Folkhälsan Institute of Genetics
Folkhälsan Research Center
Helsinki, Finland

and

Division of Nephrology
Department of Medicine
Helsinki University Central Hospital
University of Helsinki
Helsinki, Finland

PHYSICAL ACTIVITY AND DIABETIC COMPLICATIONS IN PATIENTS WITH TYPE 1 DIABETES

Johan Wadén

ACADEMIC DISSERTATION

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Supervisor Per-Henrik Groop, MD, DMSc
Folkhälsan Institute of Genetics
Folkhälsan Research Center
Helsinki, Finland
and
Division of Nephrology, Department of Medicine
Helsinki University Central Hospital
University of Helsinki
Helsinki, Finland

Reviewers Docent David E. Laaksonen
Department of Medicine
Kuopio University Central Hospital
University of Eastern Finland
Kuopio, Finland

Professor Tapani Rönnemaa
Department of Medicine
Turku University Central Hospital
University of Turku
Turku, Finland

Opponent Professor Edwin Gale
Department of Diabetes and Metabolism
Division of Medicine
University of Bristol
Southmead Hospital
Bristol, UK

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"I reject your reality and substitute my own"

Adam Savage, Mythbusters, Discovery Channel (1967-)

"If there is any deficiency in food or exercise the body will fall sick"

Hippocrates (460-370 B.C.)

Contents

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I Wadén J, Forsblom C, Thorn LM, Saraheimo M, Rosengård-Bärlund M, Heikkilä O, Lakka TA, Tikkanen H, Groop PH, FinnDiane Study Group: Physical activity and diabetes complications in patients with type 1 diabetes: The Finnish Diabetic Nephropathy (FinnDiane) study. *Diabetes Care.* 31:230-232, 2008

II Wadén J, Tikkanen H, Forsblom C, Fagerudd J, Pettersson-Fernholm K, Lakka T, Riska M, Groop PH, FinnDiane Study Group: Leisure-time physical activity is associated with poor glycemic control in type 1 diabetic women: The FinnDiane study. *Diabetes Care.* 28:777-782, 2005

III Wadén J, Forsblom C, Thorn LM, Gordin D, Saraheimo M, Groop PH, on behalf of the FinnDiane Study group: A1C variability predicts incident cardiovascular events, microalbuminuria, and overt diabetic nephropathy in patients with type 1 diabetes. *Diabetes*, 58: 2649-2655, 2009

IV Wadén J, Thorn LM, Forsblom C, Lakka T, Saraheimo M, Rosengård-Bärlund M, Heikkilä O, Wessman M, Turunen JA, Parkkonen M, Tikkanen H, Groop PH, FinnDiane Study Group: Leisure-time physical activity is associated with the metabolic syndrome in type 1 diabetes: Effect of the *PPARγ* Pro12Ala polymorphism: The FinnDiane study. *Diabetes Care.* 30:1618-1620, 2007

V Wadén J, Peltonen J, Rosengård-Bärlund M, Aho J, Groop PH, Tikkanen H: Aerobic physical fitness and muscle strength in patients with type 1 diabetes. VO_{2max} and association with glycemic control, *Submitted manuscript*

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ABBREVIATIONS

ACE	Angiotensin converting enzyme
ACR	Albumin-to-creatinine ratio
AGE	Advanced glycation end-product
AMI	Acute myocardial infarction
AT	Anaerobic threshold
ATP	Adenosine triphosphate
BMI	Body-mass index
CGMS	Continuous glucose measurement systems
CI	Confidence interval
CV	Coefficient of variation
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
eGDR	Estimated glucose disposal rate
ESRD	End-stage renal disease
FEV1	Forced expiratory volume during one second
FVC	Forced vital capacity
GLUT4	Glucose transporter 4
GFR	Glomerular filtration rate
HbA_{1c}	Haemoglobin A_{1c}
HDL	High-density lipoprotein
HLA	Human leukocyte antigen
HR	Hazard ratio
IQR	Interquartile range
LDL	Low-density lipoprotein
LTPA	Leisure-time physical activity
MET	Metabolic equivalent
MetS	Metabolic syndrome
OR	Odds ratio
PPAR	Peroxisome proliferator activated receptor
RAS	Renin-angiotensin system
RCP	Respiratory compensation point
SBP	Systolic blood pressure
SD	Standard deviation
UAER	Urinary albumin excretion rate
VE	Ventilation
VEGF	Vascular endothelial growth factor
VO_{2max}	Maximal oxygen uptake
WHR	Waist-to-hip ratio

ABSTRACT

Background

Type 1 diabetes is associated with the risk for late diabetic complications which are divided into microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular (cardiovascular disease, CVD) diseases. The risk for diabetic complication can be reduced by effective treatment, most importantly the glycaemic control. Glycaemia in type 1 diabetes is influenced by the interplay between insulin injections and lifestyle factors such as physical activity and diet. The effect of physical activity in patients with type 1 diabetes is not well known, however.

Aim

The aim of this thesis was to investigate the physical activity and the physical fitness of patients with type 1 diabetes with special emphasis on glycaemic control and the diabetic complications.

Subjects and methods

The patients included in the study were all part of the nationwide, multicenter Finnish Diabetic Nephropathy (FinnDiane) Study which aims to characterise genetic, clinical, and environmental factors that predispose to diabetic complications in patients with type 1 diabetes. In addition, subjects from the IDentification of EArly mechanisms in the pathogenesis of diabetic Late complications (IDEAL) Study were studied. Physical activity was assessed in the FinnDiane Study in 1945 patients by a validated questionnaire. Physical fitness was measured in the IDEAL Study by spiroergometry in 86 young adults with type 1 diabetes and in 27 healthy controls. All patients underwent thorough clinical characterisation of their diabetic complication status. Four substudies were cross-sectional using baseline data and one study additionally used follow-up data.

Results

Physical activity, especially the intensity of activities, was reduced in patients affected by diabetic nephropathy, retinopathy, and CVD. Low physical activity was associated with poor glycaemic control, a finding most clear in women and evident also in patients with no signs of diabetic complications. A higher level of physical activity was also associated with better insulin sensitivity. Furthermore, low physical activity was associated with a higher HbA_{1c} variability, which in turn was associated with the progression of renal disease and CVD during follow-up. The prevalence of the metabolic syndrome in type 1 diabetes was also lower the higher the physical activity. The aerobic physical fitness level of young adults with type 1 diabetes was reduced

compared with healthy peers and in men an association between higher fitness level and lower HbA_{1c} was observed.

Conclusions

In patients with type 1 diabetes, a higher physical activity was associated with better glycaemic control and may thus be beneficial with respect to the prevention of diabetic complications.

1. INTRODUCTION

Diabetes, defined as elevated blood glucose concentrations, is a major health problem in today's society as the number of patients with diabetes is growing continuously. Globally, the number of adult patients with diabetes is 285 million (6.6% of the population) according to the 2009 figures of the International Diabetes Federation, and is thought to increase to 435 million (7.8%) by the year 2030 [1]. In Finland, it is estimated that the current number of patients with diabetes is close to 300 000 individuals, and another 200 000 is estimated to have yet undiagnosed diabetes. Hence half a million, or close to 10% of the total Finnish population, is thought to be affected by diabetes. Of these, 40 000 patients have type 1 diabetes, and the majority of the remaining patients have type 2 diabetes. The hallmarks of type 1 diabetes are onset at younger age and an unconditional dependence on insulin treatment for survival due to the cessation of the insulin production of the pancreas. Type 2 diabetes is usually characterised by a later age at onset and the patients are usually not dependent on insulin treatment for survival. Even though the patients with type 1 diabetes represent a minority of all patients with diabetes, the proportion of patients with type 1 diabetes is exceptionally high in Finland, and in fact Finland has the highest incidence of type 1 diabetes in the world [2]. The incidence of type 1 diabetes has clearly increased in Finland during the last decades [3] for reasons that are not fully understood. This thesis will focus on type 1 diabetes.

Before the discovery of insulin by Frederick Banting and Charles Best during 1921-1922 in Canada at the University of Toronto; type 1 diabetes was a fatal illness and the patients died from dehydration and disturbances in their acid-base- and electrolyte balance. This acute condition, referred to as diabetic ketoacidosis, is caused by a failure of the endogenous insulin production and subsequent hyperglycaemia and increased lipid oxidation followed by high ketone body formation. This life-threatening condition can nowadays be successfully treated with exogenous insulin and replacement of fluid and electrolytes and the disease can be kept under control by subcutaneous injection of insulin. Such treatment has been available since 1922, when insulin was for the first time successfully administered to a patient with diabetes.

However, type 1 diabetes was not a fully solved problem, because it soon became obvious that the patients, despite their insulin treatment, developed a uniform array of symptoms and illnesses such as impaired renal function, decreased vision and blindness, decreased skin sensation and ulcers in the lower extremities, heart disease and, importantly, a clearly decreased lifespan. These diabetes-related illnesses have been recognised as diabetic kidney disease (nephropathy), eye disease (retinopathy),

and nerve disease (neuropathy) and collectively constitute the classical microvascular complications of diabetes. In addition, macrovascular complications manifest as atherosclerotic lesions in the coronary and the peripheral arteries, for instance in the lower extremities and the brain. The diabetic complications probably arise because the exogenous delivery of insulin does not adequately restore the physiologic effects of a properly functioning endogenous insulin secretion.

Even though the insulin treatment has undergone much improvement since 1922, the diabetic complications continue to be a major problem for patients with type 1 diabetes. Even today, diabetic complications confer a reduced quality of life and a shortened lifespan. In addition, the treatment of diabetic complications is expensive for the society and 9.2% (1.35 billion euro) of the total health care budget of Finland was used for the treatment of diabetes and its complications [4] in 2007, and similar relative expenses have been reported from the USA [5]. Diabetic nephropathy can be considered the most severe diabetic complication, because diabetic nephropathy is strongly associated with an increased risk of end-stage renal disease with subsequent need of dialysis and renal transplantation for survival, and also with increased risk of cardiovascular disease [6] and premature death [7, 8]. The presence or absence of albumin in the urine is a major determinant of the prognosis of patients with type 1 diabetes, and recent data suggest that patients with normal urinary albumin excretion rate (<30 mg/24h) in fact have a standardised mortality rate that is no different from that of the general population [8].

Hyperglycaemia is a prerequisite for the development of diabetic complications, but is not a sole causative factor. Hypertension and blood lipids are also known to be important players in the cascade leading to diabetic complications. More recently endothelial dysfunction, low-grade inflammation, oxidative stress, and insulin resistance have also been implicated in the pathogenesis. Accordingly, interventions aimed to reduce the development and progression of diabetic complications should target multiple factors.

Physical activity is known to have many health benefits and has been implicated as part of the treatment and prevention of a vast number of diseases [9]. From studies in patients with type 2 diabetes we know that physical activity improves the glycaemic control [10], but in type 1 diabetes the true effect on glycaemic control is still an open question, even though a positive effect has been demonstrated on insulin sensitivity [11-13]. Physical activity may also reduce the blood pressure [14], improve the blood lipid profile [15, 16], and may have positive effects on the endothelial function [17, 18]. Therefore, physical activity could be an effective means to prevent diabetic complications through multiple mechanisms. If proven effective, more financial resources need to be directed towards the enhancement of physical activity of patients with type 1 diabetes. There is undoubtedly a need for data on the effect of physical activity on health outcomes in these patients, because there are only a small number of

available studies with low numbers of patients. The main aim of this thesis was to examine the relationship between physical activity and glycaemic control in a large set of patients with type 1 diabetes.

2. REVIEW OF THE LITERATURE

2.1 DIABETES MELLITUS

Diabetes mellitus, henceforth referred to as diabetes, is a group of metabolic conditions characterised by elevated blood glucose concentrations. According to the World Health Organisation (WHO), diabetes is currently defined as a fasting plasma glucose concentration of ≥7.0 mmol/l in repeated measurements, or a 2 h oral post-glucose load (75g) plasma glucose concentration of ≥11.1 mmol/l [19]. Additionally, a random plasma glucose value of ≥11.1 mmol/l in conjunction with symptoms of diabetes (thirst, polyuria, weight loss) also leads to a diagnosis of diabetes. The fasting glucose value for diabetes has been lowered several times during the past 30 years. The 1980 WHO criterion of ≥8.0 mmol/l [20] was lowered first in 1985 to ≥7.8 mmol/l [21] and again in 1999 to the current definition of ≥7.0 mmol/l [22]. A HbA_{1c}-based (see section 2.1.3) definition of diabetes, however, is currently a matter of debate [23].

Historically, the word diabetes comes from the Greek word “diabainein”, which means “to pass through” and refers to the large urine volume that is associated with diabetes. Mellitus comes from the Latin word “mel” which means honey, and refers to the sweetness of urine. As a disease, diabetes was known already in Egypt about 3500 years ago. In the first century AD, Aretaeus of Cappadocia described the condition as the “melting down of the flesh and limbs into urine”; a horrific but accurate description of untreated diabetes. Diagnosis in the early days was based on the sweetness of the urine, and involved either tasting of the urine by the doctor or evaluating the degree of attraction of ants to the urine specimen.

Diabetes is not a single disease, but rather a group of diseases with different pathophysiological mechanisms that result in elevated plasma glucose concentrations. The underlying problem is a disturbed effect of insulin, which is secreted from the β-cells of the islets of Langerhans in the pancreas. This disturbance may be due either to a decreased production or to a decreased effect (or both) of insulin in the target tissues, which are the skeletal muscles, the adipose tissue, and the liver. In addition to disturbances in the glucose metabolism, a defective insulin action also leads to disturbances in the lipid and the amino acid metabolism.

2.1.1 CLASSIFICATION OF DIABETES

There are two main types of diabetes, which are called type 1 and type 2 diabetes according to the current terminology set by the National Diabetes Data Group in 1979 [24] and the WHO in 1980 [20]. Type 1 diabetes, or insulin-dependent diabetes, is the clinical manifestation of the abovementioned ancient descriptions of diabetes, and is due to a failure of the pancreas to produce insulin. Type 2 diabetes, or non-insulin-dependent diabetes, is closely related to obesity and decreased insulin sensitivity, but accumulating data indicate that also insulin secretion defects are fundamental in the pathogenesis of type 2 diabetes. Most susceptibility genes for type 2 diabetes known at the moment, for instance the *TCF7L2* [25], are in fact involved in the regulation of insulin secretion, not insulin sensitivity. The distinction between type 1 and type 2 diabetes is often anything but clear, and in the clinical setting there is a considerable overlap between these two conditions. Patients with "double diabetes" [26] have also been reported, which refers to features of type 2 diabetes, such as obesity and insulin resistance, in patients diagnosed with type 1 diabetes. In line with this, Finnish patients with type 1 diabetes frequently fulfil the criteria for the metabolic syndrome [27].

There are also other forms of diabetes, which constitute a minority of patients with diabetes. Latent autoimmune diabetes in adults (LADA) falls between type 1 and type 2 diabetes because it involves autoimmune destruction of the β-cells of the pancreas, however at an older age than that of classical type 1 diabetes and the patients are usually not insulin-dependent at the time of diagnosis [28]. Another part of the spectrum of diabetes is maturity-onset diabetes of the young (MODY), which is characterised by lower age at onset than in type 2 diabetes, but absence of both ketoacidosis and β-cell autoimmunity typical for type 1 diabetes [29]. MODY differs from the other forms of diabetes by an autosomal dominant inheritance with high penetrance. The primary causal defects have been identified at the molecular level for the subtypes MODY 1 to 6. For instance, the most common form in Finland is MODY-3 which is caused by a mutation in the hepatocyte nuclear factor 1α [30] causing defects in the insulin secretion but is still associated with normal insulin sensitivity [31]. There is also a heterogeneous group of secondary forms of diabetes, which may be caused by pancreatitis, pancreatic cancer, surgery, or other trauma to the pancreas with subsequent insulin deficiency. Long-term use of glucocorticoid medication can induce diabetes by reducing insulin sensitivity [32] and another iatrogenic form of diabetes is related to the use of cyclosporine treatment as immunosuppressant after organ transplantations [33]. Finally, gestational diabetes refers to hyperglycaemia manifesting during pregnancy, which is a state of relative insulin resistance, and is a risk factor for future type 2 diabetes [34].

2.1.2 TYPE 1 DIABETES

Type 1 diabetes is characterised by an autoimmune destruction in the β-cells of the pancreas and has in many cases an acute onset with ketoacidosis [35]. Type 1 diabetes usually manifests before the age of 30 years, but can present at any age.

2.1.2.1 Epidemiology

The incidence rates of type 1 diabetes show striking geographical differences. Data from the years 1990-1999 showed that the countries with the highest yearly incidence per 100 000 individuals aged <15 years were Finland (40.9), the Italian island of Sardinia (37.8), and Sweden (30.0) [36]. The most recent numbers from Finland show that the incidence has risen even more rapidly than expected, now 64.2 new cases per 100 000 aged <15 years in 2008 [2]. These numbers are in sharp contrast to countries like Venezuela, Peru, and China where the incidence rate is <1 per 100 000 [36]. Thus, the worldwide regional differences in the incidence of type 1 diabetes is more than 60-fold. These remarkable regional differences may also give some clues to the etiological factors of type 1 diabetes. The increase in the incidence during the last decades has been too large to be explained only by genetic factors, and thus strongly implies the involvement of environmental factors [37]. The temporal aspect can also be informative as the sharp rise in incidence began in the mid 1950s in several populations (Finland, Norway, Denmark, Sardinia, and USA) in a seemingly synchronized fashion [38, 39], however with the caveat that early incidence estimates may be unreliable due to death of patients with undiagnosed diabetes.

The age at onset of type 1 diabetes seems to be getting younger in many populations, especially the proportion diagnosed before five years of age [40]. According to the spring harvest theory [39, 41], the apparent increase in the incidence of type 1 diabetes is attributable to a decrease in the age at onset of the disease. In support of this, the cumulative incidence of type 1 diabetes before the age of 39 years has been unchanged in Sweden [42] and in Belgium [43].

Finland appears to be a hot-spot for type 1 diabetes. The increase in incidence continues in a linear [3] or even exponential [2] fashion, while a plateau is seen in other countries with already high incidence of type 1 diabetes [44]. Moreover, the spring-harvest theory does not fully explain the situation in Finland where an increase in the incidence is observed not only in young age groups (<15 years) [2], but also in the age group of 15-39 years [45, 46]. The reason(s) for this exceptional situation in Finland is unknown, but if solved could lead to better understanding of the mechanisms behind type 1 diabetes.

2.1.2.2 Pathogenesis

Type 1 diabetes is caused by an autoimmune attack directed against the β-cells of the islets of Langerhans in the pancreas. The disease usually manifests when 80-90% of the total β-cell population is lost [47]. As a marker of autoimmunity, certain autoantibodies against islet antigens can be found in the serum before, at, and after the diagnosis of type 1 diabetes. These are glutamic acid decarboxylase (GAD), islet cell (IC), insulin (IA), and protein tyrosine phosphatase IA-2 antibodies. Autoreactive T-lymphocytes are also important in the destruction of pancreatic β-cells. The clinical course of type 1 diabetes varies from a fulminant, rapidly evolving disease [48] to one with a slower onset during several years. After the diagnosis has been made and the insulin treatment initiated, a "honey-moon period" that is due to a partial recovery of the β-cell function can be seen. The autoimmune attack, however, will ultimately lead to total dependence on exogenous insulin.

The cascade leading to β-cell destruction by a misdirection of the body's own defence mechanisms involves both genetic predisposition, environmental triggers, and modifying factors [49]. All these factors act in concert in a cascade of differing order and timing of events that is probably unique for each patient who develops type 1 diabetes.

Of the genetic predisposition, about half is thought to derive from the human leukocyte antigen (HLA) region on chromosome 6p21 [50]. Especially HLA DR3/DR4 is enriched in patients with type 1 diabetes. Other (non-HLA) genes that have been convincingly associated with type 1 diabetes include the variable-number tandem repeat (VNTR) variant of the insulin gene [51], the cytotoxic T-lymphocyte-associated protein 4 (*CTLA-4*) [52], and the protein tyrosine phosphatase-22 (*PTPN22*) [53]. Large-scale genome-wide association studies for type 1 diabetes have recently been performed [54, 55] discovering several previously unknown susceptibility genes, but there are certainly many more yet unknown genetic loci that confer risk.

There are, however, several observations indicating that type 1 diabetes is not a purely genetic disease. First, the predisposing genes have a low penetrance, because most carriers of risk alleles do not develop type 1 diabetes and the concordance for type 1 diabetes in monozygotic twins is only 13-33% [56, 57]. Second, the prominent increase in the incidence of type 1 diabetes during the last decades cannot be explained by genetic factors as the required changes in the gene pool cannot occur within such a short time. Third, the proportion of high-risk HLA genotypes in patients with newly diagnosed type 1 diabetes has decreased with time, and conversely the proportion of low-risk, and even protective, genotypes has increased, suggesting a stronger environmental contribution [37]. Migration studies are scarce, but there is some support of increased risk of type 1 diabetes in those who have moved from a region of low to one of high incidence of type 1 diabetes [58]. Finally, seasonal variation with higher incidence of type 1 diabetes during the winter months also points to an environmental impact [59].

There have been a number of suggested environmental factors associated with the onset of type 1 diabetes. The prime suspects have been viral infections, such as Coxsackie A and B viruses [60]. In this context, a puzzling fact is that there has been a declining trend of enterovirus infections alongside an increase in the incidence of type 1 diabetes in the developed countries. This observation may, however, be explained by a decreased transfer of maternal antibodies against enteroviruses to children, who will thus have a reduced protection. This phenomenon was called the "polio hypothesis" [61] as an analogy to the increase of paralytic poliomyelitis in parallel with the decrease in polio infections. Recently, the enterovirus hypothesis was strengthened by high occurrence of the enteroviral capsid protein in the pancreas of patients with recent-onset fatal diabetes [62]. In Finland the seasonal variation in the appearance of type 1 diabetes autoantibodies shows a peak at the same time as the highest occurrence of enterovirus infections which is during the winter [49]. Vitamin D deficiency during the dark period of the year may be another explanation for the seasonal variation on the northern hemisphere [63]. Dietary factors have also been implicated, such as cow's milk [64, 65], a short period of breast feeding [66], and gluten [67]. Interestingly, countries with high consumption of root vegetables have high incidence of type 1 diabetes, and the common potato scab disease is caused by strains of Streptomyces-bacteria which produce toxins that are harmful for the β-cells [68]. Streptozotocin is such a toxin which, in fact, is widely used for experimental induction of insulin-dependent diabetes in animal models.

The growing obesity problem is another aspect of the rising incidence and younger age at onset of type 1 diabetes. The "accelerator hypothesis" states that factors that decrease insulin sensitivity stress the ß-cells of the pancreas, especially if they are already weakened by autoimmunity, leading to an accelerated pathogenesis of type 1 diabetes [69]. Obesity and insulin resistance have traditionally been considered crucial in the pathogenesis of type 2 diabetes, but also seem to play a role as a modifying factor in the chain of events leading to type 1 diabetes [70, 71]. Even though an increase in childhood obesity and insulin resistance may explain the spring harvest phenomenon mentioned in section 2.1.2.1 it is not likely to explain the absolute increase in incidence of type 1 diabetes. It is noteworthy that the clear distinction between type 1 and type 2 diabetes seems to fade away, and it has been proposed that type 1 and type 2 diabetes are in fact one and the same disease, but that the crucial difference is merely the rate of β-cell loss [69].

2.1.2.3 Treatment

Patients with type 1 diabetes are treated with exogenous insulin which is available as long- and short-acting preparations to cover the basal and postprandial need of insulin, respectively. Insulin is delivered subcutaneously, either as injected boluses or

as a continuous infusion by an insulin pump. The insulin treatment regimen should be flexible and insulin dose adjustments according to diet and physical activity, but also other factors such as infections are important. Islet cell transplantation as a potential cure for type 1 diabetes and with the aim to restore β-cell function is under development [72]. Pancreas transplantation has also been performed in combination with renal transplantation in patients with renal failure [73]. Another approach is to initiate immunosuppressive treatment and perform autologous stem-cell transplantation in newly-onset type 1 diabetes [74], but there is an ethical dilemma due to long-term safety concerns. GAD-treatment in recent-onset type 1 diabetes was further shown to preserve residual insulin secretion, but long-term treatment effects are uncertain [75].

2.1.3 GLYCAEMIC CONTROL IN DIABETES

The long-term glycaemic control of patients with diabetes is clinically determined by the HbA_{1c} test, which is a measure of the proportion of haemoglobin molecules with glycosylation of the N-terminal valine residue of the haemoglobin β-chain. HbA_{1c} is usually expressed as % but more recently as mmol/mol. In this thesis, HbA_{1c} is given as %. Glycaemic control in diabetes has been estimated by HbA_{1c} since the late 1970´s [76]. HbA_{1c} is generated by non-enzymatic glycosylation at a rate proportional to the blood glucose concentration. The lifespan of the haemoglobin-containing red blood cells is about 120 days; thus the HbA_{1c} value reflects the average glycaemic control of the past three months of a patients with diabetes. The past month, however, may have a relatively larger impact on the HbA_{1c} value [77]. There is some evidence that also hereditary factors affect the HbA_{1c} level in patients with type 1 diabetes [78].

2.2 DIABETIC COMPLICATIONS

The diabetic complications are divided into microvascular (nephropathy, retinopathy, and neuropathy) and macrovascular (cardiovascular) disease. Once considered a part of type 1 diabetes itself, the observation that similar complications also arise in patients with secondary diabetes led to the view that complications are caused by the hyperglycaemic milieu. The blood glucose values that define diabetes itself are set by the risk of diabetic microvascular complications [19].

2.2.1 DIABETIC NEPHROPATHY

In the year 1936 eight adults, of which seven had known diabetes, were described as having similar findings of high blood pressure, oedema, and proteinuria. Post-mortem histological evaluation of their kidneys revealed that the patients had lesions of glomerulosclerosis [79]. This condition, occasionally referred to as the Kimmelstiel-Wilson syndrome, is the first description in the literature of diabetic renal disease, or diabetic nephropathy. Besides glomerulosclerosis, the histological picture of diabetic nephropathy involves thickening of the glomerular basement membrane, and mesangial matrix expansion [80].

2.2.1.1 Definitions

Diabetic nephropathy, or diabetic kidney disease, is defined based on the urinary albumin excretion rate (UAER) determined by a timed urine collection. Another method suitable for screening is a spot urine sample from which the albumin-to-creatinine ratio (ACR) is measured. Normal values for UAER are <30 mg/24h or <20 μg/min (in an overnight collection), also referred to as *normoalbuminuria*. Diabetic nephropathy, or *macroalbuminuria*, is diagnosed when the UAER is ≥300 mg/24h or ≥200 μg/min, which is the level of dipstick-positive albuminuria, in two out of three consecutive urine collections. The intermediate range of UAER, or ≥30 but <300 mg/24h or ≥20 but <200 μg/min is called *microalbuminuria*, and is regarded as a risk factor for overt nephropathy. *End-stage renal disease* (ESRD) refers to renal failure requiring dialysis treatment or kidney transplantation for survival. The arbitrary cut-off values of the UAER represent clinical consensus, and it has to be recognised that the degree of risk related to renal involvement in type 1 diabetes probably spans the whole range of UAER. Albuminuria is a marker of renal injury, but has also been implicated in the pathogenesis of diabetic nephropathy [81]. In itself UAER does not determine the level of renal function, which also is needed to fully assess the degree of renal involvement in patient with diabetes.

2.2.1.2 Renal function

Renal function, usually expressed as the glomerular filtration rate (GFR), can be measured or estimated in several ways. The golden standard is the chromium EDTA method (Cr^{51}-EDTA) [82] which, however, is a laborious method that is mostly used for research purposes. Clinically, the measurement of serum creatinine is the most frequently used method. Creatinine is a waste turnover product of skeletal muscle creatine (see section 2.4.1.1) which is freely filtered and secreted by the kidneys.

Creatinine has, however, limitations due to an active tubular secretion which counteracts a rise in serum creatinine at early stages of decreased renal function. A rise in serum creatinine is therefore a rather late manifestation of renal disease. Muscle mass may additionally influence the creatinine production. A relatively new serum marker of renal function is cystatin C [83] which is produced at a constant rate and is freely filtered in the kidneys without active tubular secretion and might therefore be a better serum marker of renal function than creatinine [84]. GFR can also be estimated by measuring the creatinine clearance in a 24h urine collection but due to the active tubular secretion, the creatinine clearance will be higher than the actual GFR. Cystatin C clearance cannot be determined because cystatin C is mostly degraded by the tubular epithelial cells and is not secreted into the urine.

GFR is clinically most often estimated from serum creatinine by formula calculations correcting for body mass, age, sex, and ethnicity which improve accuracy. The most commonly used are the Cockcroft-Gault [85] and the Modification of Diet in Renal Disease (MDRD) [86] formulas. The Cockcroft-Gault formula was developed based on creatinine clearance, and thus does not strictly speaking calculate GFR. The Cockcroft-Gault formula was derived from a population of men with a wide range of creatinine clearance, while the MDRD formula was derived from patients with chronic kidney disease. These formulas have limitations especially in populations without established chronic kidney disease [87]. The recently developed CKD-EPI formula performed better than the MDRD at normal GFR values [88]. GFR is usually adjusted for body-surface area, and expressed as ml per minute per 1.73 m^2. Reference values for GFR are as follows: normal: >90, mildly reduced: 60-89, moderately reduced: 30-59, severely reduced: 15-29, and kidney failure <15 ml/min/1.73 m^2.

2.2.1.3 Epidemiology

Diabetic nephropathy is becoming a major problem as the incidence of diabetes is increasing. Diabetes is currently the most common cause of renal failure in the Western World [89, 90]. In patients with type 1 diabetes, the classical view is that about one patient out of three develops kidney disease [7, 91]. There is, however, data indicating a decline in the incidence of diabetic nephropathy over time in some [92, 93], but not all [94] studies. The early studies which established microalbuminuria as the most important early marker for diabetic nephropathy in type 1 diabetes indicated that up to 80% of patients with microalbuminuria progress to macroalbuminuria, or overt nephropathy, over 6-14 years [95-97]. These studies used inconsistent definitions of microalbuminuria, and later a joint effort [98] unified the cut-off level to that of today (see section 2.2.1.1). More recent reports indicate that the fate of patients

with type 1 diabetes and microalbuminuria is not as grim as initially thought. In patients with a diabetes duration >15 years, 28% of patients with microalbuminuria progressed to macroalbuminuria during 10 years [99]. In addition, regression of micro- to normoalbuminuria is common, in up to 58% of patients with microalbuminuria [100]. Of special value is a report from a Danish inception cohort of 286 consecutive patients with newly diagnosed type 1 diabetes who were followed for 18 years [101]. This study showed a cumulative incidence of persistent microalbuminuria of 33.6% and macroalbuminuria of 14.6%, and 35.4% of the patients with microalbuminuria regressed back to normoalbuminuria. Therefore, the impact of microalbuminuria in the prediction of diabetic nephropathy in patients with type 1 diabetes is not as large as initially reported, but is still the best early non-invasive predictor in clinical use [102].

2.2.1.4 Risk factors

The pathways leading to diabetic nephropathy are thought to involve both genetic and environmental factors intertwined in a complex fashion. Fundamental risk factors for diabetic nephropathy are: glycaemic control [103], blood pressure [104,105], male gender [91,106], smoking [107], and ethnicity (blacks having higher risk than Caucasians) [108,109].

Glycaemic control is important for the development of diabetic nephropathy. This fact was established by the landmark Diabetes Control and Complications Trial (DCCT) which showed a 54% reduction in the incidence of diabetic nephropathy in patients with type 1 diabetes by lowering of the HbA_{1c} by 2%-units [103]. Previously the Steno [110], the Oslo [111], and the Stockholm [112] studies had also shown reductions in renal outcomes by lowering of the HbA_{1c}. In addition to the mean level of glycaemia, it has been discussed that glycaemic variability might confer additional risk of diabetic complications [113]. The thought is that hyperglycaemic peaks may have detrimental cellular effects [114-117] which ultimately might cause kidney damage. In patients with type 2 diabetes, variability of fasting plasma glucose was associated with the incidence of retinopathy [118]. In the DCCT, variability of quarterly measured, one-day 7-point glucose profiles did not predict incident nephropathy or retinopathy [119], and this was also observed in the Epidemiology of Diabetes Interventions and Complications (EDIC) [120], which is an extended follow-up of the patients in the DCCT. Variability in HbA_{1c} was, however, associated with the incidence of both diabetic nephropathy and retinopathy in the DCCT [121], thus linking the variability of long-term glycaemic control to the risk of microvascular complications in type 1 diabetes.

Insulin resistance is another factor that has been implicated in the pathogenesis of diabetic nephropathy [122,123]. It has been discussed that pathway-selective insulin

resistance is important for the development of diabetic nephropathy [124]. In line with this, there is some evidence that the metabolic syndrome is associated with the prevalence [27] and the incidence [125] of nephropathy in type 1 diabetes. Low birth weight [126] and short adult stature [127, 128] are other factors related to insulin resistance that have been linked to the risk of diabetic nephropathy. Furthermore, the presence of type 2 diabetes in a first-degree relative increases the risk of diabetic nephropathy in a patient with type 1 diabetes [129].

It is, however, obvious that the abovementioned clinical risk factors do not fully explain the risk for diabetic nephropathy in type 1 diabetes as there are undoubtedly patients with good glycaemic control who still develop this complication. In patients with type 1 diabetes, diabetic nephropathy clusters in families [130-132], which indicates that there might be an inherited component for the risk of diabetic nephropathy. The search for susceptibility genes for diabetic nephropathy using the candidate gene approach has identified a number of genes which, however, in most cases have not been replicated in other populations. A genome-wide microsatellite scan for diabetic nephropathy in type diabetes found linkage to a susceptibility locus on chromosome 3q [133]. Interestingly, this particular region of the genome has also previously been linked to diabetic nephropathy in another linkage analysis that also compared siblings with type 1 diabetes but discordant for diabetic nephropathy [134].

2.2.1.5 Pathogenesis

The molecular mechanisms leading to diabetic nephropathy are probably the result of an interaction between genes and the environment. The renin-angiotensin system (RAS) seems to play an important role in the pathogenesis of diabetic nephropathy. The RAS mediator angiotensin II, which is formed from angiotensin I by the angiotensin converting enzyme (ACE), stimulates the production of transforming growth factor β (TGF-β). TGF-β may in turn cause extracellular matrix overproduction [135] and induce harmful reactive oxygen species [136] in the kidneys. Hyperglycaemia induces renal angiotensin II production [137]. It is also of note that there is both a systemic RAS and a local RAS in the kidneys. Medication that blocks the RAS system is used in the treatment of diabetic nephropathy (see section 2.2.1.7).

Another potential source of tissue damage in diabetes is the accumulation of advanced glycation end products (AGEs) by glycation of proteins and lipids and these are considered important in the development of diabetic complications [138]. Besides structural and functional effects, AGEs also induce cellular responses via activation of the receptor for AGEs (RAGE) [139] which activates cell signalling pathways [140] that may ultimately cause kidney damage. A unifying hypothesis for the development of diabetic microvascular complications was suggested by Michael

Brownlee, that integrates AGEs, oxidative stress, the polyol pathway (reduction of glucose to sorbitol which accumulates in the cells), protein kinase C activation, and the hexosamine pathway (glucose conversion to fructose derivates which leads to increased production of TGF-β)[141]. The core of this hypothesis is that hyperglycaemia causes excessive production of mitochondrial superoxide which initiates these pathways ultimately leading to tissue damage.

2.2.1.6 Clinical course

In patients with type 1 diabetes, nephropathy typically presents after 15-20 years of living with diabetes, and is preceded by a variable time of microalbuminuria. Macroalbuminuria at short duration of type 1 diabetes should raise suspicion of the presence of nondiabetic renal disease. Some patients with type 1 diabetes develop hyperfiltration defined as a GFR >125-140 ml/min/1.73 m^2 at the early stages of diabetes. Some studies have even suggested that hyperfiltration is a risk factor for future diabetic nephropathy [142, 143]. Some, but not all, of the patients with macroalbuminuria will develop ESRD. Patients with macroalbuminuria, and especially those with ESRD, are at great risk of cardiovascular events and premature death [6-8]. Premature death is thus a competing outcome to ESRD in patients with type 1 diabetes and macroalbumniuria.

2.2.1.7 Treatment

The treatment of patients with diabetic nephropathy aims to halt the deterioration of the kidney function and to reduce the cardiovascular risk. Optimal control of glycaemia and blood lipids as well as smoking cessation are important. Dietary protein restriction may not only slow down the progression rate of diabetic nephropathy [144, 145], but also the progression to ESRD, and may increase survival [146]. The blood pressure should be treated effectively. Optimal blood pressure control has repeatedly been shown to slow down the progression of renal disease. In the kidneys, ACE-inhibitors reduce the intraglomerular pressure by vasodilation of the efferent arteriole because angiotensin II, the main mediator of the RAS system, preferentially increases the resistance of the efferent arteriole. ACE-inhibitors and angiotensin receptor blockers are the most important antihypertensive medications for these patients, because inhibition of the RAS-system in patients with diabetes is thought to have beneficial renal effects beyond blood pressure reduction [147, 148]. Of note are, however, two recent studies that showed no reduction in the incidence of microalbuminuria by inhibitors of the RAS-system in patients with type 1 diabetes

and normoalbuminuria [149, 150], suggesting no benefit of RAS-blockers at least for primary prevention of renal disease in diabetes. Renal failure is associated with a decrease in the erythropoietin (EPO) secretion from the kidneys and subsequent renal anaemia [151], which can be corrected by injections of recombinant human EPO or its derivate darbepoetin alfa. Excess correction of the anaemia should however be avoided due to concerns for adverse events in patients with kidney failure [152]. Renal failure also causes profound changes in the calcium and phosphate metabolism with deleterious effects on the skeletal tissue due to a decrease in the vitamin D activation by the kidney. This condition, referred to as renal osteodystrophy [153], can be treated by calcium and vitamin D supplementation and phosphate binders, but sometimes there is need for surgical excision of the parathyroid glands to halt the breakdown of the skeletal tissue by the secondary and tertiary increase in the secretion of parathyroid hormone. Renal failure requires renal replacement therapy; initially dialysis (haemodialysis or peritoneal dialysis) followed by kidney transplantation.

2.2.2 Diabetic retinopathy

The retinas of the eyes are commonly affected by diabetes. The first report on diabetic retinopathy is from 1935 [154]. Most patients with type 1 diabetes of 20 years duration develop some degree of *background retinopathy* that most frequently involve microaneurysms of the retinal blood vessels. A proportion (up to 42% by 25 years of type 1 diabetes duration [155]) of patients with type 1 diabetes will progress to *proliferative retinopathy*, a condition which is characterised by the growth of new blood vessels (neovascularisation) in an attempt to compensate for local ischaemia in the retina. If left untreated, proliferative retinopathy can cause blindness. Diabetic macular oedema is another diabetes-related entity and this condition is caused by hyperpermeability of the retinal capillaries, and may also cause visual impairment and blindness. It is of note that diabetic retinopathy is the most common cause of blindness in the Western World, but that other causes dominate in the developing counties [156]. In 1998 in the USA, the cumulative 14-year incidence of blindness due to diabetes was 2.4% [157]. There is a strong comorbidity with diabetic nephropathy in the sense that almost all patients with nephropathy also have retinopathy, but retinopathy frequently occur as the sole complication of diabetes.

Risk factors for diabetic retinopathy are diabetes duration, poor glycaemic control, hypertension, and an unfavourable blood lipid profile. In the DCCT, intensive glycaemic treatment reduced the incidence of retinopathy by 76% and slowed the progression of existing retinopathy by 54% [103, 158], and the protective effect seemed to persist at least 10 years after the end of the trial [159]. Blood pressure lowering reduces the risk of diabetic retinopathy in type 1 diabetes [160], but the superiority of inhibitors

of the RAS-system has been questioned [161, 162]. The lipid-lowering agent fenofibrate has been shown to reduce the incidence of proliferative retinopathy in type 2 diabetes [163]. Regarding hereditary factors, proliferative retinopathy seems to cluster in siblings with type 1 diabetes [164]. The pathogenesis of diabetic retinopathy seems to share several mechanisms with nephropathy (see section 2.2.1.5): accumulation of sorbitol and AGEs, oxidative stress, and PKC activation [165]. Neovascularisation, however, is a distinct feature of retinopathy and is induced by growth factors, such as the vascular endothelial growth factor (VEGF) [166].

The diagnosis of diabetic retinopathy is based on direct inspection of the retina, and does not rely upon intermediate markers such as albuminuria as for the case of diabetic nephropathy. Thus, the retina is a true window into the microcirculation. Retinas may be examined by fundus photography or direct ophtalmoscopy. Active screening and treatment for retinopathy is cost-effective [167] and can reduce the incidence of diabetes-related blindness up to 98% [168].

The treatment of diabetic retinopathy involves the clinical management of risk factors. If needed, retinal changes can be treated with laser photocoagulation, a procedure that prevents visual loss in patients with proliferative diabetic retinopathy [169] and/or diabetic macular oedema [170]. Inhibition of VEGF is a potential now therapy for diabetic proliferative retinopathy [171].

2.2.3 DIABETIC NEUROPATHY

Diabetic neuropathy is a disease of the peripheral nervous system which can be a disabling diabetic complication and may reduce the quality of life and increase the risk of premature death. Diabetic neuropathy is believed to be a disease of the vasa nervorum, the small blood vessels of the nerves; hence neuropathy is considered a microvascular complication of diabetes. The clinical definition of diabetic neuropathy is symptoms or signs of nerve disease in a patient with diabetes when other causes than diabetes are excluded [172]. Screening for neuropathy in patients with diabetes should be performed annually by testing tendon stretch reflexes and sensation in the lower extremities [173]. Prevalence estimates for diabetic neuropathy differ greatly according to the population studied and the definitions used. Clinical risk factors for diabetic neuropathy include poor glycaemic control, diabetes duration, obesity, smoking, lipid profile, hypertension, and tall stature [173-175].

There are several types of neuropathies in diabetes. The most common is a symmetric chronic sensory polyneuropathy which mainly involves the lower extremities and causes loss of sensation and predisposition for ulcers in the feet, which ultimately may require limb amputation. There is also an acute, often painful,

sensory neuropathy which infrequently occurs at sudden changes in glycaemic control. Mononeuropathies also rarely occur. Autonomic diabetic neuropathy affects the system that for instance regulates cardiovascular and gastrointestinal reflexes. Cardiac autonomic neuropathy is important due to the increase in the risk of sudden death, and is characterized by tachycardia at rest (heart rate >100/min) and a drop in blood pressure (>20 mmHg) when standing up [173]. Cardiac autonomic neuropathy frequently causes exercise intolerance (see section 2.4.2.2) and increases the risk of sudden death during exercise. Therefore, exclusion of this condition prior to the initiation of a heavy exercise program is therefore warranted in patients with diabetes [176]. Other types of autonomic diabetic neuropathy involve gastroparesis, constipation, diarrhoea, erectile dysfunction, and urinary bladder dysfunction.

There are several measures of cardiac autonomic neuropathy. The classical Ewing tests constitute non-invasive interventions to test cardiovascular reflexes during the valsalva manoeuvre, deep breathing, standing up, and during sustained handgrip [177]. A more sensitive method is measurement of the baroreflex sensitivity [178] which allows detection of autonomic neuropathy even before signs of clinical organic neuropathy are evident. The basic principle behind measurement of the baroreflex sensitivity is the feedback loop from a change in blood pressure into a change in heart rate by regulating the vagal nerve activity.

2.2.4 CARDIOVASCULAR DISEASE

Cardiovascular disease (CVD) is the term for clinical manifestations of atherosclerotic changes of the large conductance arteries. The clinically most relevant arteries are in the heart (coronary heart disease), in the brain (stroke), and in the lower extremities (claudication). CVD is not, as the microvascular complications, specific to diabetes but patients with diabetes have a considerably increased risk thereof. Type 2 diabetes may even be a risk factor for a first CVD event equal to that of a previous CVD event [179]. The risk of CVD in patients with type 1 diabetes is also increased compared with nondiabetic subjects [180,181]. Diabetic nephropathy markedly increases CVD morbidity [6] and mortality [7] compared with the absence of nephropathy in type 1 diabetes. The reason why renal impairment strongly increases CVD risk – a phenomenon called the chronic renocardiac syndrome [182] – is unclear. Diabetic microvascular complications and CVD might share common risk factors and according to the Steno hypothesis, albuminuria is a marker of general vascular damage in diabetes [183]. Also in nondiabetic subjects, microalbuminuria seems to be a CVD risk factor [184] thereby extending this phenomenon beyond diabetes.

CVD in patients with diabetes is associated with some special features that are not seen in nondiabetic subjects. Diabetes conferred higher CVD relative risk in

women than in men in the Framingham study [185] and subsequent reports [181, 186], thus suggesting a loss of gender-associated protection from CVD in patients with diabetes. Equal relative CVD risk in both genders with type 1 diabetes has, however, also been reported [187, 188].

The risk factors for CVD in diabetic and nondiabetic subjects do to a large extent overlap and include age, blood pressure, blood lipid profile, smoking, and a family history of CVD. The lipid profile of patients with type 1 diabetes differs from that of nondiabetic subjects [189]. In patients with well-controlled type 1 diabetes, total and LDL-cholesterol are often similar to that of the nondiabetic subjects, but HDL-cholesterol is often higher. The LDL-cholesterol in patients with type 1 diabetes has increased atherogenic properties because of its oxidation [190] and glycation [191] in the diabetic milieu. The role of glycaemic control in the context of CVD in patients with diabetes is a matter of debate at the moment. In type 2 diabetes, recent randomised clinical trials to lower HbA_{1c} by intensive treatment failed to show a reduction in the incidence of CVD [192-194], and in one study [193] there was even an apparent increase in mortality in the intensive treatment group. A subsequent meta-analysis, however, indicated that intensive treatment reduced the incidence of CVD events [195]. These studies may have been influenced by the fact that the patients had a relatively long duration of type 2 diabetes at randomisation [196]. Notably, in newly diagnosed patients with type 2 diabetes, the U.K. Prospective Diabetes Study (UKPDS) showed a near-significant (P=0.052) reduction in the incidence of myocardial infarction by lowering of HbA_{1c} [197], and in a follow-up report intensive treatment showed a clear CVD benefit [198]. In the UKPDS risk engine for CVD in patients with type 2 diabetes, HbA_{1c} is included as a CVD predictor [199].

2.3 THE METABOLIC SYNDROME

The metabolic syndrome is defined as a cluster of cardiovascular risk factors that frequently occur together. Initially called syndrome X [200], this condition was recognised as the simultaneous finding of a disturbed glucose metabolism, hypertension, and dyslipidaemia. Insulin resistance has been considered the key feature of what today is referred to as the metabolic syndrome [201]. The first clinical definition was presented by the WHO in 1998 [202]. Since then, several definitions of the metabolic syndrome have emerged. In 2001, the National Cholesterol and Education Program Adult Treatment Panel III (NCEP) introduced a definition [203] and in 2005 the International Diabetes Federation (IDF) introduced the latest definition to be widely used [204]. Table 1 presents the definitions in detail. The IDF definition differs fundamentally from the others by the demand of abdominal obesity. The WHO definition, on the other hand, is the only to include microalbuminuria.

	Requirement	Multiple options
WHO	Diabetes/IGT plus two out of:	1.BMI >30 and/or WHR >0.90 (M) or >0.85 (W) 2. SBP ≥140 and/or DBP ≥90 mmHg 3. HDL-chol. <0.9 (M) or <1.0 mmol/l (W) and/or triglycerides ≥ 1.7 mmol/l 4. Microalbuminuria (UAER ≥20 μg/min)
NCEP	Three out of:	1. Diabetes/IGT 2. Waist >102 (M) or >88 cm (W) 3. SBP ≥130 and/or DBP ≥85 mmHg 4. HDL-chol. <1.0 (M) or <1.3 mmol/l (W) and/or triglycerides ≥ 1.7 mmol/l
IDF	Waist ≥94 cm (M) or ≥80 cm (W) and two out of:	1. Diabetes or fasting glucose ≥5.6 mmol/l* 2. SBP ≥130 and/or DBP ≥85 mmHg* 3. HDL-chol. <1.03 (M) or <1.29 mmol/l (W) and/or triglycerides ≥ 1.7 mmol/l*
Joint statement [205]	Three out of:	1. Elevated waist circumference (population-specific cut-off values, see IDF for Europe) 2. Triglycerides ≥1.7 mmol/l* 3. HDL-chol. <1.0 (M) or <1.3 (W)* 4. SBP ≥130 mmHg and/or DBP ≥85 mmHg* 5. Fasting blood glucose ≥5.6 mmol/l*

Table 1. Common definitions of the metabolic syndrome. M: men. W: women. *Or treatment for hyperglycaemia, hypertension or hyperlipidaemia.

Type 2 diabetes is in close relation to the metabolic syndrome as one of the criteria mention in table 1. The prevalence of the metabolic syndrome increases abruptly with the degree of glucose tolerance. In a Finnish cohort, the prevalence of the WHO definition of the metabolic syndrome was 14.4% in men with normal glucose tolerance, 74.0% in case of impaired fasting glucose, and 91.5% if type 2 diabetes had been diagnosed [206]. The metabolic syndrome has also been described in Finnish patients with type 1 diabetes, even though the commonly used definitions do not take type 1 diabetes into consideration. Using the NCEP definition, 38% of men and 40% of women with type 1 diabetes fulfilled the criteria for the metabolic syndrome which furthermore was associated with poor glycaemic control and a higher prevalence of diabetic nephropathy in a cross-sectional setting [27]. A similar prevalence was reported in Italian patients with type 1 diabetes [207]. The cross-sectional association between the metabolic syndrome and diabetic nephropathy was challenged by two prospective studies that showed little predictive value of the

metabolic syndrome for cardiovascular disease and diabetic nephropathy in type 1 diabetes [208, 209]. Nevertheless, a recent follow-up study indicated that the NCEP definition of the metabolic syndrome was predictive of CVD events and mortality, and even though the predictive value for development and progression of diabetic nephropathy was low, the presence of the metabolic syndrome added upon the predictive value of an elevated UAER for CVD events [125].

It can be argued, though, that the metabolic syndrome is redundant. There are totally opposing views as to whether the metabolic syndrome serves a purpose in clinical medicine [210, 211]. The metabolic syndrome can be regarded as merely an arbitrary condition with varying cut-off values of a continuous risk relationship between the blood glucose concentration and cardiovascular disease and that the metabolic syndrome is not an entity of its own [212]. As a practical tool for the physician treating patients with metabolic disorders, however, the metabolic syndrome is at least useful as a reminder that when one cardiovascular metabolic risk factor is present, the physician should also test for the rest and treat them.

2.4 PHYSICAL ACTIVITY

Herodicus (born 480 B.C.), a Greek physician, is considered to have been the first to use exercise as a therapy for various diseases [213]. Hippocrates, inspired by Herodicus, early acknowledged the importance of lifestyle in his work *Regimen* around 400 B.C.: "For food and exercise, while possessing opposite qualities, yet work together to produce health".

Physical activity is the general term for any energy expenditure that result from skeletal muscle contractions. *Exercise* is a component of physical activity and refers to planned physical activities aimed to increase the level of *physical fitness*, which in turn has components of *endurance* and *strength*. Activities have a *frequency* (how often performed), an *intensity* (performed at what level of exertion), and a *duration* (time per session). In this thesis, the term physical activity has been preferred to describe the activity behaviour of the patients.

Quantification of physical activity involves objective measurements, diaries, and questionnaires. Each of these methods has distinct benefits and limitations. Physical activity can be measured by step counters, accelerometers, or heart rate monitors. Doubly labelled water is a method using deuterium (^{2}H)- and ^{18}O-labelled water to determine the metabolic rate in free-living condition [214]. The energy expenditure exceeding the basal metabolic rate gives an estimate of the physical activity. Doubly labelled water can be used for the validation of physical activity questionnaires [215]. Objective measurements of physical activity may be regarded more accurate than self-report questionnaires [216]. Devices measuring activity, however, are generally more applicable only to smaller study populations. Such devices also have the

potential bias of increased physical activity during days of measurement. Physical activity diaries may also have such a bias of increased activity during surveillance. For epidemiological studies involving large numbers of patients, physical activity questionnaires have often been used due to practical and economical reasons.

2.4.1 GLUCOSE RESPONSES DURING PHYSICAL ACTIVITY

2.4.1.1 Energy supply

Skeletal muscle tissue has a tremendous energy output potential as the metabolic rate in the muscle cells can increase 200-fold during strenuous physical activity [217]. Accordingly, an efficient glucose transport system into the muscle cells is essential. Muscle glycogen is the principal source of glucose, but glucose from the blood circulation is also important. Muscle glycogen stores are usually regenerated in 0.5-2 h after a prolonged activity and are fully restored within one day by glucose from the blood, thus preparing the muscles for the next bout of activity.

For the exercising skeletal muscle tissue, adenosine triphosphate (ATP) is the principal fuel currency, and energy is released by the breaking of high-energy phosphate bonds resulting in adenosine diphosphate (ADP). The main source of ATP during a bout of physical activity varies. Existing ATP in the muscles is depleted within one second, and the maintenance of skeletal muscle work depend on constant replenishment of ATP by three principal systems [217]. The first system is creatine phosphate stored in the muscle as a rapid source for rephosphorylation of ADP to ATP. This system is independent of oxygen (anaerobic) and only operates for up to nine seconds of activity. The second system involves breakdown of muscle glycogen to glucose and further to lactic acid during anaerobic glycolysis, a process during which ATP is obtained. This lactic acid system is most critical during ten seconds up to two minutes of physical activity. This system is limited by accumulation of lactic acid in the muscle, which causes a burning sensation. The third and most sophisticated system is initiated when the activity exceeds two minutes of duration. Hereby, glucose obtained mainly from muscle glycogen deposits, but also from the blood, is oxidised in the citric acid (Krebs) cycle to produce ATP. Likewise, free fatty acids are degraded and oxidised during β-oxidation to yield ATP. These oxygen-dependent processes take place in the mitochondria and the ATP yield per molecule of glucose is tenfold to that of the anaerobic lactate system. In real life, the temporal boundaries between these three systems is somewhat overlapping. Furthermore, a sudden intensity increase during prolonged endurance activities

requiring extra ATP may temporarily reactivate the lactate system. The skeletal muscle tissue, unlike the liver, lacks the enzyme glucose-6-phosphatase which means that muscle glycogen is consumed locally and glucose is not released into the bloodstream. The liver, on the other hand, can release glucose into the blood by glycogenolysis, and can further synthesise glucose from amino acids and lactate, a process called gluconeogenesis. Muscle glycogen constitutes about 130 g and typically lasts for 90 minutes of endurance activity without carbohydrate ingestion. The ratio of carbohydrate-to-fat energy use by the body varies, from about 40% during rest but increases during high-intensity exercise up to 100%. Many factors such as the diet content prior to activity, muscle glycogen store depletion, and hormone (such as insulin and adrenalin) levels also influence the source of energy during physical activity.

Skeletal muscle tissue is insulin sensitive. Insulin binds to the insulin receptor which causes autophosphorylation of intracellular tyrosine residues. This further activates the signal transduction cascade ultimately leading to translocation of the glucose transporter 4 (GLUT4) to the cell surface whereby glucose can enter the muscle cell. Physical activity causes an acute increase in insulin sensitivity of the skeletal muscle cells which facilitates glucose entry [218]. Even more important, however, is that muscle contractions themselves increase glucose influx independently of insulin [219]. This effect of contraction is also mediated via an increase in GLUT4 translocation to the muscle cell surface, but via other intracellular signalling pathways than insulin [220]. Moreover, the GLUT4 molecules recruited by insulin and contractions seem to derive from separate pools of intracellular GLUT4-containing vesicles [221]. Insulin resistance does not seem to impair the contraction-dependent glucose uptake of glucose to the skeletal muscle [222]. The mechanism behind increased glucose uptake by muscle contractility is somewhat unclear, but nitric oxide produced by the contracting muscle is considered important either through increased blood flow or through a direct effect on GLUT4 translocation to the cell surface [223]. Another suggested mechanism in contracting skeletal muscle cells is via increased 5'AMP-activated protein kinase (AMPK) levels. AMPK is activated by alterations in the ATP/AMP- and creatine/phosphocreatine-ratios [224].

2.4.1.2 Hormonal responses in nondiabetic subjects

During physical activity, the secretion of several hormones differ greatly from the resting state [217]. The extent of these changes depends upon duration and intensity of the activity and on the fitness level of the individual. The main purpose is to provide contracting skeletal muscles with nutrients and to maintain a normal blood glucose level. The insulin secretion from the pancreas diminishes from the initiation

of physical activity and remains lowered throughout the activity. At moderate intensity, insulin levels decrease to about half of the level at rest. The levels of counterregulatory hormones of insulin, on the other hand, increase during activity. Neuroendocrine (sympathetic) reflexes cause a pre-emptive rise in adrenaline and noradrenaline already at mental preparation for physical activity. During activity, the adrenal medulla continues to secrete adrenaline and noradrenaline. These catecholamines stimulate glycogenolysis in the muscles, lipolysis in the adipose tissue, and to some extent glucose output from the liver by glycogenolysis and gluconeogenesis. Simultaneously, glucagon secretion from the pancreas increases, with similar metabolic effects as the catecholamines, especially glycogenolysis and gluconeogenesis in the liver. This results in glucose output from the liver, decreased peripheral glucose uptake (apart from the skeletal muscles), release of free fatty acids to the circulation, and glucose release from glycogen stores in the skeletal muscles. Cortisol secretion from the adrenal cortex increases during activity which promotes protein catabolism to provide substrates for gluconeogenesis. Growth hormone secretion from the pituitary gland increases when the activity exceeds 40-50 minutes, in turn stimulating lipolysis and also working through multiple mechanisms to raise the blood glucose concentration. Water- and electrolyte balance during activity is facilitated by an increase both in aldosterone and antidiuretic hormone secretion leading to retention of water and sodium ions in the kidneys.

2.4.1.3 Hormonal responses in patients with type 1 diabetes

Insulin is administered exogenously in patients with type 1 diabetes. During physical activity, therefore, the physiologic decrease in insulin secretion is absent and the amount of circulating insulin depends on the amount and the timing of recent insulin injections. Insulin absorption from the site of injection is more rapid during physical activity than at rest due to increased blood circulation, especially in the thigh [225]. Also in patients with diabetes, physical activity increases insulin sensitivity. If these aspects are not taken into consideration, there is considerable risk of hypoglycaemia during or after physical activity in a patient with type 1 diabetes. Relative hyperinsulinaemia during physical activity increases the skeletal muscle glucose uptake from the circulation disproportionately in relation to the use of muscle glycogen as the source of glucose. Insulin also inhibits lipolysis in the adipose tissue, thus increasing the carbohydrate demand as the skeletal muscle fuel source. In addition, with increasing duration of type 1 diabetes, the glucagon response is blunted [226] which in concert with relative hyperinsulinaemia impairs an adequate glucose output from the liver to the circulation during physical activity. Autonomic neuropathy may in turn damage the sympathetic nervous system resulting in a

decreased adrenergic response during physical activity, which can also cause hypoglycaemia during activity. Symptoms of hypoglycaemia can further be silenced by autonomic neuropathy, which can result in severe hypoglycaemia.

2.4.1.4 Acute effect on blood glucose

During physical activity the blood glucose concentration in a patient with type 1 diabetes can remain unchanged, decrease, or increase depending on numerous factors that influence the amount of circulating insulin and the availability of glucose. Before the start of physical activity the blood glucose concentration should be between 5.6 and 16.7 mmol/l and in case of ketosis no higher than 13.9 mmol/l [176]. If the blood glucose is below 5.6 mmol/l, carbohydrate supplementation (15-30g depending on activity) should be ingested to prevent hypoglycaemia but this is not an unconditional rule for all types of activities, especially those of high intensity and short duration. Hyperglycaemia should be corrected with short-acting insulin, because physical activity during hypoinsulinaemia can cause a further rise in blood glucose concentration and, in a worst case scenario, even lead to ketoacidosis.

In nondiabetic individuals, the blood glucose concentrations are strictly controlled during physical activity resulting in normal blood glucose levels both during and after activity. An exception is high intensity activities, which can cause transient hyperglycaemia mostly due to a massive adrenergic activation of the liver glucose output. A rise in the insulin secretion at the cessation of activity rapidly normalises such elevated blood glucose levels in nondiabetic subjects. In patients with type 1 diabetes, however, the absence of a physiologic insulin response may cause blood levels to rise substantially during activities of high intensity [227]. A bolus of short-acting insulin may be required after such activities, with subsequent frequent blood glucose measurements to prevent hypoglycaemia.

2.4.1.5 Hypoglycaemia

Hypoglycaemia during or after physical activity, or fear thereof, is often a barrier for a patient with type 1 diabetes to engage in physical activity [228, 229]. Hypoglycaemia can be defined as a blood glucose concentration ≤3.9 mmol/l [230], but the threshold for symptomatic hypoglycaemia varies both inter- and intraindividually. Hypoglycaemia can in the broad perspective be regarded as the main limiting factor for achievement of good glycaemic control in type 1 diabetes [231] and this is true for all interventions that lower blood glucose, mainly insulin treatment and physical activity.

The mechanisms of hypoglycaemia during or after physical activity mainly include the circulating insulin concentration, the carbohydrate availability, the

replenishment of muscle glycogen stores, the effect of the counterregulatory hormones, and the awareness of symptoms of hypoglycaemia.

To prevent hypoglycaemia, one should remember that the peak effect of most short-acting insulin preparations appears 1.5-2 h after the injection, and rapid-acting insulin analogues peak at 60 min after injection. The site of injection [225] and the ambient temperature [232] may affect insulin absorption during activity. Prandial insulin doses previous to physical activity may be reduced to mimic the physiologic decrease in insulin secretion during physical activity in nondiabetic subjects. After the activity, the next prandial insulin dose may also have to be reduced to account for the acute increase in insulin sensitivity. The magnitude of reduction in insulin dose depends upon the type, intensity, and duration of physical activity and probably also on the individual responses to physical activity. For instance, a moderate intensity activity for 30 min may require only 10-20% reduction in prandial insulin, but a high-intensity activity may require up to 60% reduction [233]. Basal insulin doses may also be reduced at days of activities with long duration. During physical activity, routine carbohydrate supplementation is usually needed only if the duration is >1 h, but can be needed for activities of shorter duration in case of relative hyperinsulinaemia. After activity, carbohydrate ingestion is usually recommended because of the increased demand of glucose to replenish muscle, and sometimes also liver, glycogen stores. The amount of carbohydrate is often 15-30 g and can be taken as solid food, fluids, or gels. Fluids containing 5-10% of carbohydrate are rapidly absorbed [233], because the carbohydrate concentration of fluids is important for the rate of gastric emptying [234].

Muscle glycogen stores are replenished from blood glucose after physical activity at a rate that is highest 0.5-2 h after activity but can continue up to 24 h, thus (together with the acute increase in insulin sensitivity) increasing the risk of hypoglycaemia many hours, typically during the night, after physical activity. Immediate carbohydrate ingestion after activity may decrease this risk. [235].

Counterregulatory hormones to insulin prevent hypoglycaemia in nondiabetic subjects, but in patients with type 1 diabetes this response may be abnormal. The glucagon response to hypoglycaemia is blunted in patients with type 1 diabetes [226]. The adrenergic response may be defective in patients with autonomic neuropathy, which leads both to a decreased ability to increase the blood glucose concentration and, perhaps even more importantly, to decreased symptoms of hypoglycaemia which are mostly mediated by the adrenergic system. Even in patients with type 1 diabetes who otherwise have a functioning counterregulatory hormone system, an episode of hypoglycaemia will reduce this response even for several days, a phenomenon called “hypoglycaemia-associated autonomic failure” [236]. An episode of hypoglycaemia will have consequences for any forthcoming (next day) physical activity in form of a reduced response of counterregulatory hormones during physical

activity and subsequent risk of hypoglycaemia during, or after, activity [237]. Moreover, physical activity in itself has also been reported to blunt these counterregulatory hormone responses during a subsequent hypoglycaemic event [238]. This may lead to a vicious cycle of recurrent hypoglycaemic episodes that can cause a highly instable glycaemic control with alternating hypoglycaemia and rebound hyperglycaemia due to the overcorrection of blood glucose concentrations. An interesting detail is that women with type 1 diabetes seems to have better counterregulatory hormone responses to physical activity than men [239].

To decrease the risk of hypoglycaemia after physical activity, a 10 s maximal sprint at the end of a session of moderate-intense aerobic activity has been proposed [240]. This approach takes advantage of the counterregulatory hormones, especially the catecholamines that are secreted during high-intense exercise. There is some evidence for higher post-exercise blood glucose concentrations using the 10 s maximal sprint both when performed at the end [240] and at the beginning [241] of a bout of moderate-intense physical activity. There are, however, no available data investigating the actual incidence of hypoglycaemic events after physical activity with the 10 s maximal sprint.

2.4.2 PHYSICAL ACTIVITY AND TREATMENT OF TYPE 1 DIABETES

According to the recommendations from the American Diabetes Association, patients with diabetes should engage in regular physical activity to improve their cardiovascular risk profile [176]. The effect of physical activity regarding the risk of microvascular diabetic complications is to a large extent unknown. Glycaemic control is a major risk factor for microvascular diabetic complications. In type 2 diabetes, the consensus is that physical activity improves the long-term glycaemic control [10, 242]. In type 1 diabetes the evidence is inconclusive, however.

2.4.2.1 Glycaemic control in type 1 diabetes

There are intervention and cross-sectional association studies investigating the relationship between physical activity and glycaemic control in patients with type 1 diabetes. Available physical activity intervention studies [11, 12, 243-258] are presented in Table 2. Most of these studies showed no beneficial effect on HbA_{1c}, HbA_1, or glucose values. These studies were performed predominantly during the 1970-80s and thus the treatment does not fully represent the insulin regimen of today. Most of the studies employed a small number of patients and short time of intervention. A

more recent cross-sectional study showed no association between a physical activity score and HbA_{1c} in adult patients with type 1 diabetes, but the validation of the score was not reported [259]. Cross-sectional data from a large paediatric population, on the other hand, showed that patients with a frequency of regular physical activity <1 time/week had higher HbA_{1c} than those with a frequency 1-2 and ≥3 times/ week, while the latter two groups had similar HbA_{1c} [260]. A limitation with these cross-sectional association studies is that the causal effect of physical activity is uncertain because physical activity is in itself usually associated with other lifestyle factors and diabetes treatment behaviour, which creates problems with collinearity.

Authors	N	Age (yrs)	Controls	Intervention	Effect on glycaemic control
Dahl-Jorgensen *et al* 1980	22	Mean 11	Yes	AeT x2/week, 20 weeks	HbA_1 decreased
Wallberg-Henriksson *et al* 1982	9	25-46	No	AeT x2-3/week, 16 weeks	HbA_1 unchanged
Campaigne *et al* 1984	19	5-11	Yes	AeT x3/week, 12 weeks	HbA_1 decreased
Yki-Järvinen *et al* 1984	32	Mean 26	Yes	AeT 6 weeks	HbA_1 unchanged
Zinman *et al* 1984	20	Mean 30	Yes	AeT x3/week, 12 weeks	HbA_1 unchanged
Wallberg-Henriksson *et al* 1984	20	25-36	Yes	AeT x3/week, 8 weeks	HbA_1 unchanged
Strickland *et al* 1984	36	-	No	AeT 2 weeks	HbA_1 unchanged
Baevre *et al* 1985	10	14-17	No	AeT 24 weeks, strenuous 2 weeks	HbA_1 unchanged
Landt *et al* 1985	15	Mean 16	Yes	AeT x3/week, 12 weeks	HbA_1 unchanged
Rowland *et al* 1985	14	9-14	No	AeT 12 weeks	HbA_1 unchanged
Wallberg-Henriksson *et al* 1986	13	26-43	Yes	AeT daily, 20 weeks	HbA_1 unchanged
Hansen *et al* 1989	16	11-16	No	AeT one week	HbA_{1c} unchanged
Huttunen *et al* 1989	32	8-17	Yes	AeT 12 weeks	HbA_{1c} increased
Bak *et al* 1989	14	18-40	Yes	Unspecified training six weeks	HbA_{1c} decreased
Lehmann *et al* 1997	20	22-48	No	AeT 12 weeks	HbA_{1c} unchanged
Mosher et al 1998	21	Mean 17	Yes	AeT and strength training 12 weeks	HbA_{1c} decreased
Laaksonen *et al* 2000	56	20-40	Yes	AeT 16 weeks	HbA_{1c} unchanged
Ramalho et al 2006	13	Mean 20	No	AeT and strength training 12 weeks	HbA_{1c} increased by AeT, unchanged by strength training

Table 2. Physical activity intervention studies involving glycaemic control in patients with type 1 diabetes. Age as range or mean. Controls refer to presence of a nondiabetic control group. AeT: aerobic training.

2.4.2.2 Diabetic complications

Physical activity and diabetic complications might have multiple interactions. First, the diabetic complications, being chronic devastating illnesses, can be thought to reduce the physical activity of affected patients. Second, physical activity results in multiple responses that all have been implicated in the pathogenesis of diabetic complications (Figure 1) and could therefore reduce the incidence and progression of the diabetic complications. A caveat, on the other hand, is that physical activity in theory also could be detrimental for patients with diabetic complications.

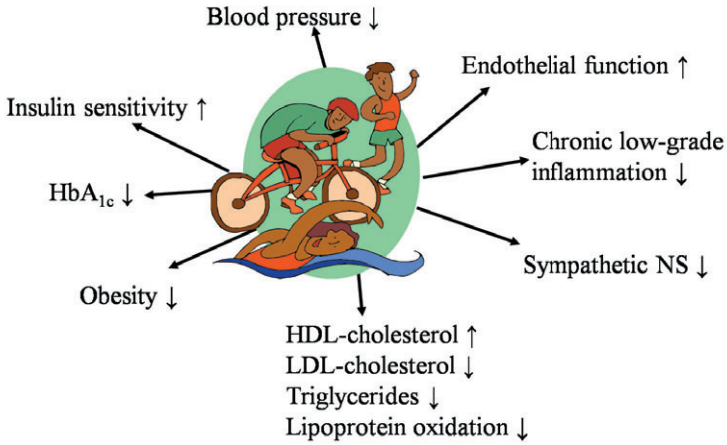

Figure 1. Potential chronic responses to physical activity

There are some specific physical activity recommendations for patients affected by diabetic complications. The American Diabetes Association recommends[176] that patients with proliferative retinopathy should avoid activities that involve valsalva manoeuvres because of the risk of vitreous haemorrhage. Loss of sensation in the feet due to diabetic neuropathy promotes the choice of non-weight-bearing activities. Autonomic neuropathy, and especially cardiac autonomic neuropathy, should be diagnosed prior to initiation of a physical activity program because of exercise intolerance and increased risk of sudden death during physical activity. Cardiovascular evaluation may also be needed depending on age, duration of diabetes, and presence of cardiovascular risk factors. Diabetic nephropathy is thought to self-limit physical activity due to a reduced capability for physical activity [176].

It has been shown that patients with type 1 diabetes have decreased aerobic work capacity if they have increased UAER, and similar impairment was shown both for patients with micro- and macroalbuminuria compared with normal UAER [261]. Conversely, exercise has been shown to induce transient albuminuria in patients with

type 1 diabetes with normoalbuminuria, and also a transiently worsened albuminuria in those with microalbuminuria was reported [262, 263]. This phenomenon is also well-known in nondiabetic subjects and is thought to result from haemodynamic factors such as increased glomerular pressure and increased glomerular permeability for albumin[264, 265]. There have been concerns that the renal function of patients with diabetic nephropathy may be harmed by moderate to heavy physical activity[266]. Recent follow-up data showed that physically active patients with type 2 diabetes and macroalbuminuria had increased total and CVD mortality [267]. Some patients with type 1 diabetes seem to have an exaggerated albuminuria response to an exercise test compared with nondiabetic subjects[263]. Improved glycaemic control by insulin pump therapy for three weeks reduced the exercise albuminuria response of patients with type 1 diabetes to that of nondiabetic controls [268]. An exercise albuminuria provocation test might reveal early signs of future renal involvement in patients with type 1 diabetes and normal UAER at rest [262, 263]. There are some data that support a prognostic value of exercise-induced albuminuria for the development of microalbuminuria in patients with normoalbuminuria, and it was even suggested that repeated albuminuria testing thus could be substituted by one single exercise test in patients with normoalbuminuria [269]. This is, however, a controversial issue as another study showed that the exercise albuminuria response do not predict new-onset microalbuminuria [270].

The physical activity of patients with type 1 diabetes and diabetic complications has not been extensively studied. A cross-sectional study in type 1 diabetes reported an association between lower physical activity and higher prevalence of diabetic neuropathy, retinopathy, and nephropathy [271]. The causal relationship between physical activity and complication status was, however, unclear due to the cross-sectional design. Higher level of past physical activity during adolescence was, interestingly, associated with lower prevalence of nephropathy and neuropathy in men, but not in women, with type 1 diabetes which could indicate a protective effect of physical activity on future risk of diabetic complications [271]. Mortality in patients with type 1 diabetes was in a longitudinal study inversely associated with physical activity even when controlling for the presence of diabetic complications [272]. Regarding diabetic complications there are no longitudinal studies, however premature mortality in type 1 diabetes is mainly attributable to the diabetic complications and therefore indirectly supports a protective effect of physical activity on development and/or progression of the diabetic complications. The hardest level of evidence for such a protective role would come from a physical activity intervention study with prospective assessment of the incidence of diabetic complications, but this is an laborious study setting requiring large number of patients undergoing intervention and extensive follow-up. There are some intervention studies on intermediary outcomes for diabetic complications. Endothelial dysfunction in the forearm of

patients with type 1 diabetes improved by 4 months of aerobic exercise training, and interestingly the vasodilatory response of the ocular vasculature also improved with theoretical implications for diabetic retinopathy [273].

2.4.3 PHYSICAL FITNESS

The aerobic capacity of patients with type 1 diabetes is either decreased [274, 275] or similar [276, 277] compared with healthy controls. There are no available studies regarding the relative strength fitness of patients with type 1 diabetes. The diabetic milieu could be thought to reduce the exercise capacity by changes in the cardiovascular, respiratory, muscular, and neural systems. Pulmonary changes due to diabetes, such as thickening of the alveolar wall, could impair the gas exchange during physical activity [278]. Diabetes could also affect the microcirculation of the skeletal muscle [279]. Diabetic complications decrease the exercise capacity. Patients with type 1 diabetes and micro- and macroalbuminuria have lower maximal exercise capacity as compared with patients with normal UAER [261]. Diabetic nephropathy and retinopathy in patients with type 2 diabetes was associated with reduced exercise capacity [280].

A higher aerobic capacity in patients with type 1 diabetes has been linked to better glycaemic control [276, 281] and to a more beneficial blood lipid profile [281]. A more recent study of consecutive patients at a diabetes outpatient clinic, on the other hand, reported that patients with higher aerobic capacity had higher HbA_{1c} while patients with higher strength capacity had lower HbA_{1c} [282].

2.4.4 GENETIC FACTORS AND RESPONSES TO PHYSICAL ACTIVITY

The magnitude of responses to physical training are individual and these differences at least partly depend on heritable factors [283]. VO_{2max}, for example, increase in response to training in a variable fashion with familial resemblance [284]. A genome-wide scan for the increase in VO_{2max} in previously sedentary healthy subjects during a physical activity intervention in the HERITAGE study revealed linkage to several genetic loci that are associated with the magnitude of improvement in aerobic fitness [285]. Some genes have been linked to responses in glucose metabolism, for instance the peroxisome proliferator-activated receptor γ (*PPARγ*) gene for which the Ala-allele of the functional Pro12Ala polymorphism has been associated with improvement of insulin sensitivity by physical training in nondiabetic subjects [286, 287] and lowering

of the fasting plasma glucose concentrations in patients with type 2 diabetes [288]. The knowledge about genes affecting training outcomes is rapidly increasing and yearly updates in this field are published [289].

3. AIMS OF THE STUDY

The role of physical activity and physical fitness for patients with type 1 diabetes has not been extensively studied and there is controversy regarding the connection with the long-term glycaemic control. The relationship between physical activity and the diabetic complications in patients with type 1 diabetes is also not well known, and especially the impact of early stages of diabetic nephropathy has gained little attention.

The main aims of the study were the following:

I To investigate the associations between diabetic complications and physical activity in patients with type 1 diabetes with special attention to the degree of involvement of renal, retinal, and cardiovascular complications.

II To evaluate the associations between physical activity and long-term glycaemic control (HbA_{1c}) in patients with type 1 diabetes

III To study the intrapersonal variability in HbA_{1c} in type 1 diabetes with respect to incidence of diabetic complications and physical activity.

IV To study the association between physical activity and the metabolic syndrome in patients with type 1 diabetes, and to assess the impact of the peroxisome proliferator-activated receptor γ (PPARγ) polymorphism.

V To compare the aerobic and strength capacities of patients with type 1 diabetes to that of healthy controls, and additionally to assess the associations between physical fitness and long-term glycaemic control.

4. SUBJECTS AND STUDY DESIGN

All patients included in this thesis are participants in the Finnish Diabetic Nephropathy (FinnDiane) Study. The subjects in study V are in addition participants in a substudy of the FinnDiane, called the IDentification of EArly mechanisms in the pathogenesis of diabetic Late complications (IDEAL) Study.

4.1 THE FINNDIANE STUDY

The FinnDiane Study was initiated in 1997 to answer an important clinical question: why do one third of patients with type 1 diabetes get diabetic nephropathy? The study uses a wide approach and is designed to identify clinical, genetic, environmental, and metabolic risk factors for diabetic late complications in patients with type 1 diabetes, with an emphasis on diabetic nephropathy. In addition, data on retinopathy and cardiovascular disease (CVD) are collected. The FinnDiane Study is a nationwide multicenter study with study centres distributed across Finland (Figure 2), which include all five university central hospitals, all 16 central hospitals, most (N=27) regional hospitals, as well as 31 primary health care centres. At the moment, about 4700 patients have been recruited. As the total number of patients with type 1 diabetes in Finland is about 30 000, a total of 16% of the Finnish type 1 diabetic population have been recruited to the FinnDiane Study.

Figure 2. Municipalities with FinnDiane Study centres in Finland.

The FinnDiane Study has a prospective design. Follow-up data has been collected since the year of 2004. The majority of the data in this thesis (studies I, II, and IV) report cross-sectional findings from the baseline visit. Study III take advantage of the prospectively collected data regarding development and progression of diabetic nephropathy and CVD. FinnDiane investigators reviewed medical files at the local study centres to verify possible occurrence of renal and cardiovascular outcomes during the follow-up time.

Type 1 diabetes was defined in all studies as age at onset of diabetes <35 years and permanent insulin treatment initiated within one year of diagnosis. In addition, in study II we required C-peptide negativity defined as <0.2 nmol/l. The age at onset has been shown to be a reliable discriminator between type 1 and type 2 diabetes in the Finnish population [290].

At baseline, patients in the FinnDiane Study fulfilling the abovementioned criteria for type 1 diabetes (N=3968) had a slight male predominance (51.2% men), mean age 37.8±11.5 years (range 18-78 years), duration of diabetes 23.0±12.0 years, BMI 25.0±3.5 kg/m^2, and HbA_{1c} 8.5±1.5%. Regarding the kidney disease status, 56.7% had normoalbuminuria, 12.5% had microalbuminuria, 14.5% had macroalbuminuria, and 6.9% had ESRD. In 9.4% of the patients the renal status could not be defined because of insufficient number of urine collections. Furthermore, 9.8% had CVD and 35.3% had laser-treated (proliferative) retinopathy.

The number of patients in studies I-IV varied due to several reasons. The major factor is the continuous recruitment of new patients as the study is ongoing. In study II the requirement of C-peptide negativity excluded also patients with missing values. The main outcomes in the studies also caused some differences in inclusion criteria, for instance the metabolic syndrome requires complete data on lipid profile, blood pressure, and obesity. Study III is prospective and includes all patients with data on renal status both at baseline and at follow-up, and available data on physical activity was not a prerequisite for inclusion in that study.

All patients in the FinnDiane study did not have data on physical activity. Of the patients that fulfilled the abovementioned criteria for type 1 diabetes, 1945 (49%) had data on physical activity. In Table 3, patients with and without data on physical activity are compared showing an overrepresentation of men and patients with diabetic nephropathy in patients with unavailable data, but no corresponding differences in age and BMI. The distribution of patients with microalbuminuria was also similar.

	Data available	Data unavailable	P-value
Patients (N)	1945	2023	-
Gender (% men)	47.8	54.2	<0.01
Age (yrs)	38.0±11.7	37.6±11.3	0.330
Duration of diabetes (yrs)	22.9±12.3	23.1±11.8	0.660
BMI (kg/m^2)	25.0±3.4	25.0±3.6	0.822
Microalbuminuria (%)	13.9	13.7	0.883
Macroalbuminuria (%)	12.9	18.7	<0.01
ESRD (%)	3.4	9.9	<0.01

Table 3. Characteristics for patients comparing the availability of data on physical activity in the FinnDiane Study.

4.2 THE IDEAL SUBSTUDY

In 2003, the IDEAL-substudy of the FinnDiane Study was launched to search for early markers for diabetic complications in patients with type 1 diabetes. The FinnDiane Study was not suitable for this purpose due to the long mean duration of diabetes in most patients. Therefore, there was a need to recruit patients with a short duration of type 1 diabetes. In addition, there was a desire for a true population-based cohort.

A query at the Finnish Social Insurance Institution, which is thought to have a registry covering 98% of all patients with type 1 diabetes in Finland [291], identified 400 patients with type 1 diabetes (code E10 in the ICD-10 classification) living in the Helsinki area with an age of 18 to 35 years and duration of diabetes 6 to 12 years. The high completeness of the registry is facilitated by full reimbursement for insulin for patients with type 1 diabetes. Of these, 165 patients responded and were willing to participate. After excluding pregnant women and using a tight definition of type 1 diabetes (unstimulated C-peptide <0.3 nmol/l and insulin treatment within one year of diabetes diagnosis), the final number of patients were 140 and constituted the IDEAL cohort. At recruitment, the patients were 54.1% men with mean age 27.1±5.7 years, duration of diabetes 9.6±4.3 years, BMI 24.9±4.2 kg/m^2, and HbA_{1c} 8.1±1.3%. No selection was made with respect to diabetic complication status, and due to the short duration of diabetes most patients were free from complications. Accordingly, of the 140 patients at baseline only three had microalbuminuria and one had macroalbuminuria, and none had clinically evident CVD.

The IDEAL Study has a prospective study design. The patients undergo a vast amount of testing for cardiac autonomous neuropathy, ambulatory blood pressure

measurements, and echocardiography. In addition, physical fitness is assessed by spiroergometry. The development of diabetic complications is followed up by quarterly performed urine collections and yearly fundus photographs of the retinas.

4.3 ETHICS ASPECTS

The FinnDiane Study and the IDEAL substudy are purely observational, and thus no interventions which could be harmful are performed. The patients give written informed consent to participate. The major disadvantage for the participants is the time consumed by the study visit and completing questionnaires, as well as possible pain from venapuncture when drawing blood samples. For the patients with diabetes, an overnight fast was not required prior to blood sampling. The research protocol was in accordance with the Declaration of Helsinki (as revised in the year 2000) and approved by the local ethical committees of the participating study centres.

5. METHODS

5.1 FINNDIANE STUDY PROTOCOL

5.1.1 PATIENT RECRUITMENT

In the FinnDiane Study, patients were recruited by nurses and physicians at general, diabetes, and renal outpatient clinics at the local study centres. As sole inclusion criterion type 1 diabetes was required (code E10 in the ICD-10 classification), with no prerequisite regarding renal disease or other diabetic complications.

5.1.2 MEDICAL HISTORY

The physician at the study centre completed, based on the patient´s medical records, a standardised check-list regarding medication, year of onset of diabetes and year of initiation of permanent insulin treatment. Renal status was categorised as microalbuminuria, macroalbuminuria, or ESRD (dialysis or kidney transplantation) and retinopathy status as any retinopathy or retinal laser photocoagulation. CVD was assessed as diagnosis of coronary heart disease as well as occurrence of any hard CVD end-point: myocardial infarction, coronary artery by-pass surgery or angioplasty, stroke (ischaemic or haemorrhagic), amputations in the lower limbs, and peripheral artery by-pass surgery or angioplasty.

5.1.3 ANTHROPOMETRIC MEASUREMENTS

Weight and height were measured with the patient wearing light clothing. Waist circumference was measured halfway between the lowest ribs and the iliac crest. Hip circumference was measured at the major trochanters of the femurs. Blood pressure was measured twice after a 10 minute of rest from the brachial artery in a sitting position using a manual sphygmomanometer or an automated blood pressure measurement device in accordance to the clinical practice at the local study centres.

5.1.4 LIFESTYLE AND SOCIOECONOMIC FACTORS

The patients completed a self-report questionnaire regarding current and previous smoking, as well as dose (cigarettes, pipes or cigars per day) and duration (years) of smoking. Current alcohol consumption was asked as average weekly amount of beer (as 33 cl bottle), wine (as glass), or strong spirits (as 10 cl). Level of education and the current occupation were asked, and in case of retirement whether it was due to disability pension and in that case also the underlying diagnosis. Possible current unemployment and its duration were also reported. The patients were categorised regarding their socioeconomic status as: unskilled/skilled blue collar workers, lower/upper white collar, farmers, and others.

5.1.5 LABORATORY MEASUREMENTS AND ASSAYS

Blood was drawn and one 24h urine collection was performed for central laboratory measurements. In addition, from the local study centres the most recent HbA_{1c} value and lipid profile (total-, HDL-, LDL-cholesterol, and triglycerides) were obtained, as well as the three most recent values for UAER in either overnight or 24h urine collections. HbA_{1c} was measured with a high performance liquid chromatography (HPLC) method. In study III, HbA_{1c} measurements by standardised assays from the local study centres were also used. C-peptide was measured with radioimmunoassay (RIA). The serum lipids were measured in Prof. Marja-Riitta Taskinen´s laboratory at the University of Helsinki, Division of Cardiology, by automated enzymatic methods with a Cobas Mira analyser. The LDL cholesterol was derived by the Friedewald formula [292]. Serum creatinine was measured with a kinetic Jaffé reaction until 7th January 2002, thereafter by a photometric, enzymatic method (Hitachi 917 or Modular analyser, Boehring Mannheim/Roche Diagnostics, Basel, Switzerland). Urinary albumin concentration was measured with RIA until 1st of November 2002, thereafter by immunoturbidimetry. Insulin sensitivity was estimated by a formula for estimated glucose disposal rate (eGDR) originally developed for patients with type 1 diabetes based on insulin clamp measurements [293]. The formula was modified for the use of HbA_{1c}, not HbA_1:

$$(1) \qquad eGDR = 24.4 - 12.97(WHR) - 3.39(hypertension) - 0.60(HbA_{1c})$$

where hypertension is designated 1 if blood pressure ≥140/90 or antihypertensive medication, otherwise 0. HbA_{1c} is given as %. WHR: waist-to-hip ratio.

5.1.6 GENETIC ANALYSES

In study IV, genetic data for the *PPARγ* Pro12Ala polymorphism is presented. Genomic DNA was extracted from leukocytes using Gentra Systems Puregene DNA isolation kits (Gentra Systems, Minneapolis, MN, USA). In some of the older samples, the standard phenol extraction method was used in order to get a better DNA yield. Extracted DNA was stored at −20°C until required for genotyping. The Pro12Ala (rs1801282) polymorphism of the *PPARγ* gene was genotyped using fluorogenic 5´ nuclease allelic discrimination chemistry (TaqMan®) with an ABI Prism® 7900 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The assay mix containing primers and probes was designed by applied Biosystems. The genotyping success rate was 99%. All duplicated samples were coherent and the marker was in Hardy-Weinberg equilibrium.

5.2 ASSESSMENT OF PHYSICAL ACTIVITY

Leisure-time physical activity (LTPA) was assessed by a self-report questionnaire. The validity and reproducibility of the questionnaire has previously been described [294, 295]. This questionnaire was originally developed from the Minnesota Leisure Time Activity Questionnaire [296] — which is validated against doubly labelled water [215] — to the Finnish setting for use in the Kuopio Ischemic Heart Disease Risk Factor Study (KIHD) [297]. The Finnish conversion had been based on results regarding physical activity from the Mini-Finland survey [298] to ensure appropriate types of activities in the questionnaire due to possible cultural and climate-related differences between Finland and USA. The questionnaire, called *12-month KIHD Leisure-Time Physical Activity Questionnaire*, contains a section of questions of general type, frequency, duration, and intensity of LTPA. Additionally, a second section asks specific details on frequency (times per month), duration per session, and intensity (graded 0-3) for 21 types of predefined activities retrospectively from the past 12 months. The intensity grading was defined as follows: 0: no shortness of breath, no sweating; 1: slight shortness of breath, no sweating; 2: shortness of breath, slight sweating; 3: heavy shortness of breath, profuse sweating.

Based on intensity level, each of the 21 activities can be assigned a specific metabolic equivalent (MET) value. MET is a widely used unit for energy expenditure both in exercise practice and in research and is defined as multiples of the energy expenditure at rest, which approximates an oxygen consumption of 3.5 ml/kg/min or about 1 kcal/kg/h [299]. For instance, light walking is assigned 3 MET, brisk walking 5-7 MET, and moderate jogging 8-10 MET. The use MET- instead of kcal-based energy expenditure has the great advantage of being independent of gender, body mass, and age because MET always relates to the resting energy expenditure.

The amount of LTPA is usually expressed as MET during a time-period, and in this thesis the total amount of LTPA is given as MET hours per week (MET*h/week). For quantification of physical activity, MET per time unit has the advantage of being a continuous variable, thus with more statistical power than for example the frequency or intensity categorical variables in the KIHD 12-month questionnaire.

In this thesis, the patients were grouped based on their LTPA into *sedentary* (<10 MET*h/week), *moderately active* (10-40 MET*h/week), and *active* (>40 MET*h/week). The cutoff value chosen to represent a sedentary lifestyle was defined as LTPA lower than the minimum given by international recommendations [300], that is moderate physical activity (about 5 MET) for 30 minutes on most days of the week (four days), which equals 10 MET*h/week.

5.3 MEASUREMENT OF PHYSICAL FITNESS

5.3.1 PRE-PARTICIPATION SCREENING FOR EXERCISE TESTING

The participants reported to the laboratory approximately 2 hours post meal consumption with normal medication, and after at least 24 hours without alcohol ingestion and ≥12 h after physical exercise. They filled and signed the Physical Activity Readiness Questionnaire to reveal possible contraindications to exercise. A 12-lead ECG in a supine position, height, weight, waist and hip circumference were measured. The lung function was determined by flow-volume spirometry testing (Medikro Spiro 2000, Medikro, Kuopio, Finland). A physician confirmed the patient´s suitability for exercise testing. A fingertip capillary blood sample was analyzed before the exercise test to maintain blood glucose concentration within an acceptable range (5.6-16.7 mmol/l) for exercise, in accordance to guidelines [176]. In case of lower blood glucose the subjects were given oral glucose supplementation, and in case of hyperglycaemia the subjects injected the insulin they routinely use. Blood glucose measurements were repeated until within acceptable range. A blood glucose measurement was repeated 10 minutes after exercise. Diabetic complication status was not an exclusion criterion because none of the patients had overt diabetic nephropathy or cardiovascular disease in this young population with short duration of type 1 diabetes.

5.3.2 SPIROERGOMETRY PROTOCOL

The aerobic physical fitness level of the patients was measured with spiroergometry, which is a clinical exercise tolerance test with simultaneous respiratory gas measurements. The cycle ergometer (Monark 839 E, Monark Exercise AB, Vansbro, Sweden) exercise test was performed with a step incremental protocol. The test began with a 3 min rest while sitting on the cycle ergometer. Then, the subjects began with 5 min baseline unloaded cycling, after which the step incremental exercise protocol was initiated (30 W/3 min for women and 40 W/3 min for men), and the subjects continued exercising until volitional fatigue. The criterion for fatigue was inability to maintain pedalling cadence at the minimum of 60 rpm. The immediate recovery of the subjects after cessation of the test was followed for 3 min during unloaded cycling.

Heart rate (HR) and ECG were continuously monitored by a 12-lead (Marquette Max-1, Marquette Electronics, Milwaukee, WI, USA). Arterial blood pressure (BP) was measured by sphygmomanometer from the brachial artery at rest, at the end of each work rate and after the 3-minute recovery period. Ventilation ($\dot{V}E$) and alveolar gas exchange were measured breath-by-breath throughout the test protocol. The subjects breathed through a facemask (Hans Rudolph Inc., Kansas City, MO, USA) connected to a low-deadspace (30 ml) low-resistance turbine (Triple V, Jaeger Mijnhardt, Bunnik, The Netherlands) for measurement of inspiratory and expiratory volumes and flow. The turbine was calibrated before each test by using a syringe of known volume (3.00 L, Hans Rudolph Inc., Kansas City, MO, USA). Inspired and expired gases were sampled continuously at the mouth and analyzed for concentrations of O_2, CO_2, N_2, and Ar by mass spectrometry (AMIS 2000, Innovision A/S, Odense, Denmark) after calibration with precision analyzed gas mixtures. Raw data were transferred to a computer, which aligned concentrations with volume data to build a profile of each breath. Breath-by-breath alveolar gas exchange was calculated by using the AMIS algorithms. The alveolar gas exchange data were interpolated to give values in 1 s intervals. The averaged values of the last 30 s at every work rate were chosen to represent a given work rate. Maximal aerobic capacity ($\dot{V}O_{2max}$) was determined as the highest value for a moving 60 s "window". Anaerobic threshold (AT) and respiratory compensation point (RCP) were determined with the V-slope method. To determine ventilatory efficiency, $\dot{V}E$ was plotted against CO_2 production ($\dot{V}CO_2$) and a linear regression slope was calculated for this ratio from rest up to RCP and to peak exercise.

5.3.3 MUSCLE STRENGTH TESTING

Hand-grip strength was tested twice from both hands by a hydraulic hand dynamometer (Baseline, Irvington, NY, USA), and the highest value was used.

Lower-limb strength was tested by three squat jumps and three counter movement jumps. In the squat jump, the jumper started from a stationary semi-squatted position and jumped as high as possible. In the countermovement jump, the subject did employ a preliminary downward phase (a countermovement) and so the jump involved pre-stretching of muscles. The maximal height reached during the jump was measured three times by an electronic contact mat (Newtest, Oulu, Finland), and the highest value was used.

5.4 STATISTICAL ANALYSES

All statistical calculations were performed with the SPSS statistical package for Windows. When comparing groups, ANOVA or Student´s t-test were used in case of normally distributed variables, otherwise the Kruskal-Wallis or Mann-Whitney´s tests were used. Continuous variables were correlated with the Pearson´s coefficient if normally distributed, otherwise the Spearman coefficient was used. Dispersion was reported as standard deviations (SD) for normally distributed variables, otherwise interquartile range (IQR) was given. Multivariate analyses in cross-sectional studies were performed by multiple logistic regression when the dependent variable was dichotomous and multiple linear regression for continuous dependent variables. In longitudinal data, Cox proportional hazard survival regression was used as multivariate analysis.

6. RESULTS

6.1 STUDY I: PHYSICAL ACTIVITY AND DIABETIC COMPLICATIONS

6.1.1 DIABETIC NEPHROPATHY

The total amount of leisure-time physical activity in patients with normo- vs. micro- vs. macroalbuminuria was (median, IQR) 20.3, 10.5-35.1 vs. 19.9, 9.4-39.7 vs. 18.0, 7.9-31.7 MET*h/week (overall P=NS). The proportion of patients being sedentary was, however, higher in patients with macroalbuminuria compared with normal UAER (Figure 3). For patients with ESRD the results were largely dependent upon treatment modality and renal function. Patients on dialysis had LTPA 14.5 (2.6-44.3) MET*h/week, while those patients who had received a renal transplant and had normal renal function (serum creatinine <150 μmol/l) had LTPA 21.9, 14.9-54.9 MET*h/week (P=NS vs. normoalbuminuria). For renal function estimated with the Cockcroft-Gault formula there were no statistically significant differences in total LTPA between groups with eGFR ≥90, 60-89, 30-59, and ≤30 ml/min/1.73m^2. The Pearson´s correlation coefficients between LTPA and UAER was -0.03 (P=0.247) and LTPA and eGFR was 0.04 (P=0.120). Age- and gender-stratification of the association between total LTPA and albuminuria revealed that patients <40 years of age and with macroalbuminuria had lower LTPA compared with those with normal UAER (Figure 3). Men, but not women, with macroalbuminuria had similarly reduced LTPA (figure 3). Participation in competitive sports, neither previous nor present was associated with albuminuria status.

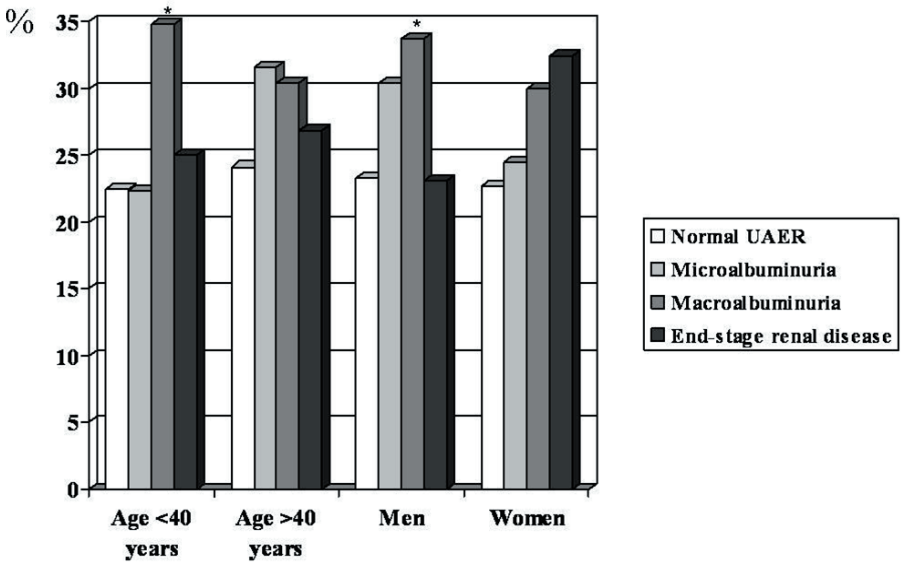

Figure 3. Proportion (%) of sedentary patients (LTPA <10 MET*h/week). *P<0.05 vs. normal UAER.

Despite surprisingly minute differences in total LTPA according to albuminuria status and renal function there were, however, differences in the composition of LTPA. Intensity was the component of LTPA most clearly associated with albuminuria status (Figure 4). Patients with macroalbuminuria, ESRD and interestingly also microalbuminuria, performed LTPA of low intensity more frequently than patients with normal UAER (30.5 vs. 21.4%, P=0.047 adjusted for age and sex for microalbuminuria).

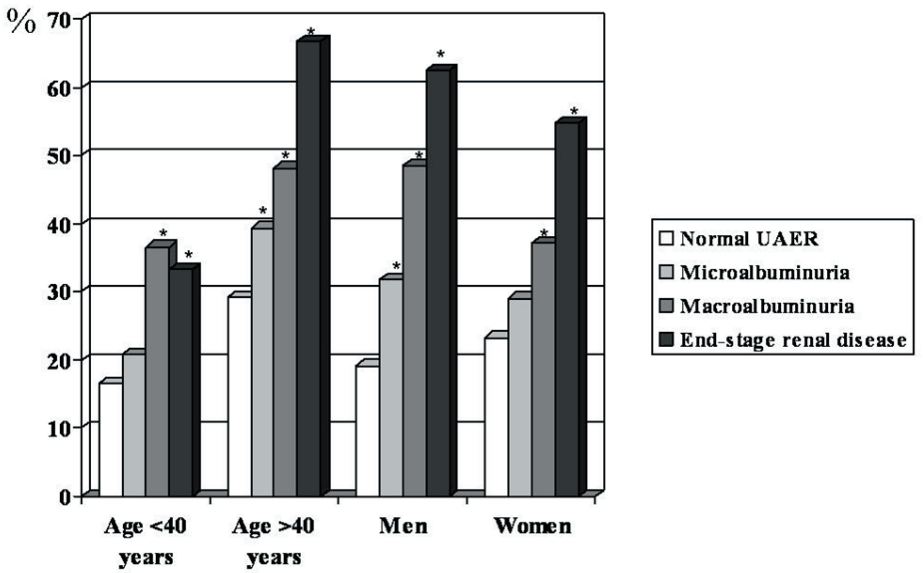

Figure 4. Proportion (%) of patients with low intensity LTPA.*P<0.05 vs. normal UAER.

Multiple regression models were performed to adjust for possible confounding factors (duration of diabetes, sex, and BMI) for the associations between LTPA and diabetic nephropathy. Low frequency (<1 session per week) LTPA (odds ratio, 95% CI: 1.88, 1.37-2.57) and low intensity LTPA (2.29, 1.71-3.07), but not total LTPA (0.89, 0.77-1.03), were independently associated with diabetic nephropathy.

6.1.2 DIABETIC RETINOPATHY

Total LTPA for patients without vs. background vs. proliferative retinopathy was 19.7, 11.0-35.6 vs. 18.9, 9.5-34.3 vs. 19.8, 8.5-35.6 MET*h/week, respectively (overall P=NS). Patients with proliferative retinopathy were, however, more frequently sedentary than patients without retinopathy (27.9% vs. 21.7%, P<0.01). The composition of LTPA was, as for nephropathy, divergent as patients with background and those with proliferative retinopathy performed LTPA of low intensity and low frequency more commonly than did patients with no retinopathy. Low intensity LTPA was independently associated with proliferative retinopathy (1.49, 1.15-1.93) in a multiple regression model correcting for duration of diabetes, sex, and BMI.

6.1.3 CVD

Patients with vs. without CVD had total LTPA 18.0, 7.7-36.4 vs. 19.7, 10.2-35.5 MET*h/week (P=NS). Low intensity LTPA was more common in patients with CVD than without (56.4 vs. 24.1%, P<0.01). Frequency of LTPA was not associated with prevalence of CVD. Low intensity LTPA was independently associated with CVD (2.58, 1.78-3.73) in a multiple regression model correcting for duration of diabetes, sex, and BMI.

6.2 STUDY II: PHYSICAL ACTIVITY AND GLYCAEMIC CONTROL

LTPA was associated with HbA_{1c} but this association was, however, found only in women (Figure 5). Sedentary patients had higher HbA_{1c} compared with moderately active patients. HbA_{1c} was, however, similar in moderately active and active patients. The Spearman´s correlation coefficients between total LTPA and HbA_{1c} were r=-0.12 (P=0.007) in women and r=-0.03 (P=NS) in men. Insulin dose (Figure 6) and eGDR (Figure 7), an estimate of insulin sensitivity was also inversely associated with LTPA.

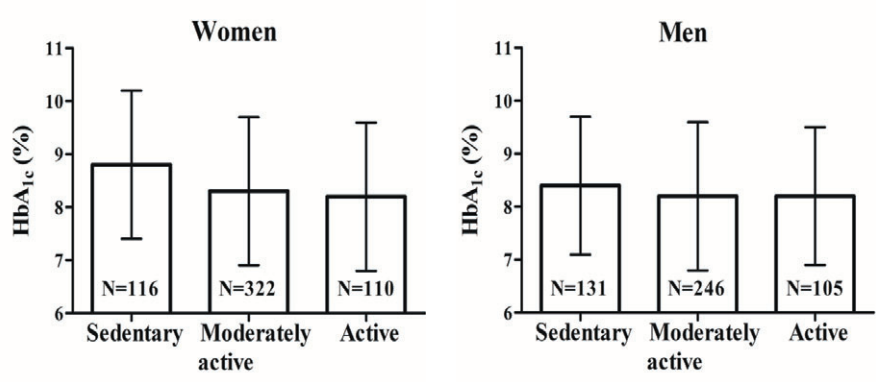

Figure 5. HbA_{1c} and physical activity in patients with type 1 diabetes. Error bars represent SD:s.

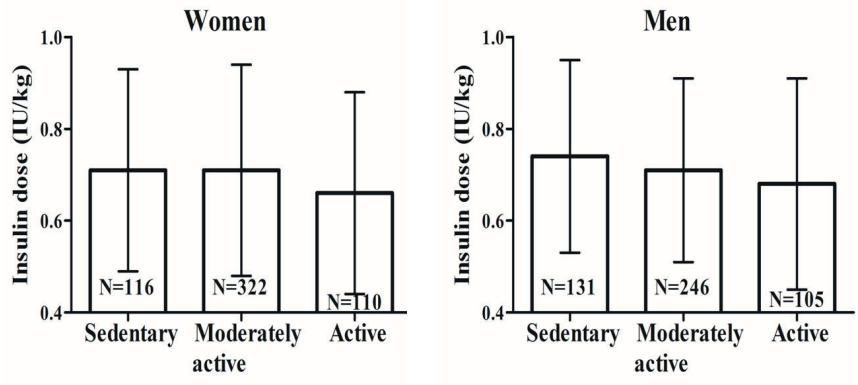

Figure 6. Insulin dose per kg body weight per day and physical activity in patients with type 1 diabetes. Error bars represent SD:s.

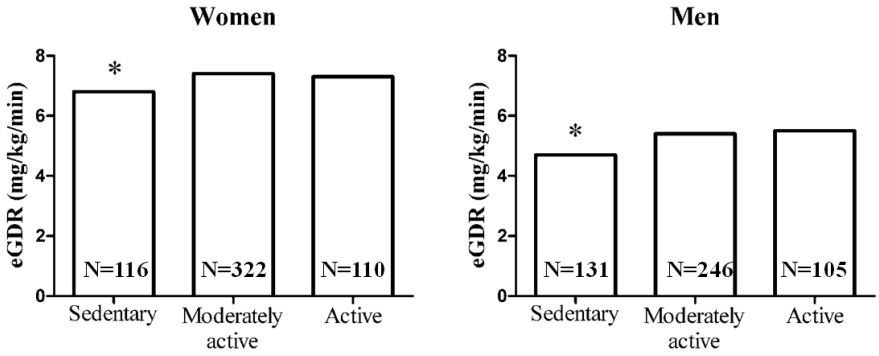

Figure 7. Estimated glucose disposal rate (eGDR)and physical activity in patients with type 1 diabetes. Values are medians. *P<0.05 vs. active.

The diabetic complication status of the patients could potentially affect the associations between HbA_{1c} and LTPA. A subanalysis was performed only with patients free from diabetic complications (UAER <30 mg/24h, no CVD, N=624). Hereby, sedentary vs. moderately active vs. active women without complications had HbA_{1c} 8.7±1.4 vs. 8.2±1.3 vs. 8.2±1.3% (P=0.042), respectively. In men without complications corresponding values were 8.3±1.3 vs. 8.1±1.4 vs. 8.1±1.2% (P=0.659), respectively. Components of LTPA were also associated with HbA_{1c} (Table 4). There were, moreover, gender differences in the components of LTPA. Men more often performed high intensity LTPA than women, which in turn performed LTPA with higher frequency (Table 4).

Intensity	**Low**	**Moderate**	**High**	**P-value**
Women	8.8±1.4 (21%)	8.1±1.2 (66%)	8.4±1.7 (13%)	0.012
Men	8.1±0.9 (21%)	8.2±1.4 (43%)	8.2±1.5 (36%)	0.724
Frequency	**<1 time/ week**	**1-2 times/ week**	**>2 times/ week**	
Women	9.0±1.4 (16%)	8.4±1.3 (23%)	8.2±1.3 (61%)	0.008
Men	8.3±1.0 (26%)	8.3±1.1 (30%)	8.1±1.7 (44%)	0.425
Duration	**<3 h/week**	**3-6 h/week**	**>6 h/week**	
Women	8.5±1.4 (36%)	8.3±1.4 (31%)	8.1±1.3 (33%)	0.156
Men	8.3±1.3 (42%)	8.1±1.5 (32%)	8.1±1.2 (26%)	0.173

Table 4. HbA_{1c} by components of LTPA, patients without complications (N=624). Percentage values in brackets represent distribution of corresponding level of component of LTPA within each gender. P-values were calculated by ANOVA.

To further explore the impact of confounding factors, a multiple logistic regression model was performed correcting for corrected for age, duration of diabetes, social class, obesity, blood pressure, serum cholesterol, diabetic nephropathy, CVD, and laser-treated retinopathy. Hereby, poor glycaemic control (HbA_{1c} >8.5%) as dependent variable. A sedentary level of LTPA (OR, 95% CI: 2.07, 1.18-3-62), insulin dose (4.82, 1.31-17.79), and smoking (2.01, 1.11-3.63) were independently associated with poor glycaemic control in women with type 1 diabetes.

6.3 STUDY III: HBA_{1C} VARIABILITY, RISK OF DIABETIC COMPLICATIONS, AND PHYSICAL ACTIVITY

Data on HbA_{1c} variability and renal status at follow-up were available for 2107 patients, and 1845 patients had data on CVD events. The median follow-up time was 5.7 years during which 214 patients progressed in renal disease status (to a higher albuminuria status or to ESRD) and 158 patients had a CVD event. The median number of serial HbA_{1c} measurements per patients was 13. The intrapersonal mean of serial HbA_{1c} was 8.5% and SD 0.78. As measures of HbA_{1c} variability, the intrapersonal SD (standard deviation) and CV (coefficient of variation) were used.

In Table 5, the mean, SD, and CV of serial HbA_{1c} measurements are given according to state of progression in renal disease and CVD events. The intrapersonal means of HbA_{1c} were higher in patients that developed microalbuminuria and macroalbuminuria, but not ESRD. Furthermore, the intrapersonal HbA_{1c} variability was higher in all subgroups of patients that progressed in renal status as both SD and CV were higher in the progressors. Patients that suffered a CVD event did not differ in mean serial HbA_{1c} from those that did not have a CVD event. The variability of HbA_{1c}, on the other hand, was higher in those with a CVD event during follow-up.

	Non-progressors			**Progressors**		
	Mean	**SD**	**CV**	**Mean**	**SD**	**CV**
Renal status						
All	8.5	0.76	8.9	9.2	1.01	11.0
Normo -> Micro	8.3	0.74	8.9	9.2	0.94	10.1
Micro -> Macro	8.8	0.77	8.7	9.6	1.08	11.2
Macro -> ESRD	9.0*	0.84	9.2	8.8	1.07	12.0
CVD event	8.5*	0.79	9.1	8.6	0.87	10.0

Table 5. Intrapersonal mean, SD, and CV of serially measured HbA_{1c} according to progression in renal disease and occurrence of a CVD event during follow-up. *P>0.05. All other values P<0.05 within each group of progression status.

In Figure 8, Kaplan-Meier survival curves for quartiles of SD of serial HbA_{1c} show a clear association between SD and any progression in renal status. For CVD events, the corresponding association was less evident as only the highest quartile diverged from the rest.

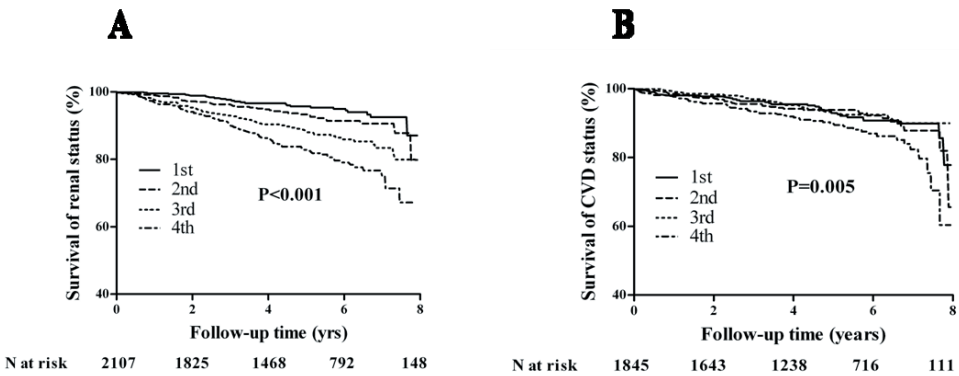

Figure 8. Kaplan-Meier survival cures for (A) any progression in renal status and (B) a CVD event during follow-up with respect to quartiles of variability (SD) of serially measured HbA_{1c}.

Figure 9 shows Kaplan-Meier survival curves which take both the mean value and the variability of serial HbA_{1c} into consideration. As can be expected those patients that had low HbA_{1c} with low variability, that is below the population median for both mean and SD, had the lowest risk for progression in renal status (fig. 9A). Both high mean and high SD of HbA_{1c} (above the population medians) was accordingly associated with the highest risk of any progression in renal status. Patients with high mean but low variability of serial HbA_{1c}, notably, had the same risk for progression in renal status as did patients that had low mean but high variability of HbA_{1c}. Corresponding analyses for any CVD event showed no separation in Kaplan-Meier survival cure analyses (Figure 9B).

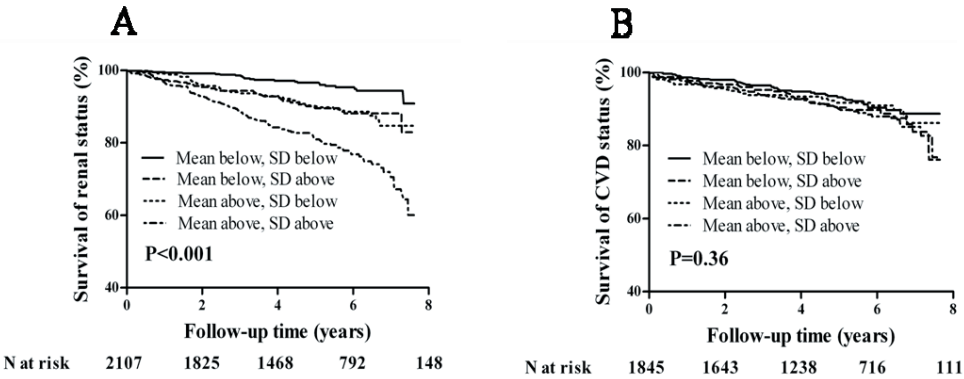

Figure 9. Kaplan-Meier survival cures for (A) any progression in renal status and (B) a CVD event during follow-up with respect to mean and variability (SD) of serially measured HbA_{1c} above and below the population medians.

The association between the variability of HbA_{1c} and risk of diabetic complications could be due to several confounding factors. Therefore, multivariate Cox proportional hazard survival regression models were undertaken to adjust for some clinically

relevant potential confounders (Table 6). The variability of HbA_{1c} was independently associated with progression in renal status and with a CVD event during follow-up even when controlling for the mean HbA_{1c} and the number of HbA_{1c} measurements. For CVD events, interestingly, the mean of serial HbA_{1c} was not an independent predictor of events.

	Renal status	**CVD event**
Duration of diabetes (years)	1.01 (0.99-1.02)	1.08 (1.06-1.10)
Male gender	1.74 (1.30-2.33)	1.17 (0.81-1.70)
Systolic blood pressure (mmHg)	1.01 (1.00-1.02)	1.02 (1.01-1.03)
Total cholesterol (mmol/l)	1.19 (1.04-1.36)	1.11 (0.92-1.34)
Ever smoking	1.22 (0.92-1.63)	1.00 (0.70-1.43)
Diabetic nephropathy	-	1.78 (1.20-2.64)
CVD event	-	3.11 (2.10-4.59)
Number of serial HbA_{1c} measurements	1.09 (0.90-1.31)	0.88 (0.71-1.09)
Intra-personal mean of serial HbA_{1c} (%)	1.34 (1.20-1.51)	1.01 (0.87-1.18)
Intra-personal SD of serial HbA_{1c}	1.92 (1.49-2.47)	1.98 (1.39-2.82)

Table 6. Multivariate Cox regression model for any progression in renal status and for any CVD event during follow-up. Values are given as HR (95% CI).

To further characterise the associations with the HbA_{1c} variability we investigated baseline lifestyle factors in comparison with quartiles of SD of serial HbA_{1c}. Comparing the lowest with the highest quartile of SD, a higher prevalence of smoking (17.5 vs. 33.1%, $P<0.01$) and low social class (60.7 vs. 76.2%) were found. A sedentary level of LTPA was also associated with higher HbA_{1c} variability (Figure 10).

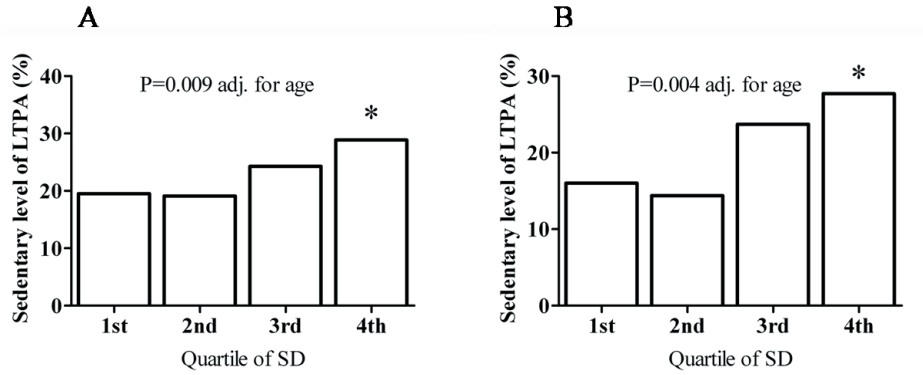

Figure 10. Variability (SD) of serial HbA_{1c} compared with the baseline physical activity level in (A) all patients (N=775) with data on LTPA and (B) only patients (N=534) with normoalbuminuria.*P<0.05 vs. 1st quartile.

In the 775 patients with data on LTPA, the proportion of patients that progressed in renal status in sedentary vs. moderately active vs. active patients were 9.2% vs. 8.1% vs. 5.0% (P=0.127 for trend). Only looking at progression from normo- to microalbuminuria, the corresponding proportions were 5.8% vs. 6.7% vs. 3.4% (P=0.435). For any CVD event, the proportions were 9.9% vs. 5.2% vs. 5.8% (P=0.122), respective.

6.4. STUDY IV: THE METABOLIC SYNDROME IN TYPE 1 DIABETES AND PHYSICAL ACTIVITY; EFFECT OF THE PPARγ PRO12ALA POLYMORPHISM

The NCEP/ATPIII criteria for the metabolic syndrome were fulfilled in 27.8% of the men and in 31.0% of the women. There was no difference in prevalence of the metabolic syndrome in subjects with various genotypes of *PPARγ* Pro12Ala polymorphism.

LTPA (median, IQR) in patients with the metabolic syndrome was 17.0, 8.6-31.6 MET*h/week while being 20.8, 10.8-34.7 MET*h/week in patients not fulfilling the criteria (P=0.038 adj. for age). Figure 11 shows the proportions of patients with the metabolic syndrome with respect to total level, intensity, and frequency of LTPA. The *PPARγ* Pro12Ala polymorphism, interestingly, affected the association between LTPA and the metabolic syndrome since the association was seen only in carriers of the Ala-allele (Figure 11D).

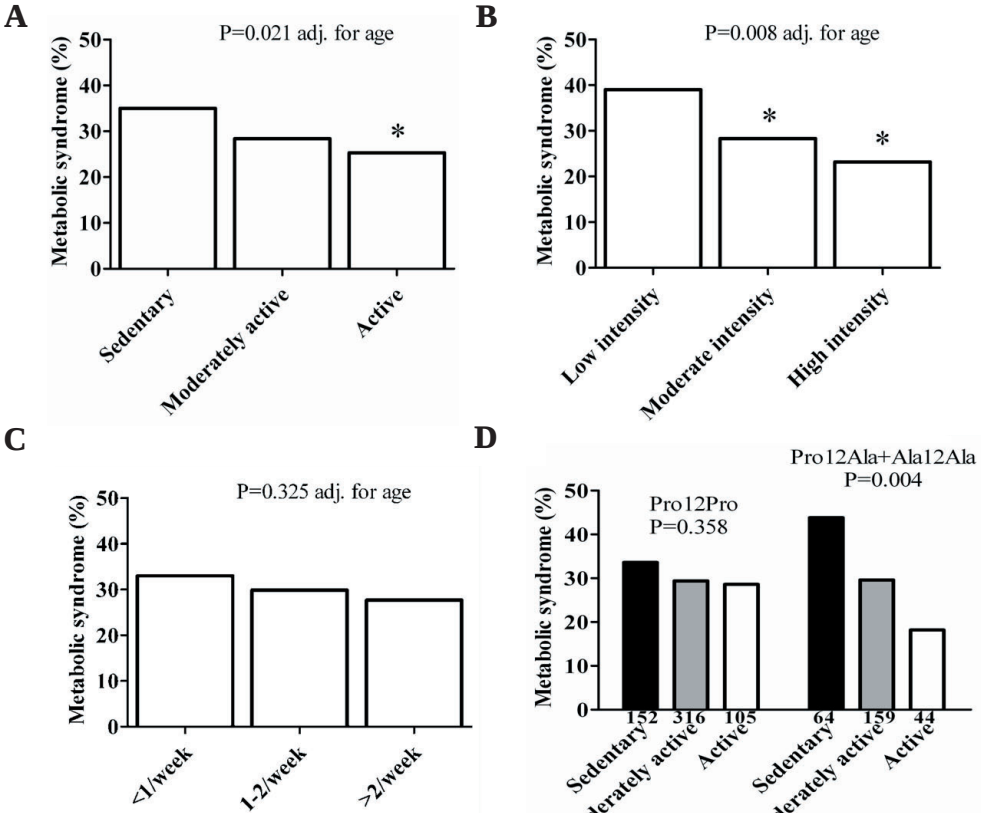

Figure 11. Prevalence of the metabolic syndrome according to (A) total LTPA, (B) intensity of LTPA, and (C) frequency of LTPA. Figure (D) shows total LTPA according to PPARγ Pro12Ala genotype. *P<0.05 vs. sedentary or low intensity.

6.5. STUDY V: PHYSICAL FITNESS AND GLYCAEMIC CONTROL

There were no differences in gender distribution, age, BMI, and smoking between patients with type 1 diabetes and nondiabetic controls (Table 7). One patient had microalbuminuria, but all were free from clinically evident CVD.

	Type 1 diabetes	Controls
Number of patients	86	27
Gender (% men)	54.7	55.6
Age (years)	28.6±5.2	28.2±6.1
Duration of diabetes (years)	11.5±1.8	-
HbA_{1c} (%)	7.4±0.9	-
BMI (kg/m^2)	25.1±3.5	23.8±3.3
Resting systolic BP (mmHg)	124±14	123±14
Resting diastolic BP (mmHg)	75±10	72±11
Resting heart rate (bpm)	70±11	66±15
Antihypertensive medication (%)	9.4	0
Current smoker (%)	17.4	22.2
Physical activity (MET*h/week)	17.2 (11.1-26.0)	28.8 (22.3-45.6)*

Table 7. Clinical characteristics for patients with type 1 diabetes and nondiabetic controls.*P<0.05 vs. patients with diabetes. Values are given as N, mean3SD, median (IQR), or %.

$\dot{V}O_{2max}$ was lower in patients with type 1 diabetes compared with controls (Figure 11). Ten patients, all with diabetes, terminated the exercise test prematurely but exclusion of these patients did not have a major impact on the association. Both the respiratory compensation point (RCP) and the anaerobic threshold (AT) were additionally lower in patients with diabetes compared with controls. Women with diabetes had especially low indicators of aerobic fitness as the AT was at a level below the oxygen consumption corresponding to brisk walking (Figure 12, dotted line).

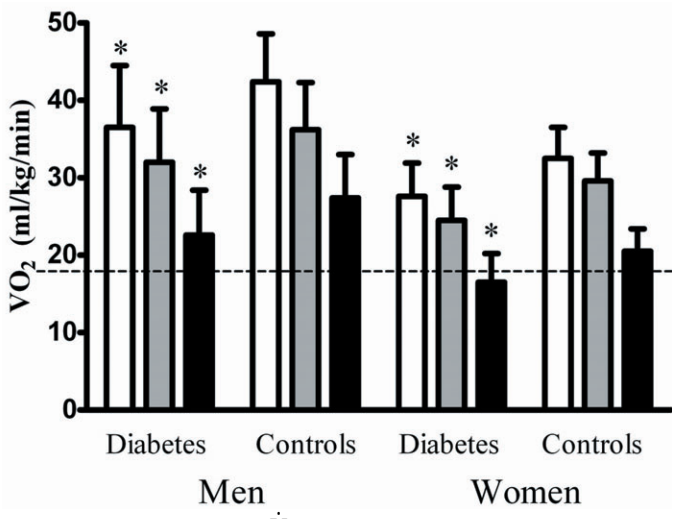

Figure 12. White bar: fatigue ($\dot{V}O_{2max}$). Grey bar: respiratory compensation point. Black bar: anaerobic threshold. Error bars: standard deviations. Dotted horizontal line: the oxygen consumption (18 ml/kg/min) corresponding to brisk walking. *P<0.05 vs. controls within the same gender.

Handgrip strength was similar in patients with diabetes and in controls. Lower extremity strength output was, however, lower in women with diabetes vs. controls due to lower height of both the squat jump (19±4 vs. 24±4 cm, *P*=0.001) and of the countermovement jump (21±5 vs. 25±5 cm, *P*=0.008). Lower limb strength was similar in men with diabetes and controls.

HbA_{1c} correlated negatively with $\dot{V}O_{2max}$ in men, but not in women, with diabetes (Figure 13). In women, the dominant hand grip strength showed a negative correlation with HbA_{1c} (Table 8), while lower limb strength correlated with HbA_{1c} in men.

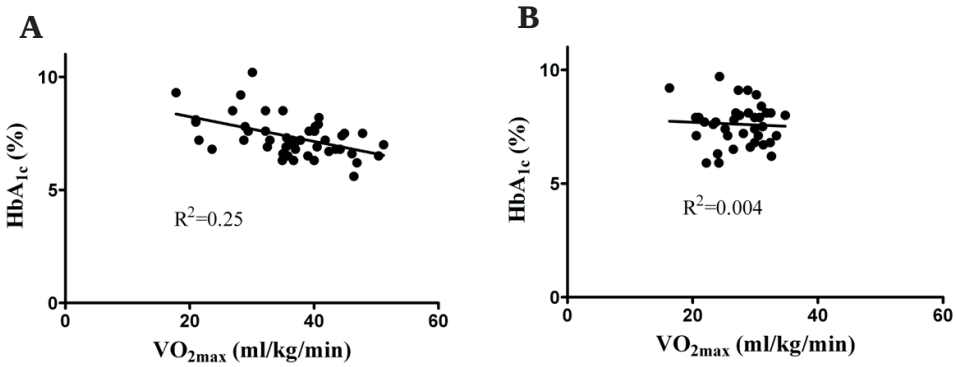

Figure 13. Scatterplot of HbA_{1c} and $\dot{V}O_{2max}$ in (A) men and (B) women with type 1 diabetes.

	Men	Women
$\dot{V}O_{2max}$	-0.50**	-0.06
Height of squat jump	-0.33*	0.10
Height of countermovement jump	-0.36*	-0.06
Dominant hand grip strength	-0.28	-0.02
Non-dominant hand grip strength	-0.26	-0.41*

Table 8. Correlation coefficients between HbA_{1c} and measures of aerobic and strength fitness in patients with type 1 diabetes.*P<0.05.**P<0.01.

To control for potential confounding variables for the association between HbA_{1c} and $\dot{V}O_{2max}$, we performed a multiple linear regression model. HbA_{1c} was chosen as the dependent variable, while the pre-test blood glucose concentration, age, BMI, and $\dot{V}O_{2max}$ were independent variables. In men, but not in women, $\dot{V}O_{2max}$ (standardized coefficient β=-0.64, *P*<0.001) was independently associated with HbA_{1c}.

7. DISCUSSION

7.1 STUDY DESIGN, PATIENTS, AND METHODS

Studies I-V include patients from the nationwide FinnDiane Study while the patients of study V were part of the IDEAL study which constitute a subpopulation of FinnDiane patients with short duration of type 1 diabetes that are more extensively studied in search of early markers for diabetic complications. The FinnDiane study recruits patients at all levels of the Finnish health care system; from primary health care up to university central hospitals. The patients are recruited simply based on a diagnosis of type 1 diabetes with no prerequisites regarding their diabetic complications. Due to the ongoing nature of the FinnDiane Study, the studies I-IV have somewhat differing sets of patients. The physical activity questionnaire was not in use at time of the launch of the study and therefore the number of patients with data on LTPA is lower than the total amount of patients. Some patients have additionally chosen not to return the questionnaire due to reasons that are not assessed in the study. Such reasons may include that the questionnaire is rendered laborious and time-consuming. Physically active patients may also be more likely than sedentary patients to complete the questionnaire. Four studies in this thesis have a cross-sectional study design which introduces difficulties to discern causal relationships. One study, however, had a longitudinal study design which takes advantage of data from two study occasions of each patient. FinnDiane is a multicenter study, thus with numerous researchers collecting clinical data. The use of standardised forms and questionnaires, as well as a written manual to ensure similar methods of measurement of clinical characteristics, decrease the possible heterogeneity caused by differences between researchers.

7.2 STUDY I

In our cross-sectional analysis, patients with diabetic micro- and macrovascular complications reported different patterns of LTPA compared with patients without complications. Patients with diabetic nephropathy and proliferative retinopathy, compared with patients without complications, more frequently reported a level of LTPA that did not meet the general physical activity recommendations. Concerning components of LTPA, low frequency was associated with diabetic nephropathy. However, the most prominent difference between patients with various degrees of

complications was the intensity of LTPA, since low intensity was associated with impaired renal function, and with increasing degree of proteinuria, retinopathy, and CVD.

The patient's ability to exercise may be reduced by diabetic complications. Macrovascular disease is an evident limitation due to exercise-induced myocardial ischaemia, systolic or diastolic cardiac dysfunction, or ischaemia in the lower limbs. Patients with CVD are frequently treated with β-adrenergic receptor blockers, which may cause exercise intolerance. Diabetic nephropathy is strongly associated with CVD [6], and patients with nephropathy have a 37-fold increased risk for an early death due to CVD [7]. A reduction in renal function, especially in diabetic nephropathy [151], is associated with a decrease in the blood haemoglobin concentration due to an impaired renal production of erythropoietin, and this "renal anaemia" may interfere with oxygen delivery during exercise. Autonomic neuropathy is a microvascular diabetic complication which shows considerable co-morbidity with other diabetic complications [301]. Autonomic neuropathy is commonly associated with exercise intolerance presumably due to inadequate responses in heart rate and blood pressure during exercise [302]. Particular attention should be paid to cardiac autonomic neuropathy, which is a risk factor for silent myocardial ischaemia and sudden death. An impaired awareness of hypoglycaemia has been attributed to autonomic neuropathy [303] which may increase the risk of exercise-induced hypoglycaemia. Peripheral neuropathy is also likely to impair physical activity due to a loss of sensation in the feet. Diabetic foot ulcers, which may develop from impaired peripheral arterial circulation or sensory neuropathy, or often from both factors acting in concert, may likewise lead to a decreased physical activity. Both diabetes and the diabetic complications are associated with endothelial dysfunction [304, 305], which could lead to impairment of vasodilation in exercising skeletal muscle tissue, possibly through reduced nitric oxide sensitivity. Finally, an increased prevalence of depression has been associated with diabetic complications [306], and which may lead to a decrease in physical activity.

The differences in LTPA habits according to the diabetic complication status are probably largely attributable to abovementioned exercise-limiting factors. However, the difference in patients with microalbuminuria compared with patients with normal UAER (lower intensity and a trend towards more sedentary patients in the former group) is an interesting finding. Microalbuminuria in patients with type 1 diabetes would probably not cause exercise intolerance because patients with microalbuminuria usually do not have decreased renal function. Therefore, low LTPA might precede the development of microalbuminuria, even though there are indications that patients with microalbuminuria might have a reduced exercise capacity compared with normoalbuminuric patients [261].

Possible mechanisms by which physical activity may prevent the development of diabetic complications are lowering of blood pressure and improvement of lipid

profile, glycaemic control, insulin sensitivity, and endothelial function. Especially an effect through insulin sensitivity is appealing, since insulin resistance is thought to play a role in the development of diabetic complications [307], including microalbuminuria [123]. Physical activity has further been shown to have anti-inflammatory effects [308], and chronic low-grade inflammation has been implicated in the pathogenesis of diabetic complications [309]. Finally, increased UAER has been proposed as a sign of global vascular dysfunction in patients with type 1 diabetes according to the Steno hypothesis [183], and regular exercise training has been shown to improve endothelial function in patients with type 1 [273] and type 2 diabetes [310].

Regarding the clinical implications of the study, a limitation is the cross-sectional study design. A longitudinal study design would have provided evidence for the role of physical activity in the development and progression rate of diabetic complications. To our best knowledge, however, there are no prospective studies available addressing this issue, not even involving patients with type 2 diabetes. Moy *et al* [272] showed in a seven-year follow-up study of patients with type 1 diabetes that a higher baseline physical activity level was predictive of lower mortality independently of diabetic complications at baseline, but unfortunately complication status at follow-up was not assessed. Prospective epidemiological studies and randomized controlled trials are needed to provide further evidence for the role of physical activity in the prevention of diabetic complications in patients with type 1 diabetes.

7.3 STUDY II

The main finding of this cross-sectional study was that for women with type 1 diabetes, physical activity was associated with glycaemic control. A sedentary level of LTPA, defined as below the general physical activity recommendations, was associated with higher HbA_{1c} compared with female patients that meet the recommendations. High levels of LTPA, on the other hand, was associated with similar glycaemic control as did moderate levels of LTPA, which suggests a non-linear relationship between LTPA and HbA_{1c}. The linear relationship between HbA_{1c} and LTPA was low, and a correlation coefficient of -0.12 for women indicates that LTPA would explain only 1.4% of the total variance in HbA_{1c} in these patients. A mean HbA_{1c} difference of 0.5%-units between sedentary and non-sedentary patients can, however, be considered clinically relevant. A similar association between LTPA and HbA_{1c} was evident also for patients free from diabetic complications, which indicates an effect independent of patients being sedentary simply due to a high disease burden which in itself associated with poor glycaemic control. Other confounders such as age, obesity, smoking, and social class did not explain the association either. It should be noted, though, that we found no association between LTPA and HbA_{1c} in men with type 1 diabetes.

Physical activity may improve the glycaemic control of patients with type 1 diabetes, and a plausible mechanism is through improved insulin sensitivity [11-13]. The effect on glycaemic control seems, however, to be smaller in patients with type 1 compared with type 2 diabetes. Most physical activity intervention studies in type 1 diabetes have failed to show any effect on glycaemic control (see section 2.4.2.1). In type 2 diabetes, on the other hand, there is evidence showing a reduction in HbA_{1c} by physical activity intervention, and a meta-analysis showed a mean reduction in HbA_{1c} by 0.66%-units [10]. The reason behind these divergent findings in type 1 and type 2 diabetes has not been adequately addressed. A potential positive effect of physical activity on glycaemic control in type 1 diabetes can be thought to be reduced by hypoglycaemia during, or more frequently after, a bout of physical activity. Patients with type 2 diabetes are not prone to hypoglycaemia to the same extent as in type 1 diabetes. Exercise-induced hypoglycaemia, or even just a fear thereof, may cause patients with type 1 diabetes to take behavioural actions to keep the blood glucose concentrations before, during and/or after physical activity at a higher level than normal by reducing the insulin doses and by excess carbohydrate loading. Such behaviour may be thought to abolish a positive effect of physical activity on glycaemic control. Our data cannot, however, address this issue since we lacked data on dietary factors. Insulin doses were lower in patients that were more physically active but whether this is due to increased insulin sensitivity or due to reduction in insulin doses due to fear of hypoglycaemia is unclear.

The reason behind the lack of association between LTPA and HbA_{1c} in men with type 1 diabetes cannot be fully determined in our study. Men tended, as compared with women, to report LTPA of higher intensity while women reported higher frequency and longer duration of their physical activity sessions. The higher the intensity, the higher is also usually the activation of the counteractive hormonal responses to insulin (most importantly adrenalin, glucagon, and cortisol) with subsequent increase in blood glucose concentrations. This response, on the other hand, may also be of benefit since a short bout of high intensity activity, for instance the 10 s maximal sprint[240], may decrease the risk of post-exercise hypoglycaemia. Longer sessions of high intensity physical activity might, however, cause a different hormonal response than a 10 s maximal sprint.

Our data regarding LTPA and glycaemic control is based on an observational study and we therefore cannot draw conclusions about the causality and about the isolated effect of physical activity independently of other lifestyle factors. There is usually significant association between different lifestyle factors such as physical activity, diet, smoking, alcohol use, and other behaviour affecting health outcomes such as diabetes treatment compliance. It is possible that the physical activity is a marker of a generally healthy lifestyle that in turn translates into a more beneficial glycaemic control.

7.4 STUDY III

We demonstrated an association between the variability of HbA_{1c} and the risk of progression in renal status and a CVD event during follow-up. We found that a higher variability, defined as the SD of serially measured HbA_{1c}, was associated with higher incidences of both renal and cardiovascular disease progression. The mean HbA_{1c} was important also for progression in renal disease, which has been shown previously in several intervention studies [110-112] including the landmark study the DCCT [103]. The findings regarding HbA_{1c} variability and progression of renal disease are in accordance with recent data from the DCCT [121]. Our data on HbA_{1c} variability differ from the data from the DCCT in the sense that the FinnDiane is a purely observational, non-interventional study whereas the DCCT was by design an intervention aimed at reducing the HbA_{1c} of the participants in the intensive treatment arm. Thus there is a possibility of iatrogenic HbA_{1c} variability due to treatment intervention in the DCCT, even though this risk was somewhat reduced by exclusion of HbA_{1c} measurements from the first 6 months of intervention, and also that an association was seen also in the conventionally treated arm.

Our serial HbA_{1c} data were gathered from the local study centres and this may introduce two potential problems. First, the measurements were accordingly not performed in a central laboratory. The HbA_{1c} variability, however, was calculated on the intrapersonal level which means that the great majority of the HbA_{1c} measurements from which the variability was derived in fact were measured in the same laboratory at each local study centre. Moreover, the concerns were also relieved by the fact that the HbA_{1c} assays in Finland has been shown to have low coefficient of variation and a high correlation with the DCCT reference method[311]. Second, due to the observational study design, the intervals between the HbA_{1c} measurements were not pre-specified and thus dependent on the clinical follow-up setting of each patient. The number of HbA_{1c} measurements could have affected the variability, but this was statistically corrected for. Another potential statistical source of error was that higher mean HbA_{1c} in the patients that progressed in renal or cardiovascular status lead to higher absolute SD values and therefore the variability of HbA_{1c} might appear greater, but this was adjusted for by the use of the coefficient of variation for HbA_{1c} and by multivariate Cox regression analyses.

Our study included prospective data on CVD events, which were not reported by the DCCT [121]. Our main finding was that the variability, but not the mean, of serial HbA_{1c} was predictive of a CVD event during follow-up. Previous data regarding the level of HbA_{1c} and risk of CVD in patients with type 1 diabetes are contradictive [312-319]. In type 2 diabetes, the effect of lowering HbA_{1c} on CVD risk has recently been debated [196] due to failure to show any benefit in large randomised clinical trials.

Glycaemic variability in patients with type 1 diabetes has previously been studied in the form of short-term glycaemic excursions and their effect on outcomes relevant to risk of diabetic complications. It has been shown that glucose variability measured by continuous glucose measurement systems (CGMS) is associated with markers of oxidative stress in patients with type 2 [320] but not type 1 [321] diabetes. The CGMS measurements are mostly performed in small patient materials and therefore large-scale epidemiological analyses have employed other measures of glycaemic variability. In the DCCT, the variability of intrapersonal, within-day measured blood glucose concentrations were not predictive of either diabetic nephropathy or retinopathy [119].

The HbA_{1c} variability and the abovementioned short-term glycaemic variability are probably two different entities with differing underlying mechanisms. It is possible that the HbA_{1c} variability is affected by behavioural factors since the HbA_{1c} variability associated with physical activity, smoking, and social class. The finding that low physical activity (<10 MET*h/week) was associated with increased variability of HbA_{1c} was somewhat unexpected. This association was also evident exclusively in patients with normoalbuminuria at baseline which indicates that the association was not due to underlying diabetic complications causing both reduced physical activity (as shown in Study I) and increased HbA_{1c} variability.

The mechanisms behind the association between HbA_{1c} variability and the increased risk of diabetic complications are unclear. The possibility of a secondary effect to variation in insulin sensitivity may imply an involvement of infections, and this speculation was reinforced by recent data from the FinnDiane Study showing an association between serum lipopolysaccharide activity, which is a marker of gram-negative bacterial infections, and increased risk of progression in renal disease in patients with type 1 diabetes [322].

7.5 STUDY IV

Low leisure-time physical activity showed an association with higher prevalence of the metabolic syndrome in patients with type 1 diabetes. This is in accordance with other studies showing an inverse relationship between physical activity and the prevalence of the metabolic syndrome in nondiabetic and type 2 diabetic patients [323, 324]. In this study, the *PPARγ* Pro12Ala polymorphism was not associated with the metabolic syndrome. We found, however, a *PPARγ* genotype-dependent association between the metabolic syndrome and LTPA, since the association was only observed in Ala-carriers. In patients with the Ala allele, the prevalence of the metabolic syndrome was 2.4-fold higher in sedentary compared with physically active patients, while patients with the Pro12Pro genotype showed no difference in prevalence of the metabolic syndrome according to level of LTPA.

Carriers of the Ala-allele have been shown to have reduced *PPARγ* receptor activity compared to subjects with the Pro12Pro genotype [325]. There is data supporting that Ala-carriers have a lower risk of developing type 2 diabetes than subjects with the Pro12Pro genotype [326]. However, it should be noticed that conflicting studies have also been published where the Ala-allele has been associated with increased risk of type 2 diabetes [327-329] and with higher prevalence of obesity [330, 331]. Such a variation in risk magnitude may reflect the presence of environmental factors modifying the association, for instance physical activity.

Interestingly, it has been shown that the Pro12Ala polymorphism of the *PPARγ* gene may modulate exercise-induced responses in insulin sensitivity and glucose homeostasis. In a Japanese study, 123 men with normal glucose tolerance were genotyped for *PPARγ* and participated in a 3-month exercise intervention [286]. Patients with the Pro12Ala genotype gained a greater reduction in fasting insulin level and HOMA index compared to patients with Pro12Pro genotype, with no significant difference in weight reduction. Similarly, Weiss et al reported that after a 6-month exercise intervention, a 4-fold greater decrease both in fasting insulin and insulin AUC (in an oral glucose tolerance test) was seen in healthy males with the Pro12Ala genotype compared to Pro12Pro, however no difference was seen in females [287]. In addition, data on type 2 diabetic patients suggest that the Pro12Ala polymorphism influences the glycaemic response to exercise; Ala-carriers had a greater reduction in fasting plasma glucose, though not in HbA_{1c}, independent of weight reduction after a 3-month exercise intervention of 139 previously sedentary type 2 diabetic patients [288]. In the Finnish Diabetes Prevention Study (DPS), 522 subjects with impaired glucose tolerance were randomized to either an exercise and diet intervention or a control group; 490 of these subjects were genotyped for *PPARγ* Pro12Ala [329]. In the intervention group, subjects homozygous for the Ala-allele lost more weight than subjects with Pro12Pro genotype. During follow-up, none of the subjects with Ala12Ala genotype in the intervention group developed type 2 diabetes even though the Ala-allele was associated with a 2.11-fold increase in risk for developing diabetes in the whole study population (intervention and control subjects combined). It was further shown that the there is a gene-environment interaction between the *PPARγ* Pro12Ala polymorphism and physical activity on the risk of developing type 2 diabetes in the DPS [332]. It has previously been shown that physiological and biochemical responses to physical activity have a hereditary component [283], and an annual review on the genetics of exercise responses is published [289]. The *PPARγ* Pro12Ala polymorphism, or another gene variant being in linkage disequilibrium, might be, based on both present and previous studies, involved in exercise-induced responses in insulin and glucose homeostasis.

In this study, waist circumference seemed to have a major impact both on the association between LTPA and metabolic syndrome and on the *PPARγ* genotype-dependent association between metabolic syndrome and LTPA. This speaks in

favour of a pivotal role of insulin sensitivity since central obesity is tightly linked to decreased insulin sensitivity. Insulin resistance is considered to be the key feature of the metabolic syndrome [201]. We previously showed that LTPA is associated with insulin sensitivity in type 1 diabetic patients (Study II). The overall prevalence of the metabolic syndrome in this study was lower (29.5%) than previously reported (39%) [27], presumably due to exclusion of patients with end-stage renal disease and/or a previous cardiovascular event.

This study has some limitations, most of which are attributable to the cross-sectional study design. The association between LTPA and the metabolic syndrome could have been caused by exercise-limiting factors themselves associated with the metabolic syndrome (for instance diabetic nephropathy). However, patients with end-stage renal disease or a previous cardiovascular event were excluded from the analyses, which reduce the risk for complication-derived physical activity bias. We found no association between the *PPARγ* Pro12Ala polymorphism and the metabolic syndrome, but we cannot exclude a type 2 statistical error due to an insufficient number of patients. The differences in association between LTPA and the metabolic syndrome according to subgroup analyses of *PPARγ* genotype cannot be explained by loss of statistical power in the Pro12Pro group because the number of patients was larger than in the group of Ala-carriers where the association was apparent.

In conclusion, physical activity might reduce the risk that type 1 diabetic patients develop the metabolic syndrome, a feature shared with the metabolic syndrome in nondiabetic and type 2 diabetic subjects. The common Pro12Ala polymorphism of the *PPARγ* gene does not seem to be related to the metabolic syndrome in type 1 diabetic patients. However, carriers of the Ala allele might especially benefit from being physically active.

7.6. STUDY V

Young adults with type 1 diabetes have reduced aerobic work capacity compared with nondiabetic controls of the same age. Not only the maximal capacity ($\dot{V}O_{2max}$), but also the RCP and AT were similarly decreased in patients with type 1 diabetes which indicates an overall lower aerobic fitness level. Previous studies of the fitness level of patient with type 1 diabetes have indicated reduced [274, 275] or normal [276, 277] aerobic fitness compared with controls. The IDEAL Study recruited patients with a population-based approach, which in contrast to earlier works means that the results may be more easily generalised. These results were especially clear in women, and the aerobic fitness level of the young women with type 1 diabetes tested in our study was surprisingly low. The finding that the AT in women with diabetes was at a level below that of brisk walking further strengthens this concern. The differences in strength fitness were not as pronounced as those of aerobic

fitness but differences were still observed. Strength fitness has not previously to our knowledge been compared in patients with diabetes and in nondiabetic peers.

HbA_{1c} correlated inversely with $\dot{V}O_{2max}$ in men with diabetes, but not in women. The distribution of $\dot{V}O_{2max}$ in women was considerably narrower in women than in men which may have decreased the statistical power to detect a true association also in women. There is some previous data showing a similar inverse association between measures of aerobic fitness and HbA_{1c} in patients with type 1 diabetes [276, 281]. A more recent study of consecutive patients at a diabetes outpatient clinic, however, showed that a higher aerobic fitness was associated with higher HbA_{1c}, but the fitness was measured with an indirect method and not by pulmonary gas exchange [282]. That study also showed an association between higher strength fitness and higher HbA_{1c}, but our study showed the opposite association because lower limb strength in men and upper limb strength in women were inversely associated with HbA_{1c}.

The reason(s) for a lower fitness level in young adult Finnish patients with type 1 diabetes compared with nondiabetic controls was not evident from our study. The physical activity of patients with diabetes was lower, which certainly explain part of the findings. The difference in physical activity was less clear in women with and without diabetes, and as the largest differences in fitness level were found in women, there are probably also other factors involved. It has been shown that elevated UAER is associated with reduced aerobic work capacity in patients with type 1 diabetes [261], but only one patient in our study had microalbuminuria and we found no correlation between UAER and $\dot{V}O_{2max}$. Chronic hyperglycaemia can, however, cause disturbances of the microcirculation of the muscles [279] and of the lungs [278], with plausible implications for the maximal work capacity of patients with diabetes. Recent data from the IDEAL Study showed signs of impaired autonomic nervous system responses, especially a reduced baroreflex sensitivity, which may cause abnormal responses in heart rate and blood pressure during physical activity [333].

8. SUMMARY AND CONCLUSIONS

I The physical activity of patients with diabetic complications was reduced when compared with patients without microvascular and macrovascular diabetic complications. This study had a cross-sectional design, and therefore the causal relationships cannot be fully determined. Patients with severe disease such as diabetic nephropathy (especially ESRD) and CVD have a reduced exercise tolerance and capability and therefore the lower level of physical activity is probably to a large extent secondary to these severe complications. Patients with microalbuminuria, on the other hand, also showed a different physical activity pattern as compared with patients with normal UAER, and in this case it is possible that the physical activity may precede the development of microalbuminuria. Future prospective studies will address the question whether a physically active lifestyle can reduce the incidence of diabetic complications in type 1 diabetes.

II Low physical activity was associated with poor glycaemic control in patients with type diabetes, and this association was evident in women. A high level of physical activity was associated with a similar glycaemic control as a moderate level of physical activity. A sedentary level was defined as physical activity below the general recommendations for regular physical activity. The association between the glycaemic control and physical activity was seen also in patients with no signs of diabetic complications, indicating that the results were not entirely due to the diabetic complication status. Insulin sensitivity was also better in patients with higher physical activity level. The results may indicate that avoidance of a sedentary lifestyle may facilitate a more beneficial long-term blood glucose control in type 1 diabetes.

III In a prospective setting, a higher variability of HbA_{1c} was associated with higher risk for progression in renal disease and with CVD events in patients with type 1 diabetes. For CVD events, the HbA_{1c} variability was predictive even though the mean level of HbA_{1c} was not. A sedentary level of physical activity was associated with higher HbA_{1c} variability. The underlying meaning of having a high HbA_{1c} variability is so far unclear and needs to be further characterised. There was a trend towards patients with low physical activity at baseline to have higher incidence of progression in renal disease and CVD events, even though the results did not reach statistical significance.

IV The metabolic syndrome in patients with type 1 diabetes has recently been described using standard definitions. A higher level of physical activity was associated with a lower prevalence of the metabolic syndrome in patients with type 1 diabetes. We further showed that the *PPARγ* Pro12Ala polymorphism — a well-known gene variant for the risk of obesity, insulin resistance, and type 2 diabetes — was not associated with the metabolic syndrome in type 1 diabetes; however we cannot fully exclude a type 2 statistical error due to a lack of power. The *PPARγ* Pro12Ala polymorphism affected the association between physical activity and the prevalence of the metabolic syndrome because the association was evident only in carriers of the Ala-allele.

V We showed in a population-based cohort of young adults with type 1 diabetes of short duration that the aerobic work capacity is reduced compared with nondiabetic controls of similar age. The golden standard of measurement of aerobic fitness, direct measurement of the alveolar ventilation, was used. Women with type 1 diabetes had an alarmingly low level of aerobic physical fitness. The strength fitness was also somewhat reduced in patients with type 1 diabetes. In men, a higher level of aerobic fitness was associated with lower HbA_{1c} and $\dot{V}O_{2max}$ explained 25% of the variance in HbA_{1c}. In women, upper limb strength, but not aerobic fitness, was inversely associated with HbA_{1c}. The underlying reasons for a decreased physical fitness in patients with type 1 diabetes warrants further studies.

General conclusions: This thesis shows that patients with type 1 diabetes should be encouraged to be physically active because an active lifestyle is associated with a more beneficial glycaemic profile that can translate into a decreased risk for diabetic complication.

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