Progressive elements in type 2 diabetes, studies on secondary failure and diabetic complications

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PROGRESSIVE ELEMENTS IN TYPE 2 DIABETES,
STUDIES ON SECONDARY FAILURE AND DIABETIC COMPLICATIONS

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ABSTRACT

Diabetes is a progressive disease with late complications, one of the most common being diabetic neuropathy (DN). DN can lead to serious complications and accounts for more hospitalization and higher costs than all other complications of diabetes combined. DN is amongst other connected to deteriorated glucose control, however no cure is available today. The prevalence and nature of peripheral DN in a Swedish type 2 diabetes (T2D) population is not known.

The aims of this thesis were to study complications in a representative population with T2D with focus on peripheral neuropathy (PN) and the associations between peripheral sensory neuropathy (PSN) and different aspects of metabolism. Moreover, the features of late complications and metabolism were studied in subjects with PSN in relation to coexistence or not of retinopathy. Since metabolic control is important for prevention of complications the effects of the length of intensive insulin treatment on beta-cell function in subjects with T2D and features of secondary failure. Special focus was on the IGF-axis in relation to complications, metabolism and weight in T2D.

RESULTS

A geographically defined population with T2D, mean age 61.7 ± 7.2 years and diabetes duration 7.0 ± 5.7 years, were investigated regarding disease and late complications.

We found despite good glucose control retinopathy in 29%, nephropathy in 22 %, cardiovascular disease in 62%, cerebrovascular lesions in 11% and peripheral vascular disease (PVD) in 26%. Peripheral neuropathy was most common (67%) and peripheral sensory neuropathy was closely connected to PVD, especially in subjects without retinopathy. This finding suggests that PVD should be carefully considered in subjects with PSN and especially when no retinopathy. Signs of peripheral autonomic neuropathy (PAN) were the most common DN and predicted foot ulceration at 5 year follow-up, which to our knowledge has not been reported previously. This underlines the necessity to include the signs of PAN in foot examination protocols. Serum IGFBP-1 levels were higher in subjects with peripheral sensory neuropathy. This has previously been reported in type 1 diabetes but to our knowledge not in T2D. This finding suggests that low bioactive IGF is associated with increased risk for peripheral DN and that this can be associated with beta cell failure. We also found that low IGF-II was associated with weight gain in normal weight T2D patients.

Intensive insulin treatment rapidly improved glucose control and beta-cell function in a group of T2D patients with secondary failure but prolongation did not elicit further enhancement. Furthermore this effect was rapidly lost when subjects were switched to less intense treatment. Subjects with higher C-peptide levels were likely to improve beta-cell function more, suggesting that the effect depends on remaining beta-cell mass. However, improvement of glucose control was less in subjects with lower IGFBP-1 which suggests other causes for deteriorated glucose control in these subjects than insulin deficiency, while high IGFBP-1
levels could indicate “true” insulin deficiency. Thus, determination of IGFBP-1 may be useful in the choice of treatment in secondary failure.

In conclusion we found macro- and microvascular complications to be common in this representative T2D population. The prevalence of peripheral neuropathy was the most common complication. The standardized foot examination used could predict future foot ulcers, especially the presence of autonomic neuropathy. Peripheral neuropathy suggested concomitant PVD if retinopathy was not present. Low HDLc, male gender and high age were other independent risk factors for DN. The IGF system seems also to be important for the development of peripheral neuropathy. Intensive insulin treatment improved insulin secretion in those with preserved beta cell function but improved metabolic control only in those with high IGFBP-1 levels, suggestive of beta cell failure

**LIST OF PUBLICATIONS**


Other;

LIST OF ABBREVIATIONS

AUC  Area Under Curve  
BMI  Body Mass Index  
BTI  Bed-Time Insulin  
CI  Confidence Interval  
CVD  Cardiovascular disease  
CVL  Cerebrovascular lesion  
DN  Diabetes Neuropathy  
GAD  Glutamic Acid Decarboxylase  
GH  Growth Hormone  
HOMA  Homeostasis Model Assessment  
hsCRP  high sensitive CRP  
IGF-I  Insulin-like Growth Factor-I  
IGF-II  Insulin-like Growth Factor-II  
IGFBP-1, -3  Insulin-like Growth Factor Binding Protein -1, -3  
IGT  Impaired Glucose Tolerance  
LADA  Late Autoimmune Diabetes of the Adult  
MI  Multiple Insulin injection  
NSS  Neuropathy Symptom Score  
NYHA  New York Heart Association  
OGT  Oral Glucose tolerance Test  
OR  Odds Ratio  
oNeph  overt nephropathy  
PAN  Peripheral Autonomic Neuropathy  
PMN  Peripheral Motor Neuropathy  
PN  Peripheral Neuropathy  
PSN  Peripheral Sensory Neuropathy  
PVD  Peripheral Vascular Disease  
RP  Retinopathy  
SD  Standard Deviation  
T1D  Type 1 Diabetes  
T2D  Type 2 Diabetes  
UKPDS  United Kingdom Prospective Diabetes Study  
UKPDS-PTM  UKPDS Post Trial Monitoring  
VEGF  Vascular Endothelial Growth Factor  
VPT  Vibration Perception Threshold
INTRODUCTION

DIAGNOSIS OF DIABETES AND CLASSIFICATION.

Diabetes mellitus is a group of metabolic disorders with the common hallmark of elevated blood glucose level, hyperglycemia, although other metabolic disturbances are associated e.g. dyslipidemia and abnormal haemostasis. Diabetes is caused by various degrees of impaired insulin secretion and altered insulin resistance/action.

A clinical diagnosis of diabetes can be made by the finding of elevated glucose in the fasting state, by high daytime glucose level together with symptoms or by the level of hyperglycemia obtained 2 h after an oral glucose tolerance test (OGT). The WHO criteria for diagnosis were changed in 1999 with regard to the fasting glucose criteria.

When based on fasting glucose a diagnosis of diabetes is presently made by fasting glucose $\geq 6.1$ mmol/L in whole blood or $\geq 7.0$ mmol/L in plasma (1999).

The ADA classification of diabetes divides diabetes into type 1 diabetes, type 2 diabetes, other specific types of diabetes and gestational diabetes. Other specific types encompass diabetes that is second to genetic defects in insulin secretion or insulin action, diseases of exocrine pancreas, endocrinopathies, drug- and chemical induced, infections, uncommon forms of immune-mediated diabetes and genetic syndromes (2008).

Type 1 diabetes (T1D) affects 5-10% of subjects with diabetes. It is a disorder marked by destruction of the beta-cells resulting in variable degree of insulinopenia, classically affecting youth. T1D has an autoimmune aetiology in up to 90% of cases were antibodies as islet cell autoantibodies (ICA), autoantibodies to insulin, autoantibodies to glutamic acid decarboxylase (GAD65), and autoantibodies to the tyrosine phosphatas IA-2 and IA-2β are demonstrated. The remaining 10% has no demonstrated autoimmune aetiology and is named idiopathic T1D.

A special form of autoimmune diabetes is called latent autoimmune diabetes in adults (LADA). LADA patients share the clinical features of T2D at debut but have antibodies against the beta-cells. LADA patients often need insulin treatment within 2 years after diagnosis (Pozzilli and Di Mario 2001; Stenstrom, Gottsater et al. 2005).

Type 2 diabetes (T2D) affects 80-85% of subjects with diabetes in the Western World. T2D displays a relative deficiency in insulin secretion and insulin resistance but no sign of autoimmune aetiology. T2D usually affects adults after 35 years of age. These subjects often have weight problem and a sedentary lifestyle. The prevalence increases markedly with age. Because of the influence of age T2D
was previously termed adult-onset diabetes. The genetic predisposition is strong and genome-wide scanning studies (GWS) using SNP (single nucleotide polymorphism) demonstrate a markedly polygenic background. However the genetic background is still poorly defined, 8 out of 10 identified genes pertain to beta-cell function and 2 to insulin resistance (Scott, Mohlke et al. 2007).

Maturity onset diabetes of the young (MODY) is caused by single gene mutations with a strong penetration. The genes causing MODY are all affecting insulin secretion. Onset is usually before the age of 25.

**DIABETES AND LATE COMPLICATIONS.**

The prevalence of diabetes is increasing worldwide. Prevalence of diabetes in Sweden is reported to be approx 3.2% with a variation between 2.2 – 4.5% depending on choice of method (Berger, Stenstrom et al. 1998). In Norway the Nord-Trondelag study reported 4.8% of residents ≥40 years of age to have diabetes (Midtjell, Bjorndal et al. 1995). A most recent report from the Danish National Diabetes register reported the diabetes prevalence to be 4.2% with an increase of prevalence with 6% per year however the incidence rate increased 5% per year before 2004 and then stabilised (Carstensen, Kristensen et al. 2008). It is also well known that a large portion of subjects with diabetes is undiagnosed.

Diabetes is a progressive disorder with late complication referred to as either macrovascular; cardiovascular disease, cerebrovascular disease and peripheral vascular disease or microvascular; retinopathy, nephropathy and neuropathy.

**EPIDEMIOLOGY**

In a cross sectional study in a health care district in northern Sweden the investigators found the prevalence of diabetes to be 3.3% in the studied T2D population with mean age 70 years, diabetes duration 9 years and HbA1c 7.1%. Reported complications were angina pectoris 23.7%, myocardial infarction 12.3%, stroke 12.3%, peripheral vascular disease 23.5%, retinopathy 18.7%, neuropathy 27.9% and nephropathy 12.7% while 2% had been amputated (Lundman and Engstrom 1998).

Another study of retinopathy in a population based primary health care setting in Sweden, subjects younger than 70 years of age and with T2D, reported the prevalence of retinopathy to be 26.5% (Falkenberg and Finnstrom 1994) which is comparable with a report from an unselected T2D population in Denmark, 31.5% (Hove, Kristensen et al. 2004).

Diabetic neuropathy (DN) is a common late complication of the disease. It is probably the complication that is least characterized and defined. Thus prevalence varies considerably depending on diagnostic criteria used (Rich 2006). DN leads to other late complications such as non-healing ulcers, amputations, osteoporosis with fractures, Charcot foot, the insensitive foot, pain and hyperaesthesia, disturbances of balance, cardiac arrhythmias, severe orthostatism, gastrointestinal symptoms, erectile dysfunction, urinary retention, hearing loss,
cognitive dysfunction and reduced quality of life.

DN accounts for more hospitalization and higher costs than all other complications of diabetes combined. It is believed that every 30 seconds a lower limb is lost somewhere in the world as a consequence of diabetes (Boulton, Vileikyte et al. 2005).

It is estimated that 80% of the neuropathies that affect diabetics are distal symmetrical neuropathy, i.e. peripheral neuropathy. Further, 8.3% of diabetic subjects are reported to display polyneuropathy at diagnosis of T2D, this prevalence increase to 41.9% 10 years later (Partanen, Niskanen et al. 1995).

Diabetic peripheral neuropathy is reported to affect 22% (the North-West Diabetes Foot Care Study), 24% (the Neuropathy Spanish study group), 32% (UK hospital study), 37% (GOAL A1C GROUP), 59% (The Rochester Diabetic neuropathy study) (Dyck, Kratz et al. 1993; Young, Boulton et al. 1993; Cabezas-Cerrato 1998; Abbott, Carrington et al. 2002; Herman and Kennedy 2005) and both lower and higher prevalence data are reported (Rich 2006).

The differences in prevalence depend mainly on differences in populations studied (e.g. hospital patients, outpatients, ethnicity), methodology (e.g. clinical examination, neurophysiological investigation, number of methods used) and definitions of neuropathy (e.g. sensory modalities only, all modalities of distal neuropathies, use of neuropathy symptom score or not) and on definition of diabetes. Studies in truly representative populations are scarce e.g. the Rochester study is based on a US population with diabetes prevalence of 1.3% of which 27% of included patients had T1D and 73% T2D (Dyck, Kratz et al. 1993). The Seattle Diabetic Foot Study enrolled patients from general internal medicine clinics (Boyko, Ahroni et al. 1999). Other studies seems more representative as The North-West Diabetes Foot Care Study (Abbott, Carrington et al. 2002) however this study reports the aggregated results for type 1 and type 2 diabetics and LADA patients were not identified. The study by the Neuropathy Spanish Study Group (Cabezas-Cerrato 1998) consists of an arbitrary, however randomised population based on health care statistics. Hence, there remains a need for further representative population studies of peripheral neuropathy in T2D.

**CARDIOVASCULAR DISEASE**

Cardiovascular disease (CVD) is a major cause of morbidity and mortality in diabetes. Subjects with diabetes have a 2-4 fold increase of the mortality risk in CVD compared with non-diabetics (Kannel and McGee 1979; Haffner, Lehto et al. 1998; Gerich 2007). CVD has multifactor background including amongst other genetic factors, lifestyle factors as smoking and over weight, hypertension and dyslipidemia (Buse, Ginsberg et al. 2007; Gerich 2007) as well as hyperglycemia (Aronson 2008). Intense treatment to achieve acceptable glucose control was reported to have a tendency to reduce the CVD risk during the UKPDS study. In a recent post-trial monitoring (UKPDS-PTM) a significant reduction of CVD events and also mortality in the intense treated group was reported (Holman, Paul et al. 2008).

An association between early development of arteriosclerosis and the IGF-sys-
tem (insulin-like growth factor system) has been reported with levels of insulin-like growth factor binding protein-1 (IGFBP-1) being positively and insulin-like growth factor-I (IGF-I) negatively correlated to the arteriosclerotic development (Boquist, Ruotolo et al. 2008) and also that the relation to IGFBP-1 differs between genders (Unden, Elofsson et al. 2005). Further high levels of IGFBP-1 at admission are associated with increased risk for cardiovascular mortality and morbidity in T2D patients with myocardial infarction (Wallander, Norhammar et al. 2007).

**CEREBROVASCULAR DISEASE**

Cerebrovascular disease affects diabetics three times as often as non-diabetics. Diabetics have higher prevalence of ischemic stroke and lacunar infarcts but comparable prevalence of haemorrhagic stroke. The pathogenesis behind the high risk for ischemic stroke is complex, including predisposing factors for arteriosclerosis, hypertension, atrial fibrillation as well as thromboembolic disposition and metabolic disturbances (Idris, Thomson et al. 2006). A connection between outcome in stroke disease with glucose control has been reported (Stevens, Coleman et al. 2004) and also between impaired glucose control and stroke incidence, which was independent of hypertension and other cardiovascular risk factors (Burchfiel, Curb et al. 1994) however intense antidiabetic treatment did not prevent cerebrovascular disease in the UKPDS (Stratton, Adler et al. 2000) or UKPDS-PTM studies (Holman, Paul et al. 2008).

**RETINOPATHY**

Retinopathy is common finding already at diagnosis of T2D and it is proposed to develop at least 7 years before diagnosis (Fong, Aiello et al. 2004). The development of retinopathy is connected to glucose control and hypertension (Stratton, Adler et al. 2000; Stratton, Kohnet al. 2001). Intense treatment to achieve glucose control during the UKPDS had long term beneficial effects on microvascular complications (Holman, Paul et al. 2008), this was in contrast to hypertension which had beneficial effects during UKPDS on retinopathy progression but not at follow up, at which time no difference in blood pressure control could be demonstrated (Holman, Paul et al. 2008). Also age, diabetes duration, albuminuria, dyslipidemia and smoking is reported as risk factors for retinopathy (Bloomgarden 2007).

Growth factors as VEGF, GH and IGF are proposed to be pathogenetic factors behind development of retinopathy (Fong, Aiello et al. 2004). There is evidence that GH and IGF-I participate in the processes that lead to retinal neovascularisation through induction of VEGF synthesis and it is also been suggested that worsen degree of retinopathy seen in intense insulin treatment may be connected to the secondary increase of serum IGF-I, however a causal connection is yet unproven (Frystyk 2005).

**NEUROPATHY**

The etiological background in peripheral neuropathy is complex with genetic and lifestyle factors as well as medical disorders contributing (Bromberg 2005). International definition of diabetic peripheral neuropathy is “ the presence of
symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after exclusion of other causes” (Boulton, Gries et al. 1998). There is no consensus about classifications of diabetes neuropathies but it is proposed to be based on clinical manifestations until further understanding of pathogenesis is obtained. One proposed clinical classification described in table 1 show the extent of somatic neuropathies (Boulton, Malik et al. 2004). Notably, peripheral autonomic neuropathy is not included but should be (England, Gronseth et al. 2008).

Table 1.

<table>
<thead>
<tr>
<th>Polyneuropathy</th>
<th>Mononeuropathy</th>
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<tbody>
<tr>
<td>Sensory</td>
<td>Acute sensory</td>
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<td></td>
<td>Isolated peripheral</td>
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<tr>
<td></td>
<td>Chronic sensory</td>
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<tr>
<td></td>
<td>Mononeuritis multiplex</td>
</tr>
<tr>
<td>Autonomic</td>
<td>Cardiovascular</td>
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<td></td>
<td>Isolated peripheral</td>
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<td>Genitourinary</td>
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</table>

A major confounder when assessing diabetic peripheral neuropathies is the increase of nerve dysfunction with normal aging (Wiles, Pearce et al. 1991). Lifetime prevalence, proportion of persons manifesting a disorder during the period of their life up to the survey date, in UK is 2/1000 for diabetic polyneuropathy and 1/1000 for polyneuropathy, excluding diabetic and alcoholic (MacDonald, Cockrell et al. 2000) and different pathogenesis exist in parallel. This demonstrates the importance of diabetes but also the difficulty in obtaining a true diabetic neuropathy population.

Another confounder is existence of familial clustering and there are also large variations among different ethnic populations despite comparable HbA1c and diabetes duration which suggests a genetic component. One study in US reported the prevalence of diabetic neuropathy to be 19% in Asians and 31-32% in Blacks, Latinos and Caucasian (Karter, Ferrara et al. 2002).

Mold et al. reported from a primary care setting that in subjects over 65 years of age 31% had peripheral neuropathy defined as a bilaterally finding of at least one of: loss of touch, vibration perception (Tuning-fork), joint-position perception or ankle reflexes. Subjects with a predisposing disease were twice as likely to have peripheral neuropathy but in 60% of this population of elderly with peripheral neuropathy no explanation was identified (Mold, Vesely et al. 2004). One source of possible error is that 59% of the subjects were diagnosed based on the single finding of loss of ankle-reflexes which inherent problems in interpretation (as discussed in the present foot examine protocol).

Also, it has been reported an association between peripheral neuropathy and peripheral vascular disease (Adler, Boyko et al. 1997; Cameron, Eaton et al. 2001) a
disorder that increase with age. In a recent population based study from Sweden the prevalence of any PVD was reported to be 7.9% in the age group 60 to 65 years and increased to 47.2% among the age group 85-90 years (Sigvand, Wiberg-Hedman et al. 2007) and it has been reported that diabetes cause even higher prevalence of PVD (Hirsch, Criqui et al. 2001). Arterial stiffening and thickness in T2D is closely associated with diabetic neuropathy (Yokoyama, Yokota et al. 2007). Peripheral vascular disease is also independently associated to HbA1c (Adler, Stevens et al. 2002; Selvin, Wattanakit et al. 2006) which in turn impairs skin capillary circulation thus causing ischemia (Jorneskog, Brismar et al. 1995; Jorneskog, Brismar et al. 1998). Hence diabetes and PVD interacts as pathogenic factors.

Peripheral neuropathy and PVD also present a similar clinical problem in so far that both are without symptoms in about 50% of the cases and that health care professionals often are unaware of the diagnosis (McDermott, Hahn et al. 2002; Boulton, Vinik et al. 2005).

It has been proposed that the mechanisms behind neuropathy in diabetes differ between T1D and T2D. Neuropathy in T1D is reported to be more severe and potentially related to insulinopenia and the lack of C-peptide in contrast to T2D with hyperinsulinemia and high levels of C-peptide (Kamiya, Murakawa et al. 2005). Hyperglycemia is however in both conditions related to neuropathy. Other proposed pathogenetic factors are adverse events second to the hyperglycemia, oxidative stress, disturbed microcirculation, hypoxia and possibly autoimmune reactions (Boulton, Malik et al. 2004).

The pathology behind neuropathy is described as dying-back axonopathy affecting not only axons and myelin but also leading to decreasing conduction velocity and in presence of regenerating nerve fibbers. Growth factors, as IGF, are reported to be important for maintenance and regeneration of neurons. IGF is reported in experimental models to facilitate nerve regeneration, regeneration rate as well as distance, an effect that can be blocked by antibodies to the IGF receptor and local IGF production is up-regulated following nerve lesions. IGF is also reported to support survival of neurons in animal models in vitro and in vivo (Chiarelli, Santilli et al. 2000). Neuropathy is reported in one study of T1D to be associated with elevated IGFBP-1 (Crosby, Tsigos et al. 1992). Also insulin has trophic effects on embryonic cells however the effects on mature cells is unclear (Brussee, Cunningham et al. 2004). Many signalling molecules are shared between IGF and nerve growth factors (Yasuda, Terada et al. 2003; Brussee, Cunningham et al. 2004).

Taken together these observations have led to hypothesis elaborated in the pathogenesis of diabetic neuropathy to involve in fact the balance between nerve degeneration and regeneration and a complex cross-talk between insulin/IGF and series of neurothropic growth factors.

In conclusion
The propensity for peripheral neuropathy is strongly connected to ageing that
coincide with increasing incidence of T2D. Peripheral neuropathy in diabetes has complex pathogenesis including ethnicity, genetic factors and environmental factors besides hyperglycemia with secondary adverse events, vascular disease, hypoxia and growth factors such as IGF:s and nerve growth factors. Finally, peripheral neuropathy is seen in several other disorders which can coexist with diabetes. Also a large proportion of neuropathies, especially in elderly populations are without known diagnosis. This makes it necessary to study diabetic peripheral neuropathy in well defined representative populations in order to investigate the impact of T2D vis-à-vis other pathogenic factors.

**Nephropathy**
Diabetic nephropathy is defined as proteinuria resulting from reversible endovascular damage of the renal filtration capacity by long-standing diabetes mellitus. Diabetic patients with albuminuria possess higher risk of developing myocardial infarctions, cerebrovascular accidents, severe progressive retinopathy, and peripheral and autonomic neuropathy (Chiarelli, Trotta et al. 2003). Risk factors for nephropathy are sustained hyperglycemia and hypertension and other putative factors are smoking and dyslipidemia as well as genetic disposition.

IGF axis is reported to play a key role in the maintenance of normal renal function and the development of diabetic nephropathy and elevated IGFBP-1 due to reduced suppression from lowered circulating insulin action in diabetes may also contribute to glomerulosclerosis (Vasylyeva and Ferry 2007).

**The Diabetic Foot**
Problems in the diabetic foot include several clinical aspects from neuropathy without perceived symptoms, approximately 50% of subjects with neuropathy, to development of foot ulcerations with subsequent need for amputation when failure to heal, prevalence 1-2% in a T2D population all ages. According to the Swedish National Health Care Board are 40-50% of non-traumatic amputations related to diabetes, 2/3 of subjects with diabetes have problems with their feet, 8% have a on-going ulcer and in the majority of cases the cause is some kind of trauma most commonly inappropriate shoes (National guidelines for diabetes care).

In addition to the genetic variation behind susceptibility for diabetic neuropathy it is also reported that incidence of amputations is higher in Mexican Americans despite a similar rate of ulceration and vascular disease compared with non-Hispanic whites (Lavery, Armstrong et al. 2003) why it is suggested to be a genetic background regarding ability to heal foot ulcerations. This view is supported by several other studies such as the North West Diabetes Foot Care Study which reported South Asians in a population based study to have substantially less problems with foot ulcerations and amputations despite having higher prevalence of all other diabetes complications compared to the general population (Abbott, Garrow et al. 2005).
The most important factors for development of foot ulceration are a combination of neuropathy, ischaemia and excessive pressure loading, often superimposed by sore infection. Hence a number of disturbances seen in diabetes coincide in the foot, metabolic, vascular, neurological and immunological to put it in danger for complications (Rathur and Boulton 2007).

Different neuropathies in the foot exert different impact on foot complications and they exist in various combinations. The order of their appearance is not known however small fibbers seems to be affected first (Malik, Tesfaye et al. 2005). Sensory Neuropathy predispose for ulceration by the loss of perception of touch and pain which increase the risk for traumatic skin lesions. Motor neuropathy causes wasting of the small muscles in the feet and is reported to be present before clinical signs of neuropathy can be detected using standard clinical protocols (Greenman, Khaodhiiar et al. 2005). Motor neuropathy results in deformities with abnormal pressure sites and callus formation that predispose for ulceration. Also the limited joint mobility in diabetes leads to further abnormal pressure on the sole of the foot however this is not a neuropathic effect. Finally, autonomic neuropathy cause deficiency in the vasosudomotor regulation and abnormal shunting of arterial blood causing warm dry skin which predispose for cracks and infections.

Ischaemia in the foot originates from both macrovascular- and microvascular disorder. Arteriosclerosis in diabetes is common and aggressive, causing stiff vessels why pulse is difficult to palpate. The autonomic dysfunction contributes with pathological shunting of arterial blood, by-passing the capillary bed and thus causing not only ischaemia but also malnutrition and dysregulation of temperature in the skin, the warm foot (1999; Boulton 2004; Dinh and Veves 2005). Further, oxygen delivery in the skin microcirculation is reduced in diabetics and this impairment is further accentuated in presence of neuropathy (Greenman, Panasyuk et al. 2005).

In conclusion

Foot ulcers are common and have a multifactor background including ethnicity, genetic factors, metabolic control, peripheral neuropathy and vascular disease. Most importantly regarding foot ulcerations, neuropathy is not only identified as a risk factor for foot ulceration but is also proposed to augment hypoxia and thus hindering healing. In the perspective of the global increase of diabetes and diabetes complications and given that there is presently no curative treatment of neuropathy it is important to develop prevailing foot examination protocols further and to validate them in prospective studies in order to identify persons at risk as early as possible. Presently most protocols include different aspects of sensory neuropathy (reflexes, vibration threshold, touch, temperature and pin-prick) but to our knowledge signs of peripheral autonomic neuropathy as potential predictor of foot ulceration is seldom tested.

**EFFECTS OF INSULIN AND INSULIN-LIKE GROWTH FACTORS.**

Glucose metabolism is regulated by a complex system including amongst other
factors the hormones insulin, glucagon, adrenalin, cortisol and growth hormone. Other factors include insulin-like growth factors.

**INSULIN**

Insulin is produced in the beta-cells of the pancreatic islets. The synthesis of insulin starts with preproinsulin which is processed to proinsulin, which is a molecule that is structurally related to insulin-like growth factor I (IGF-I) and insulin-like growth factor II (IGF-II). Proinsulin is enzymatically split into insulin and C-peptide. Both peptides are stored in secretory granules and are released when the beta-cells are stimulated by glucose or other secretagogues. When secreted also some proinsulin is cosecreted. When the beta-cells are stimulated by secretagogues (glucose being the key regulator of secretion), insulin is secreted in a biphasic manner. Insulin increase rapidly to peak after 2-4 min and then decline to nadir at 10-15 min followed by progressive increase during 2-3h. The initial spike of release is generally referred to as first-phase insulin release and the subsequent insulin release as second-phase insulin release (Gerich 2002) (Fig 1).

![Figure 1](image)

Insulin is the major regulator of the circulating level of glucose. Insulin inhibits gluconeogenesis in the liver and facilitates glucose disposal in other tissues, such as skeletal muscle and adipose tissue. Insulin secreted from pancreas must first pass through the liver via v.portae. A major part of insulin is taken up by the liver whereas C-peptide passes on to the systemic circulation. Hence the liver extraction of insulin is a major confounder when assessing insulin secretion why the level of circulating C-peptide is used as surrogate measurement for insulin release.

Insulin inhibits the hepatic production of insulin-like growth factor binding protein, IGFBP-1, which in turn regulates the amount of bioactive IGF-I. Consequently in a state of hepatic insulin deficiency or uncompensated insulin re-
sistance not only the gluconeogenesis will increase but also the production of IGFBP-1, hence IGFBP-1 signals insulin deficiency (Brismar, Fernqvist-Forbes et al. 1994; Brismar, Hilding et al. 1995).

Insulin also regulates the lipid metabolism, facilitating the storage of lipids and inhibiting lipolysis in adipose tissue.

**GH-IGF-IGFBP-AXIS (fig 2)**
The IGF system is also associated with diabetes complications though a direct connection is unproven. IGF-I and IGFBP-I are markers for cardiovascular risk and disease (Gibson, Westwood et al. 1996; Ruotolo, Bavenholm et al. 2000; Heald, Cruickshank et al. 2001; Unden, Elofsson et al. 2005) and may be involved in diabetic nephropathy, retinopathy and neuropathy (Ishii 1995; Chiarelli, Santilli et al. 2000; Bronson, Reiter et al. 2003; Wilkinson-Berka, Waight et al. 2006; Vasylyeva and Ferry 2007). However uncertainty about the importance of the differences between bioactivity on local and systemic level remains.

**Figure 2**
The GH-IGF-IGFBP axis
IGF:s, IGF-I and IGF-II, are peptides with endocrine, auto- and paracrine effects on growth and metabolism. The biosynthesis of IGF are stimulated by growth hormone (GH) but is also regulated by other hormones as insulin and nutrition, physical activity and inflammatory substances. The main source for circulating IGF is the liver but IGF is also produced in several extra-hepatic tissues (Brismar and Lewitt 2004).

The most studied of the two peptides is IGF-I which binds to both the IGF-I receptor, the insulin receptor and the hybrid receptor. IGF-I has only one-hundredth potency to bind to the insulin receptor compared to insulin and importantly, the liver and adipose tissue lack IGF-I receptors why the IGF-I effect on these tissues are small. However IGF can bind to the insulin receptor and the hybrid receptor of the IGF and insulin receptors.

IGF-II is reported to be important for prenatally growth in rodents with diminished effects after birth while IGF-I has the opposite pattern with the most important effects after birth and significantly increased during puberty. However in humans both IGF-I and IGF-II are biosynthesized throughout life and IGF-II concentration is several-fold higher than IGF-I. Some studies support the idea that the effects in humans depends on different affinity for different isoforms of the insulin receptor and that IGF-II has high affinity for IR-A, a insulin receptor that predominantly mediate proliferative effects (Adamo, Wang et al. 2004). IGF-II is also reported to be elevated in several cancer forms (LeRoith and Roberts 2003; Renehan, Zwahlen et al. 2004).

IGF stimulates glucose uptake in muscle with a potency that is approx. 5% of insulin. Most of the IGF, >99%, are bound to six binding proteins, IGFBP-1-6, that protects the IGF:s in the circulation but also regulates the bioavailability and bioactivity of IGF. Besides inhibition or enhancing the IGF action the binding proteins have IGF independent effects on cell growth and survival. The main source of IGFBP is the liver and the production is regulated by insulin and nutrition. IGFBP-3 is the main carrier of IGF in the circulation and the biosynthesis is mainly regulated by GH. The IGFBP-3 complex can not pass extravascular in contrast to the complex with IGFBP-1 or IGFBP-2. The biosynthesis of IGFBP-1 is mainly regulated by insulin but also cytokines, glucagon, stress and fasting while biosynthesis of IGFBP-2 is mainly regulated by nutrition (Brismar and Lewitt 2004).

In physiological conditions IGF is regulated by GH, insulin and nutrition. The hypoglycaemic potential of circulating IGF (Zachrisson, Dahlquist et al. 2000) is reported to be 50 times that of insulin although most of it is inhibited by IGFBP:s (Lewitt 1994). IGFBP-1 has a diurnal rhythm and is regulated by insulin and counter regulatory hormones (Baxter and Cowell 1987; Yeoh and Baxter 1988).

IGF is probably also important for maintenance of insulin sensitivity. It has been reported from animal studies that transgenic mice with inactivated IGF-I receptor in the skeletal muscle develops insulin resistance and beta-cell dysfunc-
tion at an early age (Fernandez, Kim et al. 2001). Also transgenic overexpression of IGFBP-1 in mice is reported to increase fasting blood glucose, the proposed explanation being enhanced gluconeogenesis and hepatic insulin resistance (Rajkumar, Barron et al. 1995; Rajkumar and Murphy 1999).

While poorly controlled T1D has decreased IGF and increased IGFBP-1 less is known about T2D. In T2D IGFBP-1 is reported from low to high, probably these findings reflects the heterogeneity of the disease an differences in beta-cell function. We have previously reported IGF-1 to be within normal range in a T2D population with a two-fold increase of IGFBP-1, levels of IGF-1 were lower in insulin treated and decreased with diabetes duration (Clauson, Brismar et al. 1998). Also the treatment is suggested to cause the diverse findings, sulfonylurea is known to cause increased proinsulin and low IGFBP-1 and those on multiple insulin injections have increased concentrations (Gibson, Westwood et al. 1995).

In conclusion. The GH-IGF-IGFBP axis is important for glucose regulation. IGFBP-1 is closely connected to the ambient levels of insulin (Brismar, Gutniak et al. 1988). Thus it could be conjectured that subjects with features of secondary failure (discussed below) if dependent on beta-cell dysfunction with insulin deficiency would display relatively higher levels of IGFBP-1. According to recent studies progress of deteriorated glucose control is associated with changes in IGF-I and insulin levels (fig 3) (Wallander, Brismar et al. 2006; Lewitt, Hilding et al. 2008).

Figure 3.
Schematic presentation of proposed relative changes in the IGF-IGFBP axis in relation to glucose control.

TREATMENT OF HYPERGLYCEMIA IN TYPE 2 DIABETES.
Treatment of T2D targets the deficient insulin secretion and sensitivity. Treatment aims to abolish diabetic symptoms, to reduce complications, to increase
life expectancy and to achieve as good quality of life as possible.

Clinical data and epidemiological data in humans have long supported the concept that late diabetes complication are caused by the metabolic control. However, randomized studies were needed to prove the concept. This was the reason for starting the UKPDS study in 1977 (UK Prospective Diabetes Study), including patients at diagnosis of T2D and randomising them to different intensity of treatment regarding metabolic control and hypertension.

UKPDS reported the importance of glucose control from diagnosis for the development of microangiopathy (Stratton, Kohner et al. 2001) in T2D while cardiovascular disease was only demonstrated to have a tendency to be connected to the achieved glucose control during the 10 year follow up (1998). However in a recent follow-up study UKPDS-PTM (UKPDS-post trial monitoring) subjects who received a more intense treatment in UKPDS showed favourable results 10 years later on mortality and late complications (Holman, Paul et al. 2008), hence long term beneficial effects is reported emphasising the need to strive to attain acceptable glucose control in T2D.

Lifestyle intervention is the first step in treatment of T2D and should continuously be discussed with the patient. Dietary measures, weight loss in case of over weight, increased physical activity and ending nicotine use usually improves the metabolic control.

If life style measures fail to achieve acceptable glucose control treatment with p.o drugs are usually started. Antidiabetic drugs either targets insulin resistance (insulin sensitizers) and/or stimulate the beta-cell to increase the insulin release (insulin secretagogues). If mono therapy fails, combinations of antidiabetic drugs are instituted. Some patients don’t reach acceptable glucose control by such pharmacological treatment or lose it later. When antidiabetic p.o. drugs are no longer effective secondary failure is said to occur. The cause for this secondary failure is usually a time-dependent decline in beta-cell function (see further below). In the case of secondary failure with otherwise good compliance, insulin treatment is started either in combination with p.o. antidiabetics or alone.

SECONDARY FAILURE

The beta-cell possesses the ability to adapt to increased demands by increasing beta-cell mass and function. Whereas in the early phase of diabetes the beta-cell can compensate for the increased demands with time the beta-cell decompensates with impaired insulin secretion, decreasing insulin gene expression and ultimately apoptosis. Thus, on case of so called secondary failure the beta-cells fail to release sufficient amounts of insulin to uphold glucose control despite maximum p.o. antidiabetic treatment.

Secondary failure may, in part, be secondary to the diabetic state per se. Chronic hyperglycaemia thus attenuates insulin secretion and lowers sensitivity to insulin (Rossetti, Giaccari et al. 1990; Leahy, Bonner-Weir et al. 1992; Yki-Jarvinen 1992; Robertson, Olson et al. 1994).” Glucotoxicity” is term frequently encoun-
In conclusion
The question arises whether beneficial effects by correction of hyperglycemia are related or not to the duration of “beta-cell rest”. If beneficial effects were to increase with duration, then a prolonged period of intensive insulin treatment could be recommended to precede other less demanding treatments. As mentioned above, it has been reported that intensive insulin treatment for 2-3 months can produce lasting results in recently diagnosed T2D (Ilkova, Glaser et al. 1997). However, the possible advantage of a long vs. short period of intensive insulin treatment for improving insulin secretion had, to our knowledge, not been specifically tested at the time for study III, nor if the same beneficial effects were to be seen in subjects with features of secondary failure.

INSULIN RESISTANCE AND WEIGHT
Insulin resistance is associated with abnormal beta-cell function (Grill, Dinesen et al. 2002) and obesity (Ferrannini and Camasta 1998) and worse outcome in antidiabetic treatment (Yki-Jarvinen, Ryysy et al. 1997) while loss of weight has favourable effects on these conditions. In addition to lifestyle and pharmacologic influences, hormonal factors play a role in the mechanisms determining weight gain in T2D (Ravussin and Gautier 1999). Little is known about the role of IGF in relation to regulating adipose tissue mass in T2D. Insulin resistance results in a marked decrease of IGFBP-1 second to increased insulin secretion thus obesity with hyperinsulinemia in humans is associated with low levels of IGFBP-1. Recent studies have suggested that IGF-II may be associated with lipid metabolism and body weight regulation. In transgenic models of IGF-II overexpression, elevated levels of circulating IGF-II are associated with reduced fat mass and greater

tered in the literature. It should be distinguished from secondary effects of long-standing hyperglycemia that are coupled to excessive stimulation of beta cells. “Beta cell exhaustion” leads to beta-cell dysfunction, including defective protein synthesis and possibly apoptosis (Grill and Bjorklund 2008). One of the proposed ways whereby chronic hyperglycemia leads to beta-cell dysfunction is through a linkage with the formation of reactive oxygen species (ROS), beta-cells have very low levels of antioxidant enzymes which can suggest that they are vulnerable for ROS (Grill and Bjorklund 2008; Poitout and Robertson 2008) while IGFs has been proposed to be protective for the beta-cell (Mabley, Belin et al. 1997). Also elevated levels of circulating free fatty acids may diminish insulin secretion (Zhou and Grill 1995; Grill and Bjorklund 2000). In line with negative influences of poor metabolic control it has been found that correction of hyperglycaemia improves insulin secretion in T2D (Kosaka, Kuzuya et al. 1980; Garvey, Olefsky et al. 1985; Glaser, Leibovich et al. 1988) and sometimes can postpone the need for antidiabetic agents in newly diagnosed T2D (Ilkova, Glaser et al. 1997). The cause of these beneficial effects has tentatively been ascribed to lesser demand on beta cell function, i.e.”beta-cell rest”. These beneficial effects may be reinforced by the improvement also of insulin sensitivity that follows reduction of hyperglycemia (Yki-Jarvinen 1992).
rates of lipid oxidation (Da Costa, Williamson et al. 1994; Rogler, Yang et al. 1994; Rossetti, Barzilai et al. 1996). Similarly, observational and experimental investigations in humans have shown that a number of allelic variants within the IGF-II gene influence body weight and body mass index (BMI) (O’Dell, Miller et al. 1997; Gaunt, Cooper et al. 2001; Gu, O’Dell et al. 2002). In an overfeeding study, the apa I polymorphism in the IGF-II gene also has been associated with increased adiposity and related metabolic changes, including insulin resistance (Ukkola, Sun et al. 2001). Taken together, these data indicate that IGF-II may influence body weight regulation and that individuals with low IGF-II levels may be more susceptible to weight gain and obesity.

IN SUMMARY

STUDY I-II
Data on peripheral neuropathy varies widely depending on definitions, methods and populations and there are few reports from representative T2D populations. Further, signs of autonomic peripheral neuropathy which are recognized as risk factors for foot ulceration (Ward 1982; Boulton, Kirsnser et al. 2004; Tesfaye 2006) are easily overlooked and is yet not assessed and reported in a representative population.

Peripheral sensory neuropathy (PSN) is a well known complication of diabetes attributed to chronic hyperglycemia (Boulton, Malik et al. 2004; Bloomgarden 2005). However the risk of PSN is also increased by advancing age and affected by height and possibly gender (Wiles, Pearce et al. 1991) and poorly defined factors, such as processes coupled to regulation of IGF-I (Chiarelli, Santilli et al. 2000; Brismar and Lewitt 2004). This makes it difficult to distinguish a specific diabetic component of the neuropathy. Retinopathy on the other hand is a specific complication of diabetes with a strict coupling to metabolic control and is also more easily investigated (Bloomgarden 2005). It could be conjectured that metabolic control would be a strong determinant of PSN in subjects with both retinopathy and neuropathy but less so in diabetic subjects with neuropathy alone. It follows that other risk factors for neuropathy would be important in the last mentioned patients. However, this concept has, to our knowledge not been fully investigated in a representative population of T2D.

Therefore we invited patients within a defined geographically area, with T2D and 40-70 years of age with the intention to follow these patients prospectively every 5th year regarding progressive features of T2D and late complications.

STUDY III
It has been reported that intense insulin treatment, “ beta-cell rest”, can postpone need for antidiabetic treatment and temporarily maintain beta-cell function in recently diagnosed T2D (Ilkova, Glaser et al. 1997).

It would be of interest to study if the same effects were to be obtain by ” beta-cell rest” in subjects with T2D and features of secondary failure and if possible
beneficial effects were to increase with duration. We therefore monitored the effects of intense insulin treatment on beta-cell function and glucose control during 9 weeks of treatment whereas another group received less intense treatment to serve as a control group.

**STUDY IV**

T2D is associated with obesity and with a predisposition to weight gain, which in many cases leads to deterioration in metabolic control and progression of diabetes complications. In addition to lifestyle and pharmacologic influences, hormonal factors play a role in the mechanisms determining weight gain in T2D (Ravussin and Gautier 1999) and a connection between IGF-II and lipid metabolism is reported.

We therefore assessed in individuals with T2D, who by the nature of their condition and its treatment are predisposed to increased adiposity, the association between circulating concentrations of IGF-II and subsequent weight gain.

**AIMS**

The aims of this thesis were to study;
- Complications in a representative population with type 2 diabetes (T2D), with focus on peripheral neuropathy (PN).
- Associations between peripheral sensory neuropathy (PSN) and different aspects of metabolism in a T2D population.
- Features of late complications and metabolism in subjects with PSN in relation to coexistence or not of retinopathy.
- Effects of the length of intensive insulin treatment on beta-cell function in subjects with T2D and features of secondary failure.
- The IGF-axis in relation to complications, metabolism and weight in a T2D population

**MATERIALS AND METHODS**

**SUBJECTS**

*Study I-II*

Subjects from the geographically defined population of Sundbyberg (a suburb of Stockholm) were asked to participate. Men and women, 40-70 years of age, with T2D diagnosed after the age of 35 were included. The upper age limit was decided upon in order to form a population that could be prospectively followed.

Of eligible residents in Sundbyberg, 89% were served by the three primary health care centres Kronan (42.7%), Rissne (26.3%) and Hallonbergen (19.9%), according to statistics from the health care authorities. The primary care centres register all patients in computerized medical records which were searched for eligible patients.
Eligible subjects were sent written information about the study and a form for reply. Subjects who did not reply were contacted by phone. Medical records for non-participating subjects, subjects refusing to participate and subjects that were not invited to participate, with known T2D were reviewed regarding age at diagnosis of diabetes, antidiabetic treatment, fasting blood glucose and HbA1c. Further details on medical histories were obtained from medical records at the primary health care centre Kronan, the largest of the three centres from which patients were recruited.

Study III
Patients with a clinical diagnosis of diabetes type 2 and secondary drug failure were eligible for participation. Other inclusion criteria were diagnosis of T2D from 1 to 11 years before the study, age 40–80 years, body mass index (BMI) 25-35 kg/m2 and HbA1c >7%. Exclusion criteria were alcoholism, severe asthma bronchiale, heart failure NYHA ≥ III, severe liver/kidney disease, cancer, chronic infection or inflammation, or inability to speak Swedish.

Study IV
A total of 224 subjects, who had been enrolled from 2 diabetes centres in Manchester, UK, and from 3 diabetes centres in Sundbyberg, Stockholm, at baseline, attended for follow-up 5 years later. The mean age of the participants at follow-up was 59.8 years (95% confidence interval [CI] 58.9-60.7). The subjects were matched for duration of diabetes: mean 8.1 years (95% CI, 7.9-8.3 years).

All subjects in study I-IV gave informed consent, and the protocols were approved by local ethics committees.

EXAMINATION PROTOCOLS

Foot examination (fig 4).

Foot examination (n=150) followed a standard protocol designed to assess the risk of future foot problems (Boulton, Gries et al. 1998; 1999; Apelqvist, Bakker et al. 2000). From this examination the following sub-classifications of neuropathy were made.

Peripheral autonomic neuropathy (PAN) was estimated arbitrary as a score, 0-4, one point given for the presence of each of the following four findings: dry skin, cracks, warm skin (Ward 1982; Boulton, Kirsner et al. 2004) and loss of hair in both feet. Loss of hair was not counted as PAN if palpable pulses were absent or the patient had known peripheral vascular disease (PVD). A score of 2 or more was considered as sign of PAN. Oedema was not included in the score since it is difficult to evaluate the significance as a neuropathic sign although autonomic neuropathy can sometimes lead to neuropathic oedema (Tefsaye 2006).

Peripheral motor neuropathy (PMN) was considered present if callus formation, muscle atrophy, planus feet or deformities were recorded symmetrically.
lus formation was considered as sign of motor dysfunction if present at pressure sites, i.e. plantar aspects of dig II-IV and metatarsophalangeal joint II-IV (Boulton, Kirsner et al. 2004; Tesfaye 2006).

Peripheral sensory neuropathy (PSN) was tested with regard to vibration and touch (Boulton, Malik et al. 2004). Vibration sensation was tested with a tuning fork (128 Hz) at the medial malleolus and the metatarsophalangeal joint of the big toe. The patient was asked to discriminate between vibration and pressure. More than three mistakes out of five were judged as pathological.

Vibration Perception Threshold (VPT) was assessed at the metatarsophalangeal joint dig I using a neurothesiometer (Horwell, NEU1501, U.K.) in a two step manner, first starting from 50V with decreasing stimulation and then starting from 0V with increasing stimulation. The subject stated when he/she lost or began to feel vibration. The mean of the two measurements in the most insensitive foot was used in further analyses.

VPT was also transformed to a Z score (ZscoreVPT) which adjusts for age, height and gender (Wiles, Pearce et al. 1991). Cut off limit for pathological VPT was a calculated ZscoreVPT above 2.0SD. When results differed between examination with tuning fork and neurothesiometer the assessed VPT was given priority to assess diminished vibration perception.

Sensibility to touch was tested using monofilament (10 gram Touch Test 5.07 Novo Nordisk, Copenhagen, Denmark) at four points on each foot, three on the plantar and one on the dorsal side. The procedure was repeated once and three mistakes out of four were considered pathological (Kumar, Fernando et al. 1991; Kamei, Yamane et al. 2005).

Peripheral neuropathy was diagnosed when the patient tested positive for at least one of the types of neuropathy recorded (PAN, PMN, PSN). Polyneuropathy was diagnosed when at least two of these three neurological sensorimotor modalities were present.

Foot pulses (aa dorsalis pedis and tibialis posterior) were palpated. Both pulses were required to be palpable for a normal macrocirculation.

The feet were finally classified as being of low, moderate or high risk of foot ulceration according to international consensus (Boulton, Gries et al. 1998; 1999; Apelqvist, Bakker et al. 2000). In short, low risk was considered present when there were no signs of neurological or vascular disorder. Subjects assigned to the middle risk group had neurological abnormalities or stiff joints. The high risk group had neurological and vascular abnormalities, a history of foot ulceration for ≥2 months and/or a history of amputation on the basis of a foot ulcer.
Figure 4.
Foot examination protocol used in study I-II, except that assessment of ankle pressure was not assessed.

FOOT EXAMINATION PROTOCOL®

<table>
<thead>
<tr>
<th>Date:</th>
<th>Sign:</th>
<th>MARKERS OF RISK TO DEVELOP FOOT PROBLEMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM type: Onset:</td>
<td>NEUROPATHY</td>
<td>ANGIOPATHY</td>
</tr>
<tr>
<td>Treatment:</td>
<td>Dry skin</td>
<td>Palpable foot pulses</td>
</tr>
<tr>
<td>Smoker: present Y/N</td>
<td>Hair missing</td>
<td>Blood pressure (mmHg)</td>
</tr>
<tr>
<td>previous Y/N</td>
<td>Oedema</td>
<td>Ankle pressure (mmHg)</td>
</tr>
<tr>
<td>Impaired vision: Y/N</td>
<td>Warm skin</td>
<td>Pigmentation</td>
</tr>
<tr>
<td>Right Left</td>
<td>Reduced sensibility (Monofilament 10g)</td>
<td>Rest pain</td>
</tr>
<tr>
<td></td>
<td>Reduced vibration (tuning fork 128Hz)</td>
<td>Walking pain (Claudicatio interna)</td>
</tr>
<tr>
<td></td>
<td>Reduced big toe extension</td>
<td>Discoloured skin blue/red</td>
</tr>
<tr>
<td></td>
<td>Deformity</td>
<td>Cold skin</td>
</tr>
<tr>
<td></td>
<td>Biothesiometer max (V)</td>
<td>Hba1c %</td>
</tr>
<tr>
<td></td>
<td>min</td>
<td>Reference (&lt; )</td>
</tr>
</tbody>
</table>

If above parameters are present write Y=Yes, N=No (R=Right, L=Left)

Risk classification

| Group 1 | ØØ other ......................... |
| Group 2 | ......................................... |
| Group 3 | ......................................... |

GROUP 1: (Low risk) No registered foot complication
GROUP 2: (Middle risk) Registered signs of peripheral neuropathy: dry skin, reduced hair on the foot, warm skin, reduced sensibility, callus (hyperkeratosis) and often foot deformities sometimes stiff joints
GROUP 3: (High risk) Previous and present foot ulcer and/or a combination of neuropathy and micro/ macroangiopathy.

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Test procedure

The glucose-glucagon tests were performed in the over-night fasted state. Patients did not take their morning medication and did not inject insulin in the evening that preceded the test. The tests were performed with the each patient resting in a bed. An indwelling cannula was inserted into each forearm. One cannula was used for injections and the other for blood sampling. After the fasting samples had been drawn, an intravenous bolus injection of 20% glucose, 300 mg/kg body weight was given during 2 min. Following the injection, samples were drawn at 1, 3, 5, 10 and 20 min, for blood glucose and hormone assays. After the 20-min sample, the patients received 1 mg of glucagon intravenously and samples were drawn 6 minutes later (Faber and Binder 1977; Ahren, Nobin et al. 1987; Snorgaard, Hasselstrom et al. 1988). The glucose-glucagon tests were performed at base-line (day 1), after three days of treatment (day 4), at the end of MI treatment (day 67) and, finally, three days after the change of MI treatment to BTI and glibenclamide (day 70). The test procedures were identical for the group which received BTI and per oral medication already from the start of the study.

The combined glucose-glucagon test is a procedure that differs from the original test (Faber, Madsbad et al. 1979) but has been used in previous studies (Gottsater, Landin-Olsson et al. 1992; Mold, Vesely et al. 2004). The obvious advantage of the combined test is that measures of insulin response to more than one secretagogue are obtained.

Glucagon stimulation test has within-subject CV 15% and is reliable marker for beta cell function however ambient hypoglycemia can interfere with reliability (Ahren, Nobin et al. 1987; Gjessing, Damsgaard et al. 1987; Madsbad, Sauerbrey et al. 1987; Snorgaard, Hasselstrom et al. 1988).

All samples were kept on ice until further processing. Samples for blood glucose were assayed immediately after the completion of each test. Samples for hormones were secured in test tubes to which had been added Trasylol (0,1 ml/ml blood) (Shepotinovskaia, Umanskaia et al. 1994). After centrifugation the plasma and sera were kept frozen at -70°C until assay.

Definitions

Cardiovascular disease (CVD) in study III-IV was defined as a history of myocardial infarction, angina pectoris or ischemic heart disease, drugs prescribed for cardiovascular disease or a pathological electrocardiogram (Minnesota code).

A cerebrovascular lesion (CVL) was considered present if diagnosed according to medical records or demonstrated on a CT of the brain.

Peripheral vascular disease (PVD) was defined as clinical macroangiopathy (no palpable pulse) present at foot examination and/or a medical history of intermittent claudication.

Assessment of retinopathy.

Records were available for most of the study patients, retinal photography (n 111) and ophthalmoscopy (n 21). The photographic records were reviewed and the se-
verity of retinopathy assessed by an experienced retina ophthalmologist (GvW). Subjects assessed from medical history alone were excluded from further interpretation because of poor reliability.

Assessment of retinopathy was based on the most afflicted eye or on one eye only when the photographs of only one eye were assessable or available.

Retinopathy was classified using an international DRP classification (15) using five categories: no retinopathy, mild non-proliferative retinopathy, moderate non-proliferative retinopathy, severe non-proliferative retinopathy, and proliferative retinopathy.

Overt nephropathy was defined as albuminuria ≥ 300 mg/L or a serum creatinine in women > 100 and men > 110 µmol/L. Incipient nephropathy was defined as albuminuria ≥ 30 mg/L – < 300 mg/L.

Hypertension was defined as ≥140/≥90 mmHg at examination or by the use of antihypertensive medication.

Hyperlipidemia was considered present when lipid lowering drugs were used or when total cholesterol was ≥ 5 mmol/L or triglycerides ≥ 1.7 mmol/L.

Late autoimmune diabetes of the adult (LADA) was defined as presence of GAD antibody ≥ 9.5 U/L (Pozzilli and Di Mario 2001; Borg, Gottsater et al. 2002).

ASSAYS
Fasting plasma glucose in study I-II (ref 4.0–6.0 mmol/L) was analysed at the hospital laboratory with a glucose oxidase peroxidase method, VITROS instrument (Johnson&Johnson, New Jersey, USA).

Blood glucose in study III was analysed with a glucose oxidase method (YSI 2300 Stat Plus, Yellow Springs Ohio, USA).

Fasting plasma glucose, English part study IV, was assayed using the hexokinase method (Cobas Integra autoanalyzer; Roche, Indianapolis, Ind).

C-peptide was assayed by RIA (Euro-diagnostica, Malmö Sweden). According to the manufacturer, the lowest detectable concentration was 0.05 nmol/l, the intra assay variation 5% and the total variation (sum of intra- and inter assay variation) 7%. Cross-reactivity with proinsulin was 41%.

Insulin C-peptide in study IV was measured using the DAKO (Ely, UK) enzyme-linked immunosorbent assay for intact insulin C-peptide. Cross-reactivity of insulin C-peptide assay for insulin is less than 0.1%.

Glucagon was determined by RIA (Euro-diagnostica, Malmö, Sweden). The lowest detectable concentration was 3 pmol/l, intra assay variation <4.8% and total variation <8.9%.

Immunoreactive insulin was determined by RIA using antibodies against porcine
insulin (raised in guinea pigs at the Endocrine laboratory, Karolinska hospital) and 125I labelled porcine insulin as a tracer. Inter assay variation was 9.7%, intra assay variation less than 5.1% and cross-reactivity with proinsulin approximately 100%.

Proinsulin was determined by ELISA, measuring intact proinsulin (Dako A/S, Copenhagen Denmark). According to the manufacturer the detection limit was <0.2 pmol/l, intra assay variation <5.7%, inter assay variation <6% and cross reactivity for both insulin and C-peptide <0.1%.

Insulin-like growth factor-I (IGF-I) (mg/L) was determined by radioimmuno-assay after separation from IGF-binding proteins (IGFBP:s) by acid-ethanol extraction and cryoprecipitation. To minimize interference of the remaining IGFBP:s, des (1-3) IGF-I was used as the radioligand. The intra- and interassay CV were 4% and 11% respectively.

IGF-II levels were determined by previously reported antibody-based assays (Crosby, Anderton et al. 1993; Gill, Whatmore et al. 1997). The assays have respective detection limits of 28 ng/mL and 30 ng/mL, and within- and between-assay coefficients of variation of less than 10%.

Samples in study IV were transported from Stockholm, Sweden, to Manchester, UK, by air, using dry ice (Dry shippers, BDHMerck, West Drayton, UK). Subsequent storage was at -70°C. All assays in the English samples were performed at Hope Hospital, Salford, UK.

IGFBP-1 (mg/L) was determined by RIA according to the method of Povo et al (Povo, Roovete et al. 1984). The sensitivity of the RIA was 3µg/L and the intra- and interassay CV were 3 and 10%, respectively.

IGFBP-3 (mg/L) was determined with a solid-phase, enzyme-labeled chemiluminescent immunometric assay, IMMULITE 2000 IGFB-3 (DPC, Los Ageles, USA). According to the manufactures the assay is highly specific with low cross-reactivity and analytical sensitivity is 100µg/L. Intra- and total-assay CV 4.2% and 7.2%, respectively.

Highsensitive CRP (hsCRP) was determined with immunonephelometri, N Highsensitive-CRP, OQIY (Dade Behring, Germany). Expected normal value is <2mg/L.

Lipoproteins were processed with 12h preparative ultracentrifugation after which the VLDL fraction was separated from an aliquot of serum by centrifugation. After quantitatively precipitation of LDL from the infranatant, remaining HDL in the solution was separated and analysed. Cholesterol and triglycerides were determined after extraction of whole serum, the VLDL fraction, the infranatant (containing LDL and HDL) and the supernatant (HDL) after precipitation (Tornvall, Olivecrona et al. 1995). LDL was calculated according to Friedewalds formula (Friedewald, Levy et al. 1972).
Cystatin C was determined with N Latex Cystatin C, OQNM (Dade Behring, Germany). The normal interval is 0.53-0.95 mg/L.

Hemoglobin A1c (ref. <5.2%) was determined with immunologic MonoS method, Unimate (Roche Diagnostics, Basel, Schweiz). To convert HbA1c MonoS into HbA1c NGSP (National Glycoprotein Standardization Programme) the formula, NGSP=0.92*MonoS+1.33, can be used.

S-Creatinine (ref for women >100 and men >110 µmol/L) were analyzed at the hospital laboratory with a Creatinine aminohydrolas method, VITROS instrument (Johnson&Johnson, New Jersey, USA)

Urinary albumin (ref <30 mg/L) were analyzed at the hospital laboratory with an immunological method, Beckman Array (Beckman Coulter Inc., Fullerton, USA)

GAD antibody titers were determined with Diamyd Anti-GAD65 RIA (Diamyd Diagnostics AB, Stockholm, Sweden) (Marcovina, Landin-Olsson et al. 2000).

STATISTICS

I
All results are expressed as mean and standard deviation (SD), unless otherwise stated. Parameters with non-normal distribution were log-normalised for significance testing. The Man-Whitney U test was used when log-normalization was not possible. Levels of significance were tested with Fisher’s exact two-tailed test for simple frequencies and Pearson Chi Square for multiple frequencies. One-way ANOVA was used for parametric variables classified in two groups or more. A p value <0.05 was considered significant.

II
All results are expressed as mean and standard deviation (SD), unless otherwise stated. Parameters with non-normal distribution were transformed and log-normalised values were used for significance testing. If not acceptable as log-normalised, the Mann Whitney U-test or Kruskal Wallis ANOVA was used. Levels of significance were tested with Fisher’s exact two-tailed test for simple frequency when n<10, otherwise Pearson Chi-Square was used. T-test and One-way ANOVA was used for parametric variables classified in two groups or more. Logistic regressions were performed with identified independent variables, and factors previous reported of significance, to study independent associations.

III
All results are expressed as mean and 95% confidence interval (CI), unless otherwise stated. Levels of significance were tested with Friedman ANOVA Chi Square and Kruskal Wallis ANOVA in cases of repeated measurements. Wilcoxon matched pairs test and Mann-Whitney U test were used in cases of paired tests. Correlations were calculated by Pearson product-moment.
Differences in IGF-II between groups were assessed using analysis of covariance. Non-normally distributed data were logarithmically transformed before analysis. Spearman correlations were calculated to investigate the relation among IGF-II concentrations, baseline weight, and weight gain. In multivariate linear regression analysis, the standardized beta (β) coefficients presented allow direct comparison (along a scale of 0-1) of the strength of each association. Univariate logistic regression was used to assess the risk of future weight gain in relation to changes in baseline IGF-II concentration.

In study I, III-IV statistical analysis was performed using the software program Statistica, StatSoft, Inc. In study II statistical package Intercooled STATA version 8.2 (College Station, Tex) was used.

RESULTS WITH COMMENTS

STUDY I
The identified prevalence of known diabetes in the geographically and age limited population that we studied was 3.5%, T2D affecting 90%. Of the included subjects 10% were redefined as LADA. LADA subjects were excluded according to exclusion criteria to achieve a T2D population as absolute as possible. The participation rate of the intended study population was 68%.

Diabetic complications were common even though the population was rather young with a moderate diabetes duration and fairly good glucose control. The prevalence of macrovascular complications was for CVD 62%, PVD 26% and for CVL 11%.

Microvascular complications were nephropathy 22% (incipient nephropathy 14% and overt nephropathy 8%) and retinopathy 29% (mild retinopathy 11%, moderate 13%, severe 2% and proliferative retinopathy 2%). Peripheral neuropathy affected 67% as follows; ≥2 signs of peripheral autonomic neuropathy 43%, signs of peripheral motor neuropathy 15% and peripheral sensory neuropathy 33%. Signs of peripheral autonomic neuropathy were: dry skin 50%, warm skin 45%, hairless without PVD 32% and cracks 9%.

Sensory loss according to monofilament affected 15% and abnormal vibration sense as judged by tuning fork 24%. A pathological VPT as judged by Zscore≥2.0SD affected 28% while VPT≥25V was assessed in 30% of the subjects. Peripheral polyneuropathy affected 25% when defined as at least two demonstrated neuropathies (PAN/PMN/PSN).

There were no differences regarding prevalence of complications between genders except men having more nephropathy and also, as expected, a higher VPT.

Additional data and comments:
Subjects with LADA were comparable in age, diabetes duration and BMI but me-
Bolistic control as judged by HbA1c was worse and fC-peptide, proinsulin and insulin levels lower. No differences were observed regarding prevalence in micro- and macro-vascular complications between study T2D population and subjects with LADA.

Besides the reported comparison between participants and non-participants in paper I we also controlled the groups regarding findings at foot examination. Of non-participants with T2D, 59% had their feet examined at the primary health care centre by experienced chiropodists using the same foot protocol, i.e. 77% of the entire identified T2D population had their feet assessed. Prevalence of feet classified at low risk were 48% vs. 37%, middle risk 40% vs. 54% and high risk 12% vs. 9% in non-participants vs. participants respectively, p=0.1. Hence no difference was observed between participants and non-participants at foot examination.

A history of foot ulceration was correlated with dry skin, p=0.005, and cracks, p=0.03, but not with warm or hairless feet. At follow-up 5 years later 121 subjects were re-examined following the same protocol as in baseline study. Of subjects that didn't participate at follow-up approximately 1/3 were deceased, 1/3 had moved out of the area and 1/3 refused participation. Eight new foot ulcerations had occurred in the reinvestigated study population, dry skin was correlated with new ulceration, p=0.02 while the arbitrary determined score at baseline for possible PAN, ≥2 signs of peripheral autonomic neuropathy, was borderline significant for new ulceration, p=0.07.

**STUDY II**

Thirty-four percent in the study population were found to have peripheral sensory neuropathy (PSN) defined as VPT≥25V and/or inability to feel the monofilament. Subjects with PSN had more macrovascular disease, both prevalence of PVD and pathological ECG was more common in subjects with PSN. Men with PSN also had more elevated systolic blood pressure. PSN was connected to other microvascular complications.

Subjects with PSN were older, had longer diabetes duration, lower HDLc and higher IGFBP-1 with lower IGF-I/IGFBP-1 ratio but with no significant difference in glucose control as judged by HbA1c compared to those without.

Retinopathy was connected to longer diabetes duration, worse glucose control as judged by HbA1c but not to age. Retinopathy did not associate with macrovascular complications but with both nephropathy and peripheral neuropathy.

In subjects without retinopathy we found PSN to be connected to gender (male), increasing age, worse kidney function and more vascular disease with a threefolded increase of pathological ECG and PVD. Further they displayed higher IGFBP-1.

Compared to subjects with both PSN and retinopathy, those with only PSN had some shorter diabetes duration with better glucose control and better preserved sensibility as assessed with having a lower VPT but with twice as much PVD.
Finally, in multivariate analysis the risk for peripheral sensory neuropathy was independently associated with gender (male) OR 2.01, increasing age OR 1.12, PVD OR 2.31, increased IGFBP-1 OR 1.03 and a risk reduction with increasing HDLc OR 0.21.

Retinopathy was independently associated with increasing diabetes duration OR 1.10, glucose control as judged by HbA1c OR 1.38 and overt nephropathy OR 2.04.

Additional data and comments:
There was no significant difference in HbA1c between subjects with and without PSN (6.6 ±1.4 vs. 6.2 ± 1.2 (%), p=0.07, PSN vs. no PSN), however women with PSN had higher HOMA (12.3 ± 5.9 vs. 9.5 ± 6.5, p<0.05, PSN vs. no PSN) and were consequently supposedly more insulin resistant.

Systolic blood pressure was comparable between subjects with vs. without retinopathy (152 ± 20 vs. 146 ± 18 mmHg, p=0.1) but in men with retinopathy systolic blood pressure tended to be significantly elevated (154 ± 18 vs. 144 ± 20 mmHg, p=0.06) and with higher pulse pressure (69 ± 15 vs. 60 ± 16 mmHg, p<0.05), retinopathy vs. no retinopathy respectively.

In contrast no such connection was seen amongst the women but women with retinopathy had higher resting pulse than those without (74 ± 12 vs. 66 ± 9 beats/min, p=0.02).

In a multivariate model for men only, HbA1c was significantly associated with PSN, OR 1.84 (1.16-2.92, 95% conf-limit), p=0.01, but inclusion of diabetes duration in the model erased this effect. Including diabetes duration instead of HbA1c in the risk model for the men resulted in OR 1.15 (1.02-1.29, 95% conf. limit), p=0.02, for PSN. HOMA was tested in a model for women but turned out insignificant.

Inclusion of pulse pressure in the multivariate model for retinopathy in men resulted in OR 1.04 (1.00 -1.08, 95% conf.limit) p=0.05, while overt nephropathy lost significance. Including resting pulse in the model for women resulted in OR 1.11 (1.00-1.23, 95%, conf.limit), p=0.04, overt nephropathy being too few to test in this model as category why CystatinC was used as surrogate variable, however without significance.

STUDY III
In study III we tested the effects of beta-cell rest in subjects with T2D and features of secondary failure, i.e. intensive insulin treatment in order to reduce stimulation of insulin secretion during a defined period. We compared effects of multiple insulin injections, i.e. insulin at mealtime, three-times daily and another insulin dose at bedtime (=MI), with a group that continued p.o antidiabetic agents but with the addition of bedtime insulin (=BTI).

Glucose control improved in both treatment arms during the first 9 weeks as judged by decreased HbA1c. MI treatment tended to improve the glucose control somewhat more but switching MI to BTI for another 8 weeks abolished this difference.
MI decreased the fasting levels of C-peptide and also the proinsulin/insulin ratio significantly but the effect was completely lost after 3 days on BTI and at that time point hormone levels were equal between the previous MI group and the continuous BTI group.

Neither of the treatments resulted in improvement of first phase insulin secretion. MI resulted in improved glucagon stimulated C-peptide level already on day 4 but the response to glucagon did not improve further. Hence no difference was observed on day 67 vs. day 4.

Baseline C-peptide was strongly correlated to the improvement in insulin secretion in both treatments. An undesired weight gain was observed in the MI treatment compared with BTI treated subjects. Notably, this effect persisted even after switching MI to BTI.

**Additional data and comments;**

IGFBP-1 at baseline in the intense insulin treated group was not correlated to age, diabetes duration, BMI, HbA1c or fC-peptide. Correlation between baseline IGFBP-1 and improvement of HbA1c for the intense insulin treated group was \( r = 0.58, p = 0.08 \) (fig 5). The aggregated results of fasting IGFBP-1 for both treatment groups was significantly correlated, \( r = 0.46, p < 0.05 \), and adjusted for BMI and fC-peptide at baseline, \( r = 0.49, p < 0.05 \). This was in contrast to fC-peptide at baseline that did not correlate with improvement of HbA1c.

**Figure 5.** Baseline level of fasting IGFBP-1 vs. \( \Delta \text{HbA1c} \) after 9 weeks of intense insulin treatment.


**STUDY IV**

Association between the IGF-IGFBP axis and weight change in T2D were investigated. We found subjects with low IGF-II and normal weight, defined as body mass index <26.0kg/m2, at entrance of study to increase ≥2.0 kg during the 5 year follow up. It was a strong inverse correlation between IGF-II and weight gain in the low IGF-II levels (Spearman rho -0.52, p<0.001), the effect was unrelated to received anti-diabetic treatment. With increasing weight the relationship no longer prevailed.

There was no difference in baseline IGF-II by treatment group and no difference between the group with weight gain and the group with stable weight in those who additionally received insulin or sulfonylurea treatment in the 5 years between the baseline visit and the follow-up.

**GENERAL DISCUSSION**

In part of this thesis we report from a representative population with T2D, study I-II, that signs of peripheral autonomic neuropathy were common and not only to correlate with a medical history of foot ulceration but also with future foot ulcerations at 5 year follow-up. Peripheral sensory neuropathy was independently connected to HDLc and PVD in addition to well known connections to age and gender. PVD was most common in conjunction with peripheral sensory neuropathy in subjects without retinopathy why we propose that peripheral sensory neuropathy, especially when without retinopathy should lead to the consideration of possible PVD. We also found neuropathy to be independently associated with high IGFBP-1 which previously is reported in T1D but to our knowledge not in T2D.

Intensive insulin treatment was observed in study III to result in a rapid improvement of glucose control and beta-cell function in secondary failure however no further improvement was observed after extension of treatment for 9 weeks. The effect was rapidly lost on cessation and resulted in a weight gain that sustained and can be detrimental for achieving future glucose homeostasis. Lastly we report IGF-II to predict weight gain in normal weighted subjects with T2D independently of treatment, study IV.

We believe the study population in studies I-II to be representative and also that we have acceptable knowledge of non-participants, those that refused participation as well as those who were not invited, since information on non-participants were gathered through review of medical records. Further, several aspects of our findings is in line with what other studies have found which attest the study population to be representative (manuscript).

Two important aspects in the interpretation of data need to be commented. First, non-participants had higher prevalence of cardiovascular disease and also hypertension and were supposedly more affected by diabetes complications. It is not uncommon in population studies that subjects that are most afflicted by disease tend not to participate. However risk classification of the feet were comparable between participants and non-participants and there was no difference in prevalence regarding high foot risk which is the most easily recognized risk
level. Second, it should be pointed out that the criteria for diagnosing diabetes were adjusted in 1999 (2008). In our study 2 patients are diagnosed according to the new criteria.

The observed prevalence of diabetes, 3.5%, and the distribution between T1D, T2D and LADA that we observed was in line with other Nordic studies (Midtjell, Bjorndal et al. 1995; Berger, Stenstrom et al. 1998; Lundman and Engstrom 1998; Midtjell, Kruger et al. 1999) Our findings of prevalence of macrovascular complications (CVD 62%, PVD 26% and for CVL 11%) and microvascular complications (incipient nephropathy 14%, overt nephropathy 8%, retinopathy 29% and peripheral sensory neuropathy 33%) are in line with a previous report from Sweden (Lundman and Engstrom 1998). The prevalence of retinopathy that we find is higher but in line with two other Nordic studies (Falkenberg and Finnstrom 1994; Hove, Kristensen et al. 2004).

Prevalence data on peripheral sensory neuropathy (PSN) in diabetes are vastly different between reports depending on the selection of populations, definition of neuropathies and methods used. We used a standardized protocol for clinical foot examination which has been in use at the Karolinska University Hospital since 1993 and that is based on international consensus formed by the International Foot Group (fig 4) (Boulton, Gries et al. 1998; Apelqvist, Bakker et al. 2000). The primary goal of the protocol is to identify persons at risk for foot complications by assessing the existence of distal neuropathy and/or peripheral vascular disease and/or risk factors in the medical history, which is also recorded in the protocol. The protocol is adjusted to be easy to use also in the primary care and assessing distal sensory function with neurothesiometer and monofilament are reliable methods besides being the most widely used (Bloom, Till et al. 1984; Kumar, Fernando et al. 1991; van Deursen, Sanchez et al. 2001; Kamei, Yamane et al. 2005; Miranda-Palma, Sosenko et al. 2005). The combination of two sensory tests is likely to catch 87% of the neuropathic subjects (Boulton, Winik et al. 2005).

Some other protocols include also reflexes and pin-prick tests. In our protocol we excluded reflexes because they are difficult to interpret even for a trained examiner. We excluded the pin-prick test to avoid potential harmful lesions in the skin of the foot during examination.

Peripheral neuropathies were the most common complication affecting 67% and signs of peripheral autonomic neuropathy (PAN) were the most frequent, 43%. We found some of the proposed signs to correlate to a medical history of foot ulceration but more importantly to predict future events as assessed at follow-up 5 years later (commented in results). Also the arbitrary decided limit for possible PAN, ≥2 signs, tended to be predictive, p=0.07. Signs of PAN is recognized as important factors for development of foot complications (Tesfaye 2006) but there are to our knowledge no cross sectional or prospective studies that have evaluated them in a representative T2D population nor are they commonly included in foot examination protocols.
We found 34% of the population to be affected by peripheral sensory neuropathy which is in line with the studies most likely to be comparable with ours (Young, Boulton et al. 1993; Cabezas-Cerrato 1998). Main differences between the studies are that Young et al (PSN prevalence 32%) reports from a hospital population and that both Young et al and Cabezas-Cerrato (PSN prevalence 24%) used an additional Neuropathy Symptom Score (NSS).

Symptom score is recommended to be used in studies of neuropathy by the San Antonio Consensus statement (1988). NSS can upgrade a single finding in some protocols to neuropathy, however only 50% of subjects with diabetes neuropathy experience problems (Boulton, Vinik et al. 2005). Identified prevalence depends on choice of methods and varies in the same population depending on method (Forouzandeh, Aziz Ahari et al. 2005). Symptom scoring is still debated (Meijer, Smit et al. 2002). Therefore we believe that the effect on PSN prevalence in our study would have been minor.

Peripheral sensory neuropathy was in our study independently associated with age, gender, peripheral vascular disease, HDLc and IGFBP-1. As commented in results there was also an independent association to HbA1c in men that was lost if diabetes duration was included in the model and vice versa, supposedly depending on the correlation between diabetes duration and HbA1c that was r 0.4, p<0.001.

The association to age and gender is well known and an association to PVD has been previously reported in other study (Adler, Boyko et al. 1997). Low level of HDLc is associated with PVD (Adler, Stevens et al. 2002) and in the metabolic syndrome also with PSN (Pittenger, Mehrabian et al. 2005). Most metabolic factors proposed to cause PSN also have vascular effects. The current opinion is that there is a complex interaction between metabolic disturbances and vascular changes leading to peripheral neuropathy (Cameron, Eaton et al. 2001).

We found subjects with PSN alone to have shorter diabetes duration and better glucose control but significantly more often PVD than subjects with both PSN and retinopathy. Hence, finding PSN at foot examination in subjects without retinopathy should alert the consideration of PVD. This is probably even more important in findings of unilateral neuropathy which can supposedly be caused by macrovascular disorders such as an arterial stenosis. The need to pay attention to the interaction between the disorders when assessing foot risk is further emphasized by the fact that 50% of those affected with either PSN or PVD have no symptoms.

The finding of independent connection between PSN and the GH-IGF axis with elevated IGFBP-1 is previously reported in T1D (Crosby, Tsigos et al. 1992) and the connection between GH-IGF axis and diabetes complications is proposed by several reports (Gibson, Westwood et al. 1996; Janssen, Jacobs et al. 1997; Chiarelli, Santilli et al. 2000; Janssen and Lamberts 2002; Sima, Li et al. 2003; Wallander, Norhammar et al. 2007; Vasilyeva and Ferry 2007; Boquist, Ruotolo et al. 2008). In addition a lower IGF-I/IGFBP-1 ratio was observed in subjects with PSN sug-
gesting lower bioactive IGF-I. Autocrine and paracrine IGF-I is postulated to be crucial for maintained nerve function (Ishii 1995; Chiarelli, Santilli et al. 2000) and the natural decline in IGF:s with age could be a factor behind the age dependent increase of PSN. IGFBP-1 is believed to down regulate the IGF:s action, hence an increase of IGFBP-1 resulting in a lower IGF-I/IGFBP-1 ratio suggests reduced IGF activity, which in turn could predispose for PSN. The higher IGFBP-1 in PSN was not correlated to inflammation as judged by hsCRP suggesting insulin deficiency with less portal insulin in the liver (Brismar, Fernqvist-Forbes et al. 1994).

In study III we report from a group of patients with T2D that exhibited signs of secondary failure. The rationale for study III was the observations that chronic hyperglycemia attenuates insulin secretion and increases insulin resistance, (Rossetti, Giaccari et al. 1990; Yki-Jarvinen 1992), and reports of improved beta-cell function and insulin sensitivity after “beta-cell rest”(Kosaka, Kuzuya et al. 1980; Garvey, Olefsky et al. 1985; Glaser, Leibovich et al. 1988). Secondary failure has implications for choice of treatment and the risk for late complications second to deteriorated glucose control. Hence it would be of clinical interest to test for positive effects in a situation of secondary failure and to investigate the influence of the length of intensified insulin treatment.

Intensive insulin treatment was observed to rapidly induce improvement in beta-cell function besides improving glucose control. However, the effect was not further enhanced by another 9 weeks of treatment and was rapidly lost when switched to less intense treatment. The failure to improve insulin secretion more markedly and with a longer half-life for the effect was in our mind not associated with low intensity in the treatment, i.e. insufficient beta-cell rest. Nor did we observe increased insulin resistance during the study as evidenced by the HOMA parameter for insulin resistance being unchanged. Studies that report a more favourable outcome by intensive treatment than experienced in our participants were newly diagnosed T2D (Ilkova, Glaser et al. 1997) whereas our subjects had a relatively long duration of diabetes and had developed secondary failure. We interpret these differences to be due to our subjects having less beta-cell mass left than newly diagnosed subjects with T2D. This notion is supported by the positive correlation that we find between a more favourable outcome and the level of FC-peptide (Clauson, Alvarsson et al. 1997). Granted that our interpretation is correct, it can be implied that insulin treatment early in the course of T2D should be considered to larger extent than presently practiced.

The improved beta-cell function during MI treatment was obtained at the cost of a moderate but significant weight gain that could spell future problems in maintaining glucose homeostasis and increase the beta-stress further.

We found subjects with higher IGFBP-1 to improve glucose control more than subjects with lower levels. There is a close connection between IGFBP-1 and ambient levels of insulin reported, higher IGFBP-1 being consistent with insulin deficiency (Brismar, Fernqvist-Forbes et al. 1994; Brismar, Hilding et al. 1995). Hence subjects with higher IGFBP-1 in the study were supposedly more insulin
deficient and in need of exogenous insulin while subjects with lower IGFBP-1 were less insulin deficient and could have other additional causes for their deteriorated glucose control than insulin deficiency. If this assumption is valid, IGFBP-1 may give valuable information for the choice of treatment in case of deteriorated glucose control, an assumption that needs to be further investigated.

In the prospective study IV we report baseline IGF-II to show a strong inverse association with subsequent weight gain in individuals with T2D and in the lowest quintile of BMI (BMI<26). The lack of association of low IGF-II with future weight gain in individuals with a baseline BMI of 26 or more suggests that compensatory changes in circulating IGF-II occur with progressively increasing fat mass. These findings is in line with findings in non-diabetics (Sandhu, Heald et al. 2002) and genetic studies (Gaunt, Cooper et al. 2001; Gu, O’Dell et al. 2002).

The cross-sectional association between IGF-II and indices of adiposity is not apparent in individuals with normal glucose tolerance (Cruickshank, Heald et al. 2001; Heald, Anderson et al. 2003). This may reflect compensatory changes in IGF-II as a result of developing T2D. Furthermore, propensity to obesity may depend not only on initial levels of circulating IGF-II but also on how IGF-II levels change in response to changes in body weight and adiposity (Weyer, Pratley et al. 2000). Thus, metabolic adaptation to caloric excess and subsequent weight change also may be important in body weight regulation. The biologic mechanisms and intracellular processes underlying the possible role of IGF-II in body weight regulation remain unclear.

The loss of the relationship between low baseline IGF-II and weight gain once BMI increases above 26 suggests that adipocyte-derived factors may disrupt the capacity of IGF-II to regulate fat mass. A possible influence may be the effect of increasing circulating levels of insulin in relation to increasing insulin resistance with increasing adiposity. Insulin is proposed to increase cellular IGF-II uptake and so decrease circulating IGF-II (Oka, Rozek et al. 1985). Conversely it could be argued that increasing adiposity leads to increasing insulin resistance with consequently diminished insulin-mediated uptake of IGF-II into adipocytes and so a falsely elevated circulating IGF-II. In any case, increasing insulin levels associated with weight gain may disrupt the regulation of fat mass by IGF-II by causing increased uptake and degradation of IGF-II.

In the light of the finding of Oka et al (Oka, Rozek et al. 1985) it might be argued that a low IGF-II at baseline is simply a surrogate for higher insulin levels that tend to predispose individuals to weight gain. However, in the population described by Sandhu et al (Sandhu, Gibson et al. 2003), a low IGF-II at baseline was predictive of weight gain at 5-year follow-up independent of circulating insulin levels. We acknowledge that activity level plays a significant role in weight gain. No account of calorie expenditure was taken in this study. The interaction between circulating levels of IGF-II, activity level, and tendency to weight gain is one that merits further investigation.
CONCLUSIONS

We report peripheral neuropathy to be common in a representative rather young T2D population with moderate diabetes duration and relatively good glucose control. Peripheral sensory neuropathy (PSN) and peripheral vascular disease were closely connected why presence of peripheral vascular disease always should be suspected in subjects with PSN especially when PSN is diagnosed in subjects with no retinopathy. Subjects with PSN and no retinopathy were the most afflicted by PVD.

We found signs of peripheral autonomic neuropathy (PAN) to be the most common of peripheral neuropathies and to correlate with a medical history of foot ulceration. Importantly, signs of PAN also associated with future foot ulcerations that subsequently occurred during 5 years, why they should be included in foot examination protocols.

Further we report data that support the proposed connection between peripheral neuropathy and the GH-IGF axis in T1D is also to be valid for T2D. This is in line with the new hypothesis for diabetic neuropathy but the mechanisms behind needs further investigations.

We report that intensive insulin treatment (MI) demonstrates beneficial effects on beta-cell function in type 2 diabetic patients with features of secondary failure. The effects are rapidly gained but prolonged treatment fails to improve beta-cell function further. We interpret these data to show that MI could be used as a short term treatment to rapidly improve glucose homeostasis in a deteriorated metabolic situation. However, after only a few days a more definitive treatment can be instituted. Higher fC-peptide when MI is instituted makes a positive effect on beta-cell function more likely which we interpret the effect to be depending on prevailing beta-cell mass. Granted that our interpretation is correct, it can be implied that insulin treatment early in the course of T2D should be considered to larger extent than presently practiced.

Improvement of glucose control as judged by HbA1c was positively correlated with IGFBP-1 hence subjects with lower IGFBP-1 improved glucose control less by intense insulin treatment. This can imply secondary failure in these subjects being comparably less dependent on insulin deficiency and more dependent on other factors such as failure to comply with diet treatment, however this interpretation needs to be further investigated. If proven valid IGFBP-1 has potential as marker to distinguish between insulin deficiency and other additional causes for deteriorated glucose control.

MI led to an undesired weight gain that potentially was larger than in subjects with less intense treatment, this can entail future problems in maintaining glucose homeostasis and increase the susceptibility for late complications. In study IV we report low IGF-II level at baseline in normal weighted subjects with T2D to predict subsequent weight gain of more than 2 kg when followed for 5 years, this was independent of treatment. This finding support the proposed connection between weight and IGF-II but many questions remain to be clarified especially the mechanisms behind the disrupted relationship when BMI was above 26 kg/m2.
FUTURE
The population in study I-II has been re-examined at a 5 year follow up. Data are waiting to be compiled and analysed but some unpublished data concerning peripheral autonomic neuropathy is included in this thesis as well as the data concerning weight gain at follow-up in paper IV. Analyses of follow-up data will concentrate on adverse events, macro- and microvascular, and progressive features of the metabolic disturbances and the proposed connection between peripheral sensory neuropathy and IGF-IGFBP axis will be further evaluated.

The population in study I-II was also investigated with several questionnaires regarding quality of life, amongst other SWED-QUAL, in which IGFBP-1 correlated with sleeping problems and also experience of ache and pain (unpublished data). It is known that IGF-IGFBP axis is influenced by depression, increased IGF-I, hence the question arose if well being has effects on the IGF-IGFBP axis. Further, acute critical ill patients have elevated IGFBP and sustained levels are unfavourable for outcome. Thus it would be of interest to know if less acute but severely ill subjects display similar changes in IGF-IGFBP axis and if experienced well-being assessed by different Quality scales is measurable in the IGF-IGFBP axis. To address this question we started a study 2006 including patients with severe cancer disease, first cerebral ischemic stroke and Alzheimer dementia.

We are presently working on a paper regarding Q10 and oxidative stress in relation to diabetic complications in the studied population, study I-II, and we plan to analyse our data concerning complications also in relation with APC resistance which is reported to be associated with coronary heart disease but very little is known if any associations are to be seen in relation to diabetes complications.

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