or exercise capacity despite pretest screening for CVD (280). Therefore, it is plausible that patients with proteinuria, who are known to be in a high risk for CVD events, might show similar or accentuated symptoms when exercising, especially with higher intensity, as in the present study. Unfortunately, this study was not able to evaluate a dose-response effect of physical activity since the number of patients at baseline was too small.

6.5.2 Physical activity, hs-CRP and mortality (II)

There are no previous studies on the preventive effect of physical activity on CVD mortality in diabetic subjects in relation to hs-CRP levels. In the present study, physical activity was significantly associated with reduced total mortality, CVD mortality and CHD mortality among patients with elevated hs-CRP levels. These associations were independent of conventional CVD risk factors, urinary protein, duration of diabetes, diabetes treatment and glycemic control. In patients with hs-CRP $\leq$3 mg/L physical activity had no beneficial effect on mortality. These findings are in agreement with the hypothesis that physical activity may counteract, at least to some extent, the adverse effects of chronic inflammation on the cardiovascular system and thus reduce CVD mortality.

CRP is produced primarily by the liver in response to inflammatory cytokines (e.g., IL-6). It is also produced in adipose tissue and atherosclerotic plaques (218, 219). CRP is involved in atherogenesis in that it promotes endothelial cell activation, macrophage recruitment and foam cell generation within the arterial wall (220). CRP levels predict CVD mortality not only in type 2 diabetes patients but also in the general population (225-227). Therefore, the question has been raised whether hs-CRP could be used as a marker of systemic inflammation - indeed, could CRP be causally related to CVD (281)? Inflammation has been associated with insulin resistance and the pathogenesis of type 2 diabetes (282).

Several studies show an independent, inverse dose-response relationship between physical activity and the systemic hs-CRP concentration (283, 284). This is in agreement with the present study: physically active subjects had significantly lower hs-CRP level than physically inactive subjects at baseline. Physically active subjects with a high hs-CRP level had reduced total, CVD and CHD mortality.

The pathophysiologic mechanisms of how physical activity reduces inflammation and suppresses CRP are not known in detail. After short-term strenuous exercise there is a transient increase in serum CRP which is mediated by the cytokine system, mainly by IL-6 (285-287). However, acute exercise also produces anti-inflammatory mediators including IL-8 and IL-10, thus leading primarily to an anti-inflammatory effect. Chronic physical activity seems to reduce CRP levels by several mechanisms, including a decrease in cytokine production (e.g., IL-1β, IL-6, TNF-α, IFN-γ and leptin) by adipose tissue, skeletal muscle, endothelial cells and blood mononuclear cells. Exercise induces synthesis of atheroprotective cytokines (e.g. IL-4, IL-10) and adiponectin, and improves endothelial function and insulin sensitivity (288). Antioxidant effects of exercise may also contribute to the reduction of CRP levels and inflammation (283, 288).

Patients with a low or intermediate hs-CRP level did not seem to benefit from physical activity as did patients with higher hs-CRP level. Physically active subjects with high hs-CRP had almost similar CVD mortality as those with intermediate or low hs-CRP concentrations and low physical activity. Maybe physically active patients with a hs-CRP level above 3 mg/L would have had an even higher hs-CRP concentration had they been sedentary. This would be in accordance with the Heritage family intervention trial, where a 20-week standardized exercise training program was undertaken by 652 sedentary subjects. The plasma CRP level sank significantly only in the subgroup of persons whose baseline CRP level was >3.0 mg/L (289).

6.5.3 Systolic blood pressure, proteinuria and mortality (III)

There are no previous large, population-based long-term follow-up studies on the impact of systolic BP on total and CVD mortality among patients with type 2 diabetes with and without proteinuria. The main finding was that a systolic BP <130 mmHg was associated with increased total and CVD mortality among patients with proteinuria compared to patients with a systolic BP 130–139 or 140–159 mmHg. Indeed, patients with proteinuria and a BP <130 mmHg tended to have a poorer prognosis compared even to patients with BP ≥160 mmHg. Patients with a systolic BP 130–139 mmHg had the best prognosis in terms of total and CVD mortality. These associations were independent of conventional CVD risk factors, diastolic BP, duration of diabetes, diabetes and BP treatment, glycemic control, estimated GFR, retinopathy and baseline CHD without MI.

Among patients with type 2 diabetes with no proteinuria, systolic BP <130 mmHg was associated with a tendency towards slightly lower total and CVD mortality. It is noteworthy that diabetes among the study patients was poorly controlled at baseline. The

level of glycemic control during follow-up was unknown, and therefore it is unclear how changes in glycemic control might have influenced the prognosis of these patients. It is known that good glycemic and blood pressure control have additive positive effects on total and CVD mortality in patients with type 2 diabetes (129, 130) but it is not known whether this additive effect is also valid among patients with proteinuria.

The present findings indicate that the mortality of patients with type 2 diabetes and proteinuria rises when systolic the BP is lower <130 mmHg. This finding does not necessarily apply to incident non-fatal CVD events, such as MI. Also, the increased risk in the low BP group could be due to chance, given the limited sample size of the proteinuria group. Nevertheless, the findings are most probably valid for several reasons. First, it has been proposed that MA and proteinuria may be indicators of generalized endothelial dysfunction (290) and therefore they would indicate extensive atherosclerotic disease causing impaired perfusion of vital organs when the patient's BP is low. Increased CVD mortality would follow. This hypothesis is supported by an additional analysis of baseline data: the prevalence of CHD as assessed by symptoms and ECG findings was only 5.2% among patients with no proteinuria and systolic BP <130 mmHg but it was no less than 5-fold or 28.8% among patients with proteinuria and systolic BP <130 mmHg. Secondly, impaired left ventricular systolic function, which is associated with chronic kidney disease and diabetes, could deteriorate further when BP sinks and this would result in reduced cardiac output (291-294). All in all, a low systolic blood pressure in patients with proteinuria may, in fact, mark underlying disease and this underlying disease may contribute to worsening of the patient's prognosis.

What has been observed in this thesis regarding patients with type 2 diabetes and proteinuria is in line with some of the finding in the IDNT trial (295), which is a post hoc study of patients with type 2 diabetes and nephropathy and a systolic BP ≤120 mmHg. In that study, increased CVD mortality was reported. Again, consistent with the present findings, two studies in patients with type 2 diabetes and coronary artery disease showed that the benefits of lowering systolic BP to <130 mmHg were driven mostly by a reduction in the incidence of strokes, while CVD and total mortality were unchanged or even increased (191, 296) In the ACCORD trial, lowering the systolic BP to <120 mmHg of high-risk patients with type 2 diabetes did not reduce mortality compared to those with a systolic BP target of <140 mmHg (135). The results of the present study and some other previous studies suggest that one target BP level may not necessarily fit patients with type 2 diabetes. There is probably some heterogeneity in the effects of blood pressure reduction on different CVD outcomes (e.g., cardiac events vs. stroke; non-fatal vs. fatal events). The effects may also vary by the presence or absence of comorbid conditions, such as proteinuria. Therefore, lowering systolic BP to <130 mmHg might not be justified for type 2 diabetic patients with proteinuria or for patients at high risk of CVD-related mortality for other reasons. Although the evidence supporting a target systolic BP level of <140 mmHg to slow the progression of kidney disease is strong, there are limited data regarding the effects of lowering BP

to <130/80 mmHg (182, 190, 191). The mode and intensity of treatment of BP should be based on the patient's individual CVD risk and any comorbid conditions rather than on aggressive BP goals recommended by global or regional guidelines alone. A meta-analysis concluded that, among patients with type 2 diabetes, the aggressive BP target of <130 mmHg has to be balanced against the benefits of lowering the risk of stroke and an increased risk of serious adverse events, e.g., life-threatening events or hospitalization, and one has also to consider the apparent lack of benefit for cardiac, renal and retinal outcomes (297). Proteinuria in type 2 diabetes is an important prognostic marker for cardiac disease alongside the conventional CVD risk factors (182). The results of the present study suggest that higher systolic BP targets may be justified for patients with type 2 diabetes and proteinuria than for patients with no proteinuria.

6.5.4 P wave duration and mortality (IV)

There are no previous studies on the predictive value of prolonged P wave duration with regard to stroke mortality in diabetic subjects with or without prevalent non-major macrovascular disease (PNMMVD). The present study shows that prolonged P wave duration is significantly associated with increased stroke mortality among patients with PNMMVD. This association is independent of conventional CVD risk factors, urinary protein quantity, duration of diabetes, diabetes treatment, glycemic control, heart rate and LVH. In patients with no PNMMVD prolonged P wave duration is not associated with stroke mortality.

There are two mechanisms that could explain the excess stroke mortality among type 2 diabetes patients with PNMMVD and prolonged P wave duration: IAB. The two mechanisms may be interrelated. First, IAB is associated with left atrial enlargement (LAE) and electromechanical dysfunction of the left atrium (LA) (298, 299). Patients with IAB have lower LA emptying fraction, lower LA stroke volume and lower LA kinetic energy (299). The degree of these abnormalities is related to the severity of the interatrial conduction delay signified by the duration of the P wave (299). These changes in LA could increase the risk of thrombosis. It has also been observed that patients who have had an embolic stroke have significantly more often IAB than non-stroke subjects (150, 151). Prolonged P wave duration is also associated with increased all-cause and CVD mortality (148). Secondly, IAB has been associated with AF (149). The deterioration of interatrial conduction in IAB results in a shorter wavelength and this, combined with the probable LAE, would increase the number of wavelets in the atrium (300, 301). These mechanisms could then increase the risk of AF and the risk of sustained AF, an obvious consequence of which would be increased stroke mortality among these patients. In our study, only patients with PNMMVD had increased stroke mortality when the P wave duration exceeded 114 ms. Probably that those patients had more advanced CHD and had thus more severe LA electromechanical dysfunction and a high risk of AF. Prolongation of the P wave could also, independently of the increased risk of atrial thrombus formation, mark advanced atherosclerosis. Therefore, a long duration of the

P wave could identify patients with a high probability of widespread vascular damage affecting the arteries, also the brain.

Which ECG lead should be used for assessment of the duration of the P wave? It has been suggested that P wave duration should be measured from all the leads and that the lead giving the longest duration should be used (261). Our study results are in accordance with this most pragmatic suggestion.

6.6 STRENGTHS AND LIMITATIONS

There are several strengths in this study. First, the baseline examination was carried out in 1982–1984. As statin treatment was not common practice until the second half of the 1990s, it is unlikely that lipid-lowering therapy has caused a major bias in our study. Secondly, unlike in many similar epidemiological studies, data regarding glucose control, diabetes duration and mode of diabetes treatment were available. Thirdly, the post-glucagon C-peptide assessment was used to exclude patients with type 1 diabetes. Finally, to avoid bias from the possibility of increased early mortality due to a severe disease at baseline, all subjects with possible or definite stroke, possible or definite MI or amputation at baseline were excluded from all analyses. In addition, the studies analyzing the impact of physical activity excluded the following subjects: all patients with a prior diagnosis of angina pectoris, possible or definite stroke, possible or definite MI, intermittent claudication or amputation at baseline and those who died or had an amputation of a leg during the first two years after the baseline study. These exclusion criteria were based on the assumption that subjects very likely had changed their exercise habits due to severe disease at baseline or during the first two years of follow-up.

The present study has also some limitations. First, only baseline measurements of the study variables are available. Therefore, there is no data on the changes in the study variables during follow-up. On the other hand, with respect to physical activity, the highest MET-value of physical activity at work or during leisure-time or in commuting was used to represent overall physical activity to minimize this limitation. hs-CRP values are relatively stable in the one and same individual over time and since there is no marked diurnal variation, this apparent limitation may not be very detrimental (302, 303).

The systolic BP tends to rise with age and there is also some day-to-day and within-day variation. To minimize the latter, BP was measured twice with the study subject in the sitting position. The mean of these recordings was used in study III and in the other studies hypertension was used as a dichotomous variable to reduce variation. Three series of quality-control measurements of BP were arranged during the 2-year period of the study. There were no statistically significant differences between the BP values measured by investigators in East and West Finland (251).

The total urinary protein concentration was measured from morning spot urine samples and not by measuring the urinary albumin excretion rate. The cut-off point of 150mg/L for urinary protein corresponds roughly to albuminuria of 60-140 µg/min. By definition, MA is a state of persistent, increased urinary excretion of albumin (20-200 µg/min) (116). The cut-off point of 300 mg/L is close to the common cut-off point for urinary albumin excretion rate of 200-300 mg/24h, at a mean daily urine volume of approximately 1.5L (265-268). These cut-off points were chosen to mimic MA and clinical proteinuria. Data was not available on the use of ACE inhibitors or ARBs, which are known to improve the prognosis of patients with kidney disease. However, as the first ACE inhibitor (captopril) was approved by the US Food and Drug Administration only in 1981, it is highly unlikely that our study participants were taking these drugs at baseline. Without follow-up data on eGFR it is not possible make assumptions about safe systolic BP levels for patients with renal impairment.

P wave duration was the only marker of atrial conduction. More complete assessments of the P wave, e.g., P wave dispersion and amplitude, would provide deeper insight into the association between P wave characteristics and mortality among patients with type 2 diabetes and PNMMVD. Previously the P wave duration has been associated with CVD and stroke mortality among general population (148-150).

6.7 CLINICAL IMPLICATIONS

The incidence of type 2 diabetes is constantly rising. The risk of CVD events among patients with type 2 diabetes is two-to-four fold compared with the general population (48-51). The growing epidemic urges us to put in effort to control the epidemic and to focus on patients at risk for CVD events. Multifactorial, intensive pharmacological and lifestyle intervention are considered to be the key elements in treating type 2 diabetes (80-84). However, recent trials have demonstrated that there may be marked heterogeneity among different subgroups of diabetic patients as regards the need for intensive multifactorial treatment (e.g., hypertension, glycemic control) with regard to effects on different CVD outcomes (e.g., non-fatal events vs. mortality; cardiac events vs. stroke) (133, 191, 273-275, 296). The effects of treatment may also vary by the presence or absence of comorbid conditions, e.g., proteinuria or inflammation (high levels of hs-CRP). Therefore, the emphasis of treatment and control of type 2 diabetes is shifting. It has been suggested that optimal type 2 diabetes treatment should be based on the patient's individual CVD risk and comorbid conditions rather than on aggressive global or national guidelines (297). The results in the present thesis also emphasize the importance of individual tailoring of the treatment and control of type 2 diabetes. The results highlight the importance of early prevention of CVD among those who get type 2 diabetes and may help to identify more patients at high-risk for CVD events.

Inflammation is present at the very onset of diabetes. It has been suggested that anti-inflammatory therapies might reduce CVD mortality. Physical activity is associated

with a decrease in the concentration of inflammation markers in the blood, e.g. hs-CRP. There have been no previous studies to investigate the association between physical activity and inflammation with respect to CVD mortality among T2DM patients. The present thesis (study II) demonstrates that patients with hs-CRP levels over 3.0 mg/L and who are physically active have reduced CVD mortality in comparison to patients who are inactive. The prognosis is similar to patients who have low levels of hs-CRP. This emphasizes the importance of regular physical activity and also suggests that one possible explanation for the reduction in CVD and total mortality events resides in the anti-inflammatory effects of exercise. Therefore physical activity should be advised as a therapy for type 2 diabetic patients.

The present thesis (study I) demonstrates also that proteinuria, a marker of generalized vascular damage, modifies the effect of physical activity. Among patients without proteinuria, physical activity is associated with a reduction in CVD and total mortality. These results imply that preventive measures to reduce CVD and total mortality in diabetic patients must be initiated early, preferably at the time of diagnosis of the disease and before microvascular complications and especially proteinuria develop. After proteinuria has developed caution is warranted when advising exercise instruction.

The present thesis (study III) demonstrates also that proteinuria and hypertension are important identificators of patients at high risk for CVD events. A multifactorial approach to treating patients with type 2 diabetes has resulted in progressive lower BP targets without the necessary data to support this. Several studies have reported that intensive BP targets may, in fact, be harmful to some patients with type 2 diabetes (133, 190, 192). This position is echoed by the newly published ADA guidelines for BP targets, where lowering the systolic BP to only <140 mmHg is recommended for high-risk patients (136). The present thesis corroborates that such an approach is prudent and implies that one, single target BP level may not necessarily fit all patients with type 2 diabetes. The present thesis also suggests that the mode and intensity of BP treatment should be based on the patient's individual CVD risk and that any comorbid conditions, such as proteinuria, should be taken into account.

Finally, the present thesis (study IV) highlights a subgroup of patients with type 2 diabetes: those with prevalent non-major macrovascular disease and whose P wave duration is prolonged have a high risk of stroke death. The duration of the P wave on the ECG is easy to measure and merits further scrutiny to evaluate its importance for estimating the risk of stroke of patients with type 2 diabetes.

6.8 FUTURE RESEARCH PROSPECTIVES

The reasons for the excess total and cardiovascular mortality of patients with type 2 diabetes are only partly understood. The complexity of the disease and the interrelated co-morbidities and MetS make it difficult to identify the specific mechanisms

underlying the excess mortality. Studies are needed to help us to understand better the pathophysiology of type 2 diabetes and MetS and their independent and joint relations with CVD outcomes and risk prediction. The role of environmental and gene interaction in the development of type 2 diabetes and CVD outcomes needs to be looked at. More studies are also needed to examine individuality of type 2 diabetes to identify high-risk patients and to describe the optimal lifestyle and pharmacological interventions to reduce the excess CVD mortality. Finally, due to the staggering increase in the incidence of type 2 diabetes, focus has recently been shifted towards earlier prevention of CVD events and type 2 diabetes. There is evidence that diabetes can be prevented or at least postponed by lifestyle intervention (80-84). However, more research is needed to define the extent, the most appropriate methods and optimal timing of the intervention.

7. CONCLUSIONS

This thesis was carried out to evaluate the associations between risk factors with future total, CVD, CHD and stroke events and to bring novel data to help to identify patients at increased risk for CVD events in a large cohort of middle-aged Finnish patients with type 2 diabetes.

The present thesis is population based and comprises 1,059 type 2 diabetic patients aged 45-64 years who were living in the Turku University Central Hospital district in West Finland or in the Kuopio University Hospital district in East Finland. The duration of the follow-up was 18 years and it ended in January 2001. Altogether, 768 of 1059 patients died during the follow-up. The main conclusions are:

1. The beneficial effect of increased physical activity on the mortality of patients with no proteinuria was independent on conventional CVD risk factors, duration of diabetes, diabetes treatment and glycemic control. Patients with type 2 diabetes with proteinuria lost the protective effect of physical activity. This finding implies that physical activity as a measure to prevent and reduce CVD mortality and total mortality of diabetic patients have to be initiated early, preferably at the time of diagnosis.

2. Physical activity reduced the cardiovascular and total mortality of middle-aged patients with type 2 diabetes who had hs-CRP levels above 3.0 mg/L. This beneficial effect was not observed in patients with lower hs-CRP levels. This observation suggests that the decrease in CVD mortality in physically active patients may be due to an anti-inflammatory effect of exercise independent of conventional CVD risk factors. If true, this observation will have clinical implications with regard to the timing and intensity of physical exercise as a therapy for patients with type 2 diabetes.

3. Systolic BP <130 mmHg tended to be associated with a slight decrease in total and CVD mortality of middle-aged patients with type 2 diabetes without proteinuria. Among patients with proteinuria, systolic BP <130 mmHg was associated with a significant increase in total and CVD mortality. Patients with a systolic BP level between 130 and 139 mmHg had the best prognosis with respect to total and CVD mortality. Further studies are needed to determine conclusively whether aggressive lowering of (systolic) BP in patients with type 2 diabetes and proteinuria results in an excess risk of CVD outcomes or death.

4. Stroke mortality was increased in middle-aged patients with type 2 diabetes and prevalent non-major macrovascular disease and prolonged P wave duration. Among patients without prevalent non-major macrovascular disease, the duration of the P wave was not associated with stroke mortality. The duration of the P wave in ECGs is simple to measure and merits further study to evaluate its importance for assessing the risk of stroke of patients with type 2 diabetes and prevalent manifestations of atherosclerosis but no major CVD events.

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