Metabolic Aspects on Diabetic Nephropathy

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ABSTRACT

Diabetic nephropathy (DN) is associated with morbidity and mortality due to cardiovascular disease and renal failure. This study focused on the impact of glycemic control on the development of DN and the metabolic consequences of DN. The euglycemic hyperinsulinemic clamp technique was used to assess insulin sensitivity and insulin clearance. Two different registries, the Diabetes Incidence Study in Sweden (DISS) and the Swedish Childhood Diabetes Registry, as well as questionnaires and data from medical records were used to study diabetic complications in population-based cohorts.

Microalbuminuria is an early marker of DN and may also be associated with impaired insulin sensitivity in diabetic and non-diabetic subjects. We studied the relationship between insulin sensitivity and the degree of albuminuria in patients with type 1 diabetes and micro- or macroalbuminuria but normal glomerular filtration rate (GFR). We did not find a direct quantitative association between the degree of albuminuria and insulin resistance, arguing against a cause-effect relationship.

With progression of DN, a decline in GFR is seen. Patients with severe renal failure have both impaired insulin sensitivity and insulin clearance. We studied insulin sensitivity and insulin clearance in type 1 diabetes patients with three different degrees of renal involvement (none, only albuminuria, and slightly reduced GFR, ~40-70 ml/min/1.73 m², respectively). A clear reduction in insulin sensitivity in vivo, but not in insulin clearance, was seen in the group with reduced GFR, and concomitant changes in the levels of PTH, IGF-1, IL-6 and TNF-α were found. In parallel, cellular insulin sensitivity and insulin degradation were examined in vitro, in subcutaneous fat cells but no differences were found between the three groups of patients.

To study the occurrence of renal involvement in patients with modern diabetes treatment we monitored a cohort of young adults from the DISS-registry with onset of diabetes in 1987-88 at age 15-34 years. We found that ~7% of the patients had signs of renal involvement, i.e. incipient nephropathy (5%) and overt nephropathy (2%), after a median follow-up of ~9 years and the strongest risk markers were poor glycemic control (HbA1c) and high blood pressure. Patients with type 2 diabetes were most prone to have renal involvement in this age group.

Retrospectively, we studied 94 patients diagnosed with type 1 diabetes in 1981-1992 at age 0-14 years at the Umeå University Hospital. Incipient nephropathy and background retinopathy occurred in 18 and 45%, respectively, of the patients, during ~12 years of follow-up. Glycemic control, also during the first five years of diabetes, was a strong risk marker. Young age at onset of diabetes prolonged the time to development of microvascular complications.

Conclusion: Despite modern diabetes treatment some patients with diabetes develop renal involvement within the first ten years. Inadequate glycemic control, also early in the disease, is a risk marker as well as type 2 diabetes and high blood pressure. In patients with type 1 diabetes and diabetic nephropathy a slightly reduced GFR, but not albuminuria, is associated with insulin resistance. Concomitant changes in insulin-antagonistic hormones and cytokines may be involved.
LIST OF PAPERS


**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ACE</td>
<td>angiotensin converting enzyme</td>
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<td>ACE-I</td>
<td>angiotensin converting enzyme inhibitors</td>
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<td>AER</td>
<td>albumin excretion rate</td>
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<td>AGE</td>
<td>advanced glycosylation end-products</td>
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<td>ARB</td>
<td>angiotensin II receptor blockers</td>
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<tr>
<td>AT-II</td>
<td>angiotensin II</td>
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<tr>
<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>(^{51})CrEDTA</td>
<td>chromium ethylenediamine tetraacetic acid</td>
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<td>DCCT</td>
<td>Diabetes Control and Complications Trial</td>
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<td>DISS</td>
<td>Diabetes Incidence Study in Sweden</td>
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<td>DN</td>
<td>diabetic nephropathy</td>
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<td>ECM</td>
<td>extracellular matrix</td>
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<tr>
<td>EGF</td>
<td>epidermal growth factor</td>
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<td>ESRD</td>
<td>end stage renal disease</td>
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<td>GBM</td>
<td>glomerular basement membrane</td>
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<td>GFR</td>
<td>glomerular filtration rate</td>
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<tr>
<td>GH</td>
<td>growth hormone</td>
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<tr>
<td>HbA1c</td>
<td>glycosylated hemoglobin HbA1c, a subfraction of HbA1</td>
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<tr>
<td>IGF-1</td>
<td>insulin-like growth factor 1</td>
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<td>IGFBP</td>
<td>insulin-like growth factor binding proteins</td>
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<tr>
<td>IL</td>
<td>interleukin</td>
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<tr>
<td>NEFA</td>
<td>non-esterified fatty acids</td>
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<tr>
<td>PKC</td>
<td>protein kinase C</td>
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<tr>
<td>PTH</td>
<td>parathyroid hormone</td>
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<td>RAS</td>
<td>renin angiotensin system</td>
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<tr>
<td>SD</td>
<td>standard deviation</td>
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<tr>
<td>SEM</td>
<td>standard error of the mean</td>
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<tr>
<td>TNF-(\alpha)</td>
<td>tumour necrosis factor (\alpha)</td>
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<td>VEGF</td>
<td>vascular endothelial growth factor</td>
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Diabetes and its complications
Diabetes mellitus is characterised by chronic hyperglycemia caused by defects in insulin secretion, insulin action or both. There are several pathogenic processes that may be involved in the development of diabetes and they range from an autoimmune destruction of the insulin producing β-cells of the pancreas to abnormalities resulting in resistance to insulin action in liver and peripheral tissue. The different forms of diabetes may be classified in accordance to their etiology. The two most common types of diabetes are type 1 diabetes, predominantly an autoimmune β-cell destruction leading to absolute insulin deficiency and type 2 diabetes, usually caused by a relative insulin deficiency, i.e. β-cell dysfunction and insulin resistance in combination (1). Type 1 diabetes is the most common form in children and adolescents and type 2 predominates in older ages, but both forms may occur almost any time in life.

The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys and nerves but also the arteries. The classical long-term complications are thus retinopathy, nephropathy, neuropathy, all of which are considered to be microvascular complications and macroangiopathy. These complications affect quality of life and/or life expectancy. Retinopathy may lead to severe retinal bleeding and has previously been the most common cause of blindness among young adults. Diabetic nephropathy (DN) can progress to renal failure and need for renal replacement therapy. A symmetric peripheral loss of sensibility and motor nerve function in the lower extremities are commonly early signs of neuropathy and increase the risk of developing foot ulcers. The combination of lower extremity arterial disease and neuropathy may contribute to an increased risk for gangrene and amputation. Autonomic neuropathy may lead to alterations in gastrointestinal, cardiovascular and urogenital function. Hyperglycemia is a common risk factor for all these complications but there are also other risk factors, some of which seem to have organ specific effects.

Before Banting and Best discovered insulin in 1921 the only therapy for diabetes was diet and more than 80% of patient died within the first ten years of type 1 diabetes. The most common cause of death was ketoacidosis. The first injection of insulin for treatment of juvenile diabetes was given in February 1922 and insulin therapy for general use was introduced a few years later. After the introduction of insulin treatment life expectancy increased and instead the problem with chronic complications evolved, cardiovascular disease and renal failure becoming the major causes of death among patients with diabetes (2). Today, patients with diabetes still have an excess morbidity and mortality when compared with the general population, the major causes still being cardiovascular disease and renal failure (3-5). In a Danish cohort of patients diagnosed with type 1 diabetes between 1933-1952 and followed up until 1982, the increased mortality in patients with type 1 diabetes was predominantly accounted for by a poor prognosis for patients with DN. On the contrary, among patients who did not develop DN, mortality was only slightly elevated at all ages (6).

Diabetic nephropathy – a historic perspective
Since the introduction of insulin therapy, DN and renal failure has been one of the leading causes of sickness and death in type 1 diabetes. In the 1940’s all patients with childhood-onset type 1 diabetes had nephropathy after 16 years duration of diabetes and no one lived longer than 21 years after onset of diabetes (7). Later studies from both from The Joslin Diabetes Center, Boston (8), and The Steno Diabetes Center, Copenhagen (9), have shown a gradually declining incidence of DN in patient cohorts with diabetes onset from the 1930’s until the
1970’s. Similar trends have later also been shown in Swedish patient cohorts with respect to overt nephropathy 1961-1980 (10) and incipient nephropathy 1976-1991 (11). However, surprisingly, an unchanged incidence of DN in type 1 diabetes was seen in Denmark during the period 1965-1979 (12). Poor glycemic control and a high prevalence of smoking compared to the patients in the Swedish studies may in part explain this.

Improved glycemic control with intensive insulin treatment and screening for microalbuminuria together with early intervention with antihypertensive medication can postpone the development of overt DN. Despite this, DN is still one of the leading causes of end stage renal disease (ESRD) in Sweden (13) as well as in other countries (14, 15). 25% of patients who began renal replacement therapy during the period 1991-2001 in Sweden had DN as their primary renal disease. However, during the same period the annual number of patients with type 1 diabetes starting renal replacement therapy did not increase (13).

Pathogenesis of diabetic nephropathy

Morphological findings
In 1936, a specific diabetic glomerulosclerosis was recognised by Kimmerstiel and Wilson. This finding is a rather late feature in the development of DN. The first morphological signs, glomerular basement membrane thickening and mesangial expansion, can be seen within two years of diabetes on examination with electron microscopy. Later on, nodular or diffuse glomerular sclerosis, exudative lesions in glomeruli, atrophic tubular lesions develop and can be seen in kidney biopsies from patients with DN (16).

Genetic factors
Epidemiological studies have shown that the incidence of DN peaks in the second decade of disease and after 25 years the risk rapidly declines, which is in contrast to other diabetic complications. The concept of a genetic predisposition for development of DN has therefore been postulated (6). This is supported by studies that have shown familial clustering in different populations (17-19), but this could also be a result of shared environmental factors. Some studies have demonstrated that a predisposition to hypertension (20, 21) and a family history of cardiovascular disease and type 2 diabetes (22, 23) may be associated with increased risk of developing DN. This also suggests a genetic association to the metabolic syndrome. Ethnic differences in the risk of developing diabetic renal disease have also been described (24, 25) supporting that genetic background has an impact.

Genes involved in the regulation of blood pressure, glucose and lipid metabolism as well as in renal embryonic development have been regarded as potential candidate genes in DN. The most extensively examined gene polymorphism in the renin-angiotensin-system (RAS) is the insertion/deletion (I/D) polymorphism in the angiotensin converting enzyme (ACE) gene. The results of previous studies on DN have been somewhat conflicting, but the progression of nephropathy may be faster in the group homozygous for the D allele (26, 27). Other polymorphisms in RAS genes have also been investigated and a mutation in the angiotensinogen (M235T) and in the angiotensin II receptor (A1166C) genes may slightly increase risk of developing DN (28). A polymorphism in the aldose reductase gene, that may be important in the regulation of the polyol pathway, has been associated with an increased risk of DN (29). There are also potential candidate genes involved in the metabolism of advanced glycosylation end-products (AGE) that are being investigated in ongoing studies (30).

An emerging new paradigm is the role of genes involved in organ development, i.e. ontogenic genes. Normal renal growth and development depend on a precise, complex and tightly regu-
lated interplay between a number of regulatory growth promoters and inhibitors. These genes may also play a role in the development and repair of tissue injury, for example in DN (31).

**Metabolic factors**

Hyperglycemia is necessary for the development of microvascular diabetic complications and it is associated with both onset of incipient and overt DN, i.e. micro- and macroalbuminuria (8) and the progression of overt DN to renal failure (32). The level of hyperglycemia seems to be quantitatively linked to the risk of developing renal lesions (8). Hyperglycemia enhances the non-enzymatic glycosylation of proteins and advanced glycosylation end-products (AGE) are formed. AGE are stable and resistant to degradation by enzymes and they injure cells by structural rearrangement of proteins. Increased serum levels of AGE seem to predict changes in kidney morphology such as expansion of mesangial cell matrix and glomerular basement membrane (GBM) thickening (33). AGE may also affect the charge selectivity on the GBM, altering the filtering capacity (34). AGE levels are well correlated to the degree of long-term glycemic control (35) and, in addition, they are increased when renal function declines (36).

Importantly, the level of these products do not return to normal when hyperglycemia is corrected since they may accumulate in the blood vessel-wall and remain there during the lifetime of the proteins. In renal failure the AGE-concentration declines moderately after treatment with hemodialysis and markedly after kidney transplantation (36). Aminoguanidine, a compound that inhibits the formation of AGE, has both in animal studies (37, 38) and in clinical trials demonstrated promising effects to limit the progression of DN (39).

Hyperglycemia also enhances formation of sorbitol via the polyol pathway, and this is facilitated by the enzyme aldose reductase. The net result is an increase in sorbitol and a decrease in myoinositol content in cells. An accumulation of sorbitol in cells is toxic and can cause both functional and structural changes. Although this pathway has been most extensively investigated in neuropathy, some studies also support a role in DN (38). In human DN, treatment with aldose reductase inhibition has slightly reduced hyperfiltration (40), but until now the results of clinical trials have been disappointing mainly due to toxic side-effects.

The hexosamine pathway, through which glucose is converted into glucosamines, may also be involved in the development of diabetic complications. An increased flux through this pathway is associated with protein kinase C (PKC) activation, increased transforming growth factor-β (TGF-β) expression and extracellular matrix (ECM) production, all of which are associated with the development of DN (41). In addition, a direct glucose-induced rise in renal TGF-β expression has been shown in the kidney (42) and may be responsible for some of the structural renal changes in DN. TGF-β closely interacts with the RAS and PKC activity and their interplay could be central in the development of DN (43).

Some investigators consider oxidative stress as a major mechanism in the development of diabetic complications (44). It may be a critical link between hyperglycemia and chronic complications (45) or a consequence of some other pathogenetic mechanism, for example AGE-formation (46).

An increased exposure of mesangial cells to lipoproteins and incorporation of lipids enhances proliferation of the mesangium. Lipoproteins bind to the polyanionic glucoseaminoglycans in the ECM and GBM and may alter charges of the GBM (47). Alterations in lipid metabolism are seen already in incipient DN (48) and may perhaps, via TGF-β, accelerate structural injuries and kidney complications (49).
Heparan sulphate proteoglycan (HSPG) is important for the function of the glomerular filter contributing to the negative charge (50) and a decreased level has been shown in DN (51). A genetic polymorphism in HSPG has also been associated with DN (52). In short-term trials, treatment with low-dose heparin was able to reduce albuminuria but the mechanism is unknown (53).

Hemodynamic factors
Early studies showed that systemic hypertension accelerates renal injury in diabetes (54) and that the rate of progression of renal disease is slow in normotensive patients with type 1 diabetes (55). An increase in the intraglomerular pressure has been suggested to promote progressive renal injury early in DN (56). This hypothesis is supported by the clinical findings that a high GFR early in diabetes is a risk factor for later development of DN as shown both in cross-sectional (57) and longitudinal studies (58, 59).

A beneficial effect of angiotensin converting enzyme inhibition (ACE-I) has been shown with respect to progression of both incipient (60) and overt DN (61) and this includes reversal of structural changes (62). This supports that the renin-angiotensin system (RAS), and particularly angiotensin II (AT II), play a central role in the intrarenal hemodynamic changes and in promoting structural changes in DN. AT II modulates glomerular filtration both by influencing afferent and efferent glomerular arteriolar tone and by a direct effect on mesangial cells and may also influence glomerular permeability (63). It also promotes renal sodium reabsorption by effects in the proximal tubuli and via stimulation of aldosterone secretion (64). In addition, AT II induces ECM accumulation (65). AT II interact with two specific receptors, angiotensin type 1 (ATR 1) and angiotensin type 2 receptor (ATR 2) and most of its actions seem to be mediated via the ATR 1 (63). Measurements of the different components of the RAS in plasma (66) and within the kidney (67) have revealed low or normal levels in diabetes but in vitro studies have shown an increased angiotensinogen expression in proximal tubular cells in a diabetic animal model (68). In overt DN there is an activation of the RAS, due to a declining glomular filtration rate (GFR), with an increased generation of AT II. This results in both systemic and intraglomerular hypertension, which accelerate the renal injury (69).

The ability of the kidney to maintain a constant GFR over a range of renal perfusion pressures is called autoregulation, and some data suggest that an impaired autoregulation is present in overt DN (70). There is an association between nephropathy, proliferative retinopathy and autonomic neuropathy (71, 72) and this could be due to the co-existence of two or more diabetic complications. On the other hand, autonomic neuropathy could possibly have an effect of its own to causing renal injury via higher blood pressure, renal vascular dilatation and an increased intraglomerular pressure, all of which could be caused by an impaired vascular autoregulation (71, 73). Studies have shown that microalbuminuria and autonomic neuropathy co-exist in patients with type 1 diabetes (74, 75) and among patients without nephropathy the prevalence of autonomic neuropathy is low (76).

In addition to components of the RAS, there are several vasoactive peptides that have been postulated to influence renal injury, e.g. vasoconstrictors such as endothelin and vasopressin, and vasodilators such as bradykinin, atrial natriuretic factor (ANF), prostaglandins and nitric oxide (77).

Growth factors and puberty
It was hypothesised already in the 1970’s that growth hormone (GH) and insulin-like growth factor-1 (IGF-1) influence the development of diabetic complications. Possibly, chronic hy-
perglycemia and/or lack of insulin in diabetes lead to impairment in the hepatic IGF-1 formation and a lower serum IGF-1 level, inducing GH hypersecretion by feedback. The increase in GH-concentration, in turn, might stimulate local IGF-1 production in the kidneys. In fact, all components of the GH/IGF-1 system, i.e. IGF-1, IGF-2, the six IGF-binding proteins and the two specific IGF-receptors are expressed locally in the kidney. Both experimental animal studies (78) and treatment studies on diabetic retinopathy in humans with hypophysectomy (79) as well as long-acting somatostatin analogues (80) have suggested beneficial effects on microvascular complications following inhibition of the IGF/GH system. Additionally, some studies have shown that puberty, partly due to the hormonal changes, e.g. high levels of growth and sex hormones together with a concomitant deterioration in glycemic control, may promote the development of microvascular complications (81, 82).

Environmental factors
Low birth weight has been associated with retardation of renal development, a reduced number of nephrons and an increased risk of systemic and intraglomerular hypertension (83). Low birth weight and intrauterine growth retardation have also been linked to cardiovascular risk factors and an increased risk of developing DN (23, 84). These findings support that perinatal factors may contribute to the development of DN.

Physical activity may have a beneficial effect in preventing or delaying macrovascular diabetic complications (85). Moreover, an impaired aerobic work capacity has been shown in patients with type 1 diabetes with incipient as well as overt nephropathy compared to those with normal renal function (86). Other potential environmental factors include diet and tobacco use and they are described in the section on clinical management.

Clinical development and presentation
Clinical stages of nephropathy
From studies performed in the 1980’s Mogensen et al (54) defined stages in the development of renal alterations that can be used in clinical classification. In **stage 1**, glomerular hyperfiltration and renal hypertrophy may be seen at onset of diabetes and shortly thereafter. With insulin treatment these changes are, at least, partly reversible. **Stage 2** is a clinically “silent” period of progression with normal albumin excretion rate (AER) but subtle morphological lesions, e.g. glomerular basement membrane thickening and mesangial expansion, are seen in kidney biopsies. Occasionally, in stress situations such as very poor metabolic control or during physical exercise, AER may increase, but this is a reversible phenomenon. Transition to incipient nephropathy, **stage 3**, occurs in 2-4% of cases per year and this is associated with poor glycemic control and high levels within the normal range of urine albumin excretion (87). Stage 3, is characterised by a persistent and usually slowly increasing microalbuminuria (AER 20-199 µg/min). Patients with incipient nephropathy have a high risk of progression to overt nephropathy. Intervention, e.g. optimised glycemic control (88) as well as antihypertensive treatment (62), may change the natural history and reverse or stabilise functional and maybe even structural changes. Overt or manifest nephropathy, **stage 4**, is characterised by persistent macroalbuminuria (AER ≥200 µg/min), increasing blood pressure and a declining glomerular filtration (89). Finally, in **stage 5**, kidney function becomes severely impaired and uremia, i.e. end stage renal disease (ESRD), evolves.

Clinical criteria for microalbuminuria and diabetic nephropathy
The following criteria have been proposed for the diagnosis of renal involvement for type 1 diabetes and they have subsequently been used in many studies (90). **Incipient nephropathy** is defined as microalbuminuria, i.e. AER 20-199 µg/min and **overt nephropathy** as macroalbu-
minuria, i.e. AER ≥200 µg/min, found in at least two out of three consecutive urine samples, preferably collected over a period of six months. Urine should be sterile. Other causes of increased excretion rate such as other renal or urogenital diseases, physical activity or fever should be ruled out. Particularly, when diabetes duration is less than 6 years, other causes should be considered. In clinical practice, 24-h or overnight urine collections may be difficult to achieve and spot collections in early morning samples can be performed as recommended by the American Diabetes Association; microalbuminuria, i.e. 3-29 mg/µmol creatinine and macroalbuminuria, i.e. ≥30 mg/µmol creatinine (91).

![Fig. 1](image)

**Fig. 1.**
Characteristics of the different stages of functional renal changes in type 1 diabetes. (AER, albumin excretion rate; GFR, glomerular filtration rate; ESRD, end stage renal disease; ACE-I, angiotensin converting enzyme inhibitors; ARB, angiotensin II receptor blockers) (Adopted from Mogensen et al (54))

**Microalbuminuria**
Different studies have reported that the progression from normoalbuminuria to micro- or macroalbuminuria in patients with type 1 diabetes during ten years is 7-17% (57, 92-94). Albumin excretion in the upper normal range indicates a greater risk of progression to microalbuminuria (95). Early studies in patients with type 1 diabetes showed that ~80% progressed from microalbuminuria to overt proteinuria within ~10 years (96). This led to a broad acceptance of microalbuminuria as a useful clinical predictor of the development of overt DN (90). More recent studies have observed only about a 30-45% risk of progression of microalbuminuria to macroalbuminuria within 10 years in patients with type 1 diabetes, whereas about 30% became normoalbuminuric and the rest remained microalbuminuric (58, 93, 94). It is unclear whether this is due to changes in the natural history of DN resulting from improved glycemic control and early intensive blood pressure treatment or whether there were overestimates in the original studies. To increase the complexity, some normoalbuminuric long-standing type 1 diabetic patients have morphological renal lesions typical for DN (97, 98). In addition, some patients with persistent microalbuminuria have quite advanced renal lesions (99) and, thus, in
these patients microalbuminuria may be a marker rather than a predictor of established structural renal damage. In type 2 diabetes, progression from normoalbuminuria to microalbuminuria has been found in 15-30%, from microalbuminuria to macroalbuminuria in 20-50% and from normoalbuminuria to macroalbuminuria in 0-8% during 6-9 years of follow-up (100, 101). However, these studies have a rather short follow-up time, less than 10 years, and probably more patients develop micro- and macroalbuminuria with time.

It is important from the clinical point of view to identify patients at risk of developing nephropathy early as this allows intervention before advanced renal damage is established. AER still remains the best available non-invasive predictor of risk of developing overt DN and should regularly be measured according to established guidelines. Currently 24-h collections (mg/24 h), timed collections (µg/min) and spot collections expressed in relation to urine creatinine are recommended by the American Diabetes Association in screening for microalbuminuria (91). However, new technologies for screening are now being validated and may change the screening procedure in the future (94).

Microalbuminuria is a predictor for development of renal failure, but also a powerful independent risk factor for cardiovascular disease and death in patients with diabetes and in non-diabetics (96, 102). In addition, microalbuminuria has been linked to insulin resistance both in patients with diabetes and in non-diabetics (103-105). However, there are other studies suggesting that there is no direct relationship between microalbuminuria and insulin resistance (106, 107). Microalbuminuria has also been considered to be a key factor in the development of glomerulosclerosis in both diabetic and non-diabetic renal disease and this can occur via so-called protein trafficking. Proteins filtered by the glomerulus may, due to the excessive reabsorption, cause injury to the tubular interstitium leading to renal dysfunction (69).

Overt nephropathy and renal failure

Overt DN is characterised by macroalbuminuria, hypertension and a variable decline, median 12 ml/min/year, in GFR, if left untreated (108, 109) until GFR <10 ml/min when ESRD evolves. ESRD, independent of its causes, e.g. diabetes, is characterised by several perturbations such as hypertension, accumulation of uremic toxins, hyperkalemia, hyperphosphatemia and anemia due to erythropoetin deficiency. Secondary hyperparathyroidism and alterations in D-vitamin metabolism, together with metabolic acidosis, are considered to be responsible for the osteodystrophy seen in uremic patients. Several risk factors for mortality among patients with uremia have been identified, and age, protein-energy malnutrition and low serum albumin, commonly considered to be an index of malnutrition, appear to be strong predictors of mortality (110). Uremia due to diabetes is associated with a higher mortality when compared to non-diabetic renal diseases. Many factors may contribute to malnutrition and low serum albumin in uremia and they include low protein and energy intake. Other concomitant diseases such as heart failure and infections, inflammation, catabolic effects of acidosis, reduced physical inactivity and loss of protein and amino acid during dialysis treatment may contribute (111). Chronic inflammation appears to be involved and aggravate malnutrition and progressive atherosclerotic disease by several pathogenic mechanisms. Available data suggest that inflammation, reflected by high levels of cytokines such as TNF-α and IL-6, plays a central role in the development of both malnutrition and cardiovascular disease in ESRD (112).

Metabolic consequences

Most studies on the metabolic and endocrine alterations during the development of renal failure have been performed in non-diabetic patients rather than those with DN. However, most of the findings can probably be generalised also to patients with DN, but further investiga-
tions on the interaction between diabetes and early renal impairment and the uremic state, respectively, are needed.

**Insulin clearance**

Approximately 40-50% of the insulin secreted by the pancreatic β-cells is removed by the liver during its first passage (113). The kidneys degrade 30-40% of the insulin entering the systemic circulation while the remainder is cleared via degradation in various tissues, mainly muscle and adipose tissue. In total, renal degradation accounts for ~20% of whole body insulin removal (114). Insulin is freely filtered by the glomerulus and ~60% is eliminated by glomerular filtration and the remaining ~40% by uptake from the peritubular capillaries into tubular cells where insulin is degraded (115). Insulin present in the tubular lumen is reabsorbed and also degraded in the tubular cells. Only 1% of the filtered insulin is excreted with the urine. Renal plasma clearance of insulin is about 200 ml/min. In renal failure, the reduction in GFR can be compensated by an increase in the uptake from the peritubular capillaries and until GFR falls to 15-20 ml/min, no reduction in insulin clearance has been detected. At lower GFR, renal clearance of insulin and probably also liver clearance, become drastically reduced (116).

**Insulin action on glucose metabolism**

It is considered that peripheral insulin resistance, with respect to glucose utilisation, is mainly located in skeletal muscle (117). It has been reported that in renal failure it occurs when GFR becomes markedly reduced, i.e. <40 ml/min/1.73m² (117-119). A decreased maximal response to insulin in vivo, suggesting a defect at the post insulin-receptor level, has been shown in patients with severe renal failure (120). Similar results were seen in studies in adipocytes from uremic subjects (121). The mechanisms for insulin resistance in severe renal failure are probably multifactorial. In addition to the accumulation of uremic toxins, there are several other abnormalities, such as impaired physical fitness, altered body composition, metabolic acidosis, hyperosmolality and medication that may contribute (119, 122). Abnormalities in circulating hormones or neuroendocrine pathways and markers of inflammation, e.g. cytokines, may also promote insulin resistance (123). Anemia can also be a factor contributing to insulin resistance, as patients with chronic anemia have been shown to be insulin resistant and one study (124) has shown beneficial effects on insulin resistance following erythropoetin treatment and correction of anemia in patients with renal failure.

Interestingly, insulin resistance has also been found in patients with polycystic kidney disease despite normal GFR. Even though the mechanism leading to insulin resistance in these patients remain unclear, it is suggested that it may be directly related to the structural protein and membrane alterations occurring in this disease (125, 126).

Many studies in patients with diabetes have demonstrated that chronic hyperglycemia, per se, may induce glucotoxicity and impair insulin sensitivity (127, 128). Near-normoglycemic diabetes control and intensive treatment with insulin improve but do not fully restore insulin action (129). In human diabetes an elevated endogenous glucose production is mainly due to a high rate of gluconeogenesis which occurs in the liver (130). However, studies have shown that the kidneys play a role in glucose counterregulation (131) as they have capacity for both glycogen formation and gluconeogenesis, and this may be compromised in renal failure. This observation may be clinically relevant since patients with advanced DN seem to be more prone to develop severe hypoglycemia (132). The kidneys also account for a small proportion, less than 10%, of the overall insulin-mediated glucose metabolism, but it is not known whether this is impaired in renal failure (131).
**Lipid metabolism**

In both type 1 and type 2 diabetes with renal involvement, even in the earlier stages of renal disease, various abnormalities in plasma concentrations of lipoproteins have been described. DN is associated with an altered lipid profile characterised by elevated triglyceride rich lipoproteins, in particular very low density lipoprotein (VLDL), but also low density lipoprotein (LDL) and, thus, plasma triglycerides are high (133). The levels of high density lipoprotein (HDL) are low as a secondary phenomenon (133). Elevated plasma concentrations of apolipoprotein B, apoC-III and lipoprotein (a) have also been reported (133, 134). However, there still seems to be uncertainty on the underlying mechanisms, but changes in lipoprotein lipase (LPL) and hepatic lipase (HL) have been suggested. An increased HL-activity and a reduced postheparin plasma LPL/HL ratio have been reported (134). These multiple lipoprotein alterations become more accentuated with declining renal function and increasing urinary albumin excretion (48). When compared with non-diabetic patients with renal failure, the lipid abnormalities are more marked in DN, probably reflecting an additional effect of the diabetic state and, in particular, the level of glycemia and the relative insulin deficiency (135). The lipid disorders seen in chronic renal failure resemble those seen in the metabolic syndrome and it has also been speculated that the insulin resistance seen in uremic patients may also be associated with some of the observed lipid disorders (217).

The total and LDL cholesterol levels usually are normal or slightly elevated in renal failure and the diameter of LDL particles has been reported to be smaller, i.e. small dense LDL, in patients with both incipient and overt DN (49). Small dense LDL are more readily oxidised and glycosylated (49) and they are also more deleterious to vessel walls than normal, larger LDL-particles. LDL cholesterol seems to have a similar effect on glomerular mesangial cells as on endothelial cells. Mesangial cells are closely related to vascular smooth muscle cells and possess binding sites for LDL and oxidised LDL. They help recruit macrophages and then secrete proliferative factors inducing glomerulosclerosis, i.e. a process similar to the role of endothelial cells in the process of atherosclerosis (136). Finally, the lipid lowering agents, statins, appear to have a beneficial effect on mesangial cells independent of their cholesterol lowering-effect (137).

Another factor that may reduce insulin sensitivity is an elevated level of non-esterified fatty acid (NEFA) (138). However, NEFA levels are not generally elevated in chronic renal failure (120) and the anti-lipolytic effects of insulin in uremic patients are comparable with those in healthy subjects (139).

**Hormones and cytokines**

Alterations in the levels of insulin-antagonistic hormones and cytokines during the development of DN and renal failure could potentially contribute to insulin resistance and to the changes in glucose- and lipid metabolism. There are several potential pathogenic mechanisms leading to endocrine perturbations during the development of renal failure. There can be a decreased renal and extrarenal hormones production of, i.e. erythropoetin, vitamin D$_3$ and testosterone, but also alterations in the conversion of prohormones into active hormones, e.g. reduced conversion of triiodothyronine (T$_3$) into tetraiodothyronine (T$_4$) and activation of vitamin D$_3$. An increase in hormone production can be a result of an adaptive response to homeostatic signals, for example the increase in parathyroid hormone (PTH) in response to hypocalcemia. An increased hormone level may also be the result of an impaired degradation both in renal and extrarenal tissue, which could be true for insulin. Changes in the activity of hormones may be due to alterations in isohormon profile, glycosylation, alterations in the
levels of binding proteins, e.g. insulin-like growth factor binding proteins (IGFBP), occurrence of inhibitors, altered receptor function (140), and there may also be post-receptor defects as for insulin. Moreover, there may also be alterations in circadian rhythm of hormone profiles in plasma (141).

**Growth hormone and IGF-1**

Poor glycemic control and low portal insulin levels in type 1 diabetes is associated with elevated GH levels and elevated levels of IGFBP-1 as well as reduced levels of free IGF-1 (142). In particular, this is observed in patients with microvascular complications and microalbuminuria. Circulating GH levels may be elevated in patients with renal failure due to both increased secretion and impaired degradation (143). This may by a compensation that occurs in response to a relative tissue resistance to the effects of GH, which in turn may be due to an altered GH-receptor function. Potentially, uremic toxins and IGFBP may act as IGF-1 inhibitors and the increase in IGFBP also results in a lower free fraction of IGF-1, promoting GH release (140).

**Glucagon**

Elevated levels of glucagon are detected in uremic subjects (139). However in patients with long-standing type 1 diabetes a reduction in the secretion of glucagon has been found and the glucagon response to hypoglycemia is subnormal or absent (144). This may partly be due to a high level of circulating insulin (145) and the glucagon response could, although not completely, be restored by continuous intraperitoneal insulin infusion (146).

Insulin resistance was not altered in uremic patients when using somatostatin to suppress endogenous insulin, glucagon and GH release thus implying that insulin resistance in uremia is not likely to be due to acute effects of glucagon or GH (147). The fact that insulin sensitivity is improved by dialysis treatment also argues against a major role of GH or glucagon, since dialysis therapy does not alter the levels of these hormones (120).

**Parathyroid hormone**

Early in renal failure there is an increase in parathyroid hormone (PTH) levels, i.e. secondary hyperparathyroidism due to both slight hypocalcemia and phosphate retention but also a decline in conversion of vitamin D₃ into its active forms. High PTH has therefore been suggested as a mechanism producing insulin resistance (148). Experimental data indicated that PTH can impair insulin secretion but it had no effect on insulin sensitivity assessed with the glucose clamp technique (149). On the other hand, intravenous administration of vitamin D, that can reduce PTH levels, improved both insulin secretion and insulin sensitivity in patients on hemodialysis (150). Both animal and human studies have suggested that D-vitamin per se may influence insulin sensitivity but the findings have been conflicting (151-153) and the role of vitamin D is not yet established.

**Glucocorticoids**

Cortisol is filtered by the glomeruli and nearly totally reabsorbed by the kidney and less than 1 % of filtered cortisol appears intact in the urine. Instead, cortisol undergoes both conversion and conjugation in the liver resulting in the excretion of cortisol and cortisone metabolites in the urine. Cortisol is also inactivated in the kidney by conversion to cortisone via the action of the type 2 isoform of 11β-hydroxysteroid dehydrogenase. Patients with renal failure have reduced levels of urine cortisol, but both normal and elevated circulating levels of cortisol have been reported. (120, 154). The diurnal variation, however, seems to be preserved (154). Dexamethasone-induced suppression of plasma cortisol was reported to be normal (155) or
blunted (156). An essentially normal response to ACTH stimulation has been found both using a standard (250 µg) and a low-dose (1 µg) ACTH test in patients with renal failure (157). It can not be ruled out that a tissue-specific dysregulation of cortisol metabolism that is linked to insulin resistance may be present in renal failure, as was previously reported in obesity (158).

**Sex hormones**

In male patients with renal failure, plasma testosterone levels are decreased or in the low normal range, despite an increase in gonadotropins, i.e. luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (159). This may be the result of a combined abnormality including a decrease in the strength of the gonadotrophins releasing hormone (GnRH)-pulse, altered feedback mechanism at the hypothalamic-pituitary level and a peripheral Leydig-cell resistance with moderately decreased testosterone production despite elevated gonadotropin levels (160). Low levels of androgens in men are associated with cardiovascular risk factors and also insulin resistance (161, 162). There is also a decreased metabolic clearance of GnRH, LH, FSH and prolactin. Hyperprolactinemia in renal failure is partly induced by a decreased metabolic clearance but also by autonomic overproduction and can contribute to the reduction in testosterone levels (163). Similar changes, i.e. elevated levels of LH, FSH and prolactin and reduced levels of oestradiol, have also been found in girls with renal failure (164).

**The autonomic nervous system and catecholamines**

Increased concentrations of circulating catecholamines have been demonstrated in patients with advanced renal failure (165). Plasma norepinephrine levels have also been reported to be elevated (166), however, levels of epinephrine do not appear to be generally increased (167). Autonomic neuropathy is also a prevalent finding in uremic patients. Studies have shown that it can be improved by long-term dialysis treatment or kidney transplantation (168, 169). Autonomic neuropathy also is a well-known complication among patients with diabetes and these abnormalities may be accentuated when renal failure evolves. An imbalance between sympathetic and parasympathetic nervous activity may potentially contribute to insulin resistance and perhaps a relative inactivity in the parasympathetic nervous system is the most important factor (170, 171).

**Adipose tissue hormones and cytokines**

Leptin is a hormone that is produced and released by the adipose tissue, and leptin levels are associated with obesity and insulin resistance (172). Similar to several other peptide hormones, leptin becomes elevated with renal impairment probably due to a decrease in renal clearance occurring via glomerular filtration followed by metabolic degradation in the renal tubular cells (173). This is supported by the finding that leptin levels in renal failure are elevated despite a lower leptin gene \((ob\) gene\) expression (174). Leptin is associated with plasma insulin levels, sympathetic nervous activation as well as obesity and may through different pathways be associated with insulin resistance (175).

Adiponectin and resistin are two other homones, secreted from adipose tissue, both of which may affect insulin resistance (176, 177). Resistin seems to impair insulin action and induce glucose intolerance. Adiponectin levels, on the other hand, seem to be associated with high insulin sensitivity and the hormone may have a protective role on the vascular endothelium but it has also been reported to be elevated in patients with severe renal failure (178).

Several cytokines are also secreted by the adipose tissue, for example TNF-\(\alpha\), interleukins (IL) such as IL-6 and IL-1. Previous studies have shown an association between TNF-\(\alpha\) and
insulin resistance (123). Elevated cytokine activity, e.g. of IL-1β, TNF-α and their specific inhibitors, has also been found in patients with renal failure and may be correlated to the serum creatinine level (179). An interesting finding in this context is that TNF-α may be suppressed by ACE inhibition as shown both in vitro and in vivo (180). IL-6 could also potentially contribute to insulin resistance. IL-6 is also interesting since it may affect nutritional status, is proatherogenic and associated with poor outcome in hemodialysis (181).

Other endocrine alterations in nephropathy that potentially could influence insulin sensitivity include erythropoetin deficiency (124) and activation of the RAS (182, 183), but at present their role is not clear.

The mentioned endocrine perturbations seem to only partly be influenced by renal replacement therapy. Chronic hemodialysis or continuous peritoneal dialysis significantly improves insulin action in subjects with renal failure (184). This is not explained by changes in the levels of GH and glucagon, since they are not altered. Dialysis also increases insulin clearance at the hepatic level (184). In patients with kidney transplants, immunosuppressive medication exhibit adverse metabolic effects on glucose tolerance and lipid metabolism and this can counteract the beneficial metabolic effects of the improved renal function.

Most of the studies on endocrine perturbations in development of renal failure have been performed in patients with severe renal failure, end stage renal disease or on renal replacement therapy. There are so far very few studies on endocrine alterations in patients with early renal disease.

**Clinical management**

Strategies for therapeutic interventions include primary prevention, in patients with diabetes and normoalbuminuria; secondary prevention, in patients with signs of incipient nephropathy, i.e. microalbuminuria; and tertiary prevention, in overt nephropathy to halt the development of end stage renal failure.

**Glycemic control**

Improved glycemic control has been shown to prevent the development of microalbuminuria in both type 1 and type 2 diabetes (185-187) and it can also stabilise or normalise established microalbuminuria (188, 189). Glycemic level is also important for the rate of progression of overt DN (32, 61). Some previous studies (190, 191) have suggested a glycemic threshold for the development of DN. However, no evidence for such a threshold was found in the Diabetes Control and Complications Trial (DCCT), although the magnitude of the absolute risk reduction is greatest with reductions in HbA1c from a high level. Thus, there is still a clinically relevant risk reduction when HbA1c is reduced further towards the normal range (192). On the other hand, an intensified glycemic control also increases the risk of hypoglycemia, particularly in type 1 diabetes (185). However, in the DCCT it was concluded that the problem of severe hypoglycemia with intensive insulin therapy is clearly outweighed by the reduction in microvascular complications (185).

A clinical observation in patients with DN is that glycemic control tends to deteriorate during the progression of nephropathy. To understand the pathophysiology with respect to glucose homeostasis, insulin action and kinetics is therefore important in order to optimise therapy. Some studies have suggested that rapid acting insulin analogues may be beneficial in patients with overt nephropathy (193) and in patients on hemodialysis treatment (194). In patients treated with continuous peritoneal dialysis, intraperitoneal insulin can by administered together
with the dialysis fluid. Insulin may be delivered into the circulation in a physiological way with high levels in the portal system and lower peripheral insulin levels (195). However, one problem is that the risk of infectious peritonitis may be slightly increased (196, 197). In patients with DN and dialysis treatment intraperitoneal insulin therapy may offer a better glycemic control and insulin sensitivity as compared to subcutaneous insulin. On the other hand, it was reported to reduce HDL levels, increase the LDL/HDL ratio significantly, and thus aggravate dyslipidemia (198) and to predispose patients to hepatic steatosis (199). Taken together, the present available results can not definitely prove whether insulin delivery via the subcutaneous or the intraperitoneal route is superior with respect to overall outcome.

Treatment of hypertension and microalbuminuria
Early in the 1980’s the progression of DN was shown to be postponed upon long-term treatment with blood pressure-lowering agents (108). A close correlation between arterial blood pressure and the rate of decline in GFR has also been shown in overt DN. Statistical analyses did not reveal a lowest threshold for the adverse effect of high systemic blood pressure on the fall in GFR (200). Hemodynamic factors regulated by the RAS and AT II seem to play a central role in the development of DN, and hence angiotensin converting enzyme inhibitors (ACE-I) have been used in many trials. A meta-analysis of trials with ACE-I in DN showed that these agents lead to a reduction in the risk of progression from microalbuminuria to overt nephropathy (201) and they seem to be superior, in terms of renal protection, to other antihypertensive agents not targeting the RAS. Treatment with ACE-I also seemed to have long-term effects with preservation of kidney function, i.e. GFR, over at least eight years (202). Early intervention with ACE-I and blood pressure reduction already in normotensive patients with microalbuminuria has been shown to be beneficial both in type 1 and type 2 diabetes (101, 203, 204). During the last years, several clinical trials using angiotensin receptor blockers (ARB) in patients with diabetes have shown similar renoprotective effects as ACE-I, both in type 1 (205) and, in particular, in type 2 diabetes (206, 207). The use of ACE-I and ARB can lead to renal protection in DN but still nephropathy may continue to progress but at a slower rate. A more effective blockade of the effects of AT II by reducing both synthesis and its binding to the AT-II type 1 receptor, i.e. a combination therapy with ACE-I and ARB has shown an additive effect on blood pressure and markers of renal function such as albuminuria (208). One of the reasons for employing “dual blockade” is that multiple pathways can generate AT II. It is debated whether the beneficial effects seen with ACE-I and ARB are due to blood pressure-lowering, or if other effects of RAS inhibition are important in patients with DN. Clinical trials have also indicated that ACE-I and ARB may be more effective than traditional antihypertensive treatment in reducing the progression towards ESRD. These differences are not explained by different effects on blood pressure (209). It is nevertheless clear that treatment of hypertension is an important task in preventing and postponing the development of DN. The choice of antihypertensive agent(s) should be based on several factors including patient compliance, plausible side-effects and other concomitant diseases. In the UKPDS trial more than 2/3 of the patients with type 2 diabetes and hypertension needed at least two antihypertensive agents to achieve a mean blood pressure of 144/82 (210). A combination of blood pressure-lowering agents will thus be required in most hypertensive patients with type 2 diabetes.

In the Swedish national guidelines for diabetes care, ACE-I has been recommended as a first choice in patients with type 1 diabetes and signs of incipient or overt DN. A blood pressure target ≤130/80, or perhaps even lower, is recommended in these patients (211, 212).
The rate of synthesis for AT II seems to play an important role in initiation and progression of DN by affecting hemodynamic and non-hemodynamic mechanisms. In the future perhaps determination of I/D polymorphisms of the ACE gene or other candidate gene polymorphisms within the RAS may improve the evaluation of the individual risk profile and help us to individualise antihypertensive treatment.

Dietary proteins
It has long been known that glomerular filtration rate is influenced by protein intake and that a high protein intake worsens the clinical manifestations of ESRD. Several experimental studies in the last decades have also indicated a potentially harmful effect of dietary protein on renal function and structure in animals with renal lesions (47) but it has been difficult to clinically demonstrate a beneficial action of protein restriction in the diet. However, a reduction of protein intake decrease GFR in hyperfiltrating young patients with type 1 diabetes (213). In 1996, a meta-analysis of clinical studies in diabetic and non-diabetic patients with chronic renal disease indicated that a low-protein diet (0.4-0.6 g/kg body weight) had beneficial effects on the reduction in GFR (214) and slowed the progression of both diabetic and non-diabetic renal disease. This was supported by a recent study (215) where a moderate dietary protein restriction improved the renal prognosis in type 1 diabetic patients with progressive DN. In addition, it has been shown that a low-protein diet has beneficial effects on the alterations in insulin sensitivity and clearance in patients with chronic renal failure (216, 217). Because of the reduction in protein intake, the proportion of carbohydrates or fat must be increased. Interestingly, studies have shown that an increase in carbohydrates in the diet of patients with DN also was accompanied by an improved insulin action (218). However, when a low-protein diet is prescribed, the nutritional status of the patient must be carefully monitored and the glycemic control in patients with diabetes should be adequate since absolute or relative insulin deficiency leads to catabolism. Another interesting observation is that not only the amount of proteins but also the type of protein (219) may be of importance in the development of microalbuminuria, and, if confirmed, this might have implications for dietary advice in the future. Today a low-protein diet is only recommended in symptomatic uremia.

Smoking and tobacco use
Studies have shown that smoking is a risk factor for initiation (220) and also for the progression of DN (221, 222). Despite this, 35% of men and 29% of women, were smokers in a large European study on diabetic complications in patients with type 1 diabetes (223). Smoking patients with type 1 diabetes have higher insulin requirements, triglyceride levels and blood pressure, all features of insulin resistance, when compared with non-smoking patients (224, 225). Cessation of smoking should be particularly important in patients with diabetes due to the high risk of developing both micro- and macrovascular complications.

Lipid lowering treatment
Hypercholesterolemia plays an important role in aggravating renal damage in experimental diabetes in animals and this can be prevented by lipid-lowering therapies (226). An independent association between hypercholesterolemia and rapid loss of renal function in patients with type 1 diabetes and DN has been demonstrated (227, 228). Similar results are seen in patients with type 2 diabetes (229). Intervention with the cholesterol-lowering agents, statins, over a long period in type 2 diabetes slowed the decline in GFR and reduced albumin excretion (230, 231). There are few trials in type 1 diabetes but in one trial lipid-lowering treatment tended to have a beneficial effect on microalbuminuria (232).

Erythropoetin (EPO) treatment
Anemia due to a relative erythropoetin deficiency may occur early in some patients with DN. The mechanisms for this have not been elucidated but an association with autonomic neuropathy (233) or early renal interstitial damage has been suggested. Anemia in chronic renal failure is normally not observed until GFR drops to 20-40 ml/min. Many of these patients are also treated with ACE inhibitors, which may cause a small decrease in serum erythropoetin level as a side-effect (234). Early EPO substitution is probably beneficial as anemia may contribute to insulin resistance (124) and left ventricular hypertrophy (235), both of which are strong risk factors for cardiovascular disease, and maybe also to the progression of renal disease (236).

Vitamins

No alterations in vitamin D metabolism have been shown in patients with diabetes and normal renal function (237) even though an early loss of bone density has been observed in type 1 diabetes (238). A more pronounced bone loss is seen in uremic patients with diabetes compared to non-diabetic patients (239). In chronic renal failure an early elevation of PTH, partly due to hyperphosphatemia and hypocalcemia is seen (240). The activation of 1-α hydroxylation of 25(OH) D-vitamin is reduced in renal failure, and hence, early substitution with active D-vitamin and dietary phosphate restriction in early chronic renal failure may be recommended, particularly in patients with diabetes. Vitamin E has been suggested to prevent microvascular complications patients with type 1 diabetes (241). On the other hand, no beneficial effect of E-vitamin supplementation on cardiovascular events could be confirmed in a large clinical trial, the HOPE study (242). Elevated levels of homocysteine are observed in more than 90% of patients with ESRD (243), but they do not seem to be altered by diabetes (244) or isolated microalbuminuria (245). High levels of homocysteine have been linked to atherosclerosis, but not to the rate of progression of renal impairment (246). It is speculated that a lowering of the homocysteine levels, using a folic acid and B-vitamin regimen in renal disease, could reduce the excess incidence of cardiovascular disease and this is explored in ongoing trials (243, 247).

ASA (acetylsalicylic acid)

Due to the elevated risk of cardiovascular disease, treatment with low-dose (75-325 mg) aspirin (acetylsalicylic acid, ASA) is recommended as primary and secondary prevention in adult patients with DN (248). No adverse or beneficial short-term effects on AER or GFR have been shown in patients with type 1 diabetes and microalbuminuria (91).

Renal replacement therapy

In the first decade after the introduction of hemodialysis, diabetes was considered an absolute or a very strong relative contraindication to dialysis treatment due to poor survival. Since then, the treatment modalities have improved considerably but the survival of patients with diabetes in hemodialysis is still poor. Among some nephrologists, continuous ambulatory peritoneal dialysis (CAPD) is a preferred as treatment in patients with diabetes and renal failure (249). This may partly be motivated by less weight gain between the dialysis treatments, a risk factor for mortality due to cardiovascular problems, but also by the possibility of intraperitoneal insulin administration and fewer hypoglycemic episodes. On the other hand, it is not known if survival is better in CAPD compared to hemodialysis in diabetic patients since it has been difficult to perform randomised studies (250).

Today the best treatment in patients with DN and ESRD is transplantation with a kidney from a living donor or a combined kidney-pancreas transplant (251, 252). However, the availability of organs for transplantation is limited and a selection of eligible patients must therefore be
performed. This process is mainly based on identification of certain risk factors, i.e. cardiovascular disease and high recipient age, predicting peri- and postoperative outcome (253). Hence, patients placed on the waiting list for transplantation are relatively healthier but long-term survival is better even better among those who eventually undergo transplantation (254), arguing that this treatment is truly beneficial. It is noteworthy that survival after kidney transplantation is still worse in diabetics as compared with non-diabetics (255).
Summary
In fig. 2, current hypothetical pathways for the development of DN are schematically depicted.

Fig. 2.
A schematic view summarising current hypotheses on the development of diabetic renal disease. Possible interactions between metabolic, hemodynamic, genetic and enviromental factors in the initiation and progression of DN. Modified from M Cooper (77).
RESEARCH QUESTIONS AND SPECIFIC AIMS

Hyperglycemia is necessary and a major risk factor in the development of DN. It has been suggested that the importance of glycemic control in relation to the development of microvascular complications may vary during different phases of the disease, i.e. early and late in disease. There are also conflicting data on the importance of age at onset of diabetes with regard to the future development of diabetic complications. Insulin resistance is a potential mechanism involved in the development and progression of DN, but it has also been demonstrated as a consequence of severe diabetic and non-diabetic renal impairment. Little is known about insulin resistance in earlier phases of DN, e.g. in patients with albuminuria alone or with a small reduction in glomerular filtration rate (GFR). We wished to further investigate these two aspects of DN, i.e. factors governing early development of DN and the metabolic consequences of slight or moderate renal impairment. Thus, the following aims were formulated.

Specific aims:

1. To elucidate whether there is a quantitative relationship between the degree of albuminuria and insulin resistance. This was addressed in patients with type 1 diabetes and micro- or macroalbuminuria but normal GFR (study I).

2. To investigate insulin resistance and insulin clearance in patients with type 1 diabetes with three different stages of renal involvement; none, albuminuria alone and slightly reduced GFR, respectively (study II).

3. To study early occurrence of renal involvement in diabetes with onset in young adults and relate the findings to potential risk markers such as glycemic control, type of diabetes, gender, smoking, and blood pressure (study III).

4. To study the occurrence of incipient DN and background retinopathy in patients with childhood-onset type 1 diabetes, and to address the impact of glycemic control (HbA1c) early in disease and the importance of age at onset (study IV).
METHODS

Study cohorts
The patients in study I and II were essentially recruited from the outpatient Diabetes Unit at the Umeå University Hospital. Eligible patients with type 1 diabetes and different degrees of renal involvement, i.e. micro- or macroalbuminuria alone or slightly reduced GFR, were consecutively asked whether they wished to participate and those who accepted were included. In study I, all 18 patients with albuminuria alone were studied to specifically address the relationship between the degree of albuminuria and insulin-resistance. In study II, eight patients with slightly reduced GFR were compared with eleven patients with albuminuria alone and ten patients without signs of renal involvement and the groups were matched for sex, BMI, diabetes duration and glycemic control. Altogether 36 patients with type 1 diabetes and different degrees of renal impairment participated in these studies. In study III, all patients diagnosed with diabetes at ages 15-34 years and reported to the Diabetes Incidence Study in Sweden (DISS) registry during the years 1987 and 1988, were invited to a follow-up study on diabetic complications and in 1994, 579 of these 806 patients accepted to participate. The process of subject recruitment is outlined in fig. 1 in paper III. The 94 patients in study IV, were 0 to 14 years old at onset of type 1 diabetes and they were identified through the Swedish Childhood Diabetes Registry. Most of the patients were later followed-up at the Department of Pediatrics and the department of Internal Medicine at the Umeå University Hospital. An outline of the study cohorts is shown in Fig. 3. The studies were approved by the Ethics Committee of Umeå University and all participating subjects gave their informed consent.

Renal function (study I and II)
Glomerular filtration (GFR) was determined by $^{51}$Cr EDTA plasma clearance and normal GFR was defined according to age-adjusted criteria as previously reported (256). Urine albumin concentration and albumin excretion rate (AER) were measured with immune turbidimetry on a Hitachi 911 multianalyser (Boeringer Mannheim Diagnostica, Mannheim, Ger-

Fig. 3.
Schematic illustration of study cohorts in studies I-IV.
many). AER was measured in two consecutive overnight urine collections and the mean value (µg/min) was used. Urine sterility was ascertained with urine dipstick and urinary sediment. No patient had hematuria or urinary casts when DN was diagnosed. The urine collections were performed without any changes in ongoing medication.

**Blood chemistry**

Blood samples were generally obtained at 08.00 h following an overnight fast unless otherwise is indicated.

In studies I and II HbA1c was measured by high-pressure liquid chromatography (HPLC, Integral 4000, BioRad, Anaheim, CA, USA, normal range 3.6-5.0%). In study III, HbA1c was measured using ion-chromatography at the local hospital laboratories (257). The mean value during the follow-up period was calculated for each patient with adjustments for the time between the measurements (258). This mean HbA1c showed a strong correlation with the HbA1c measured in the blood samples from the study participants at follow-up (r=0.70, p<0.001) analysed at a central laboratory (Malmö University Hospital). All HbA1c values used in study IV were measured at the Department of Clinical Chemistry, Umeå University Hospital. Between 1981-85 an isoelectrical focusing technique (normal range 5-8%) (259) was used. During 1985-91 glycosylated hemoglobin was analysed by using chromatography on boronateagarose gel (normal range 4.2-6.6%). 1991-97 HbA1c was measured by high-pressure liquid chromatography (HPLC) (normal range 3.2-4.7%). In 1997 a national Swedish calibration of HbA1c analyses was made and the normal range in Umeå was adjusted to 3.6-5.0%. Finally, HbA1c values were calculated to correspond to this method by using the regression equations between the different methods described and a mean HbA1c was estimated for each patient as previously described (258).

In study I and II blood glucose was measured by the HemoCue® glucose system (HemoCue AB, Ångelholm, Sweden). Non-esterified fatty acids (NEFA) were analysed by an in vitro enzymatic colorimetric method (NEFA, Wako, Neuss, Germany). Serum insulin was measured by microparticle enzyme immunoassay (MEIA, Abbot Imx, Abbott Laboratories, Abbott Park, IL, USA). In one patient, unexpectedly high insulin levels were found and insulin antibodies were suspected. Serum free insulin was therefore measured after PEG precipitation. It can not, however, be excluded that also other patients in study I and II, might have insulin antibodies that could affect the measurements of insulin levels or interfer with the serum insulin assay. Intact parathyroid hormone (PTH) and cortisol were measured by chemiluminescent enzyme assays on Immulite 2000 (Diagnostics Product Corporation, Los Angeles, CA, USA). Human leptin was measured by a radio immunoassay (RIA, Linco Research, inc. St Charles, MI, USA). Insulin-like Growth Factor-1 (IGF-1) was analysed by an immunoradiometric assay (IRMA, The Nichols Institute Diagnostics San Juan Capistrano, California, U.S.A.). Human Tumour Necrosis Factor-α (TNF-α) and Interleukin-6 (IL-6) were measured by enzyme-linked immunsorbent assays (ELISA, Quantikine HS, R&D systems Europe, Ltd, Abingdon, U.K.). All other blood and urine analyses were performed by routine methods at the Department of Clinical Chemistry, Umeå University Hospital.

**Insulin action in vivo**

In study I and II, the hyperinsulinemic euglycemic clamp technique was utilised to assess insulin sensitivity (260). The patients with type 1 diabetes were hospitalised the evening before the glucose clamp and given an overnight intravenous insulin infusion to normalise glycemica. They arrived to the laboratory after overnight fasting since 22.00 h. A 2h-euglycemic hyperinsulinemic clamp was then started at 08.00 h and after initial priming, a constant infu-
sion of short-acting insulin (Actrapid®) was administered at 56 mU/m² body surface/min. The glucose infusion was adjusted to maintain blood glucose at 6.0 mmol/L. Insulin sensitivity was assessed as glucose uptake at steady state, during the time period 60-120 min. Glucose uptake was expressed as the M-value, which was related to either body weight or lean body mass (mg glucose infused /kg/min). Insulin sensitivity index (ISI) is a measure of tissue sensitivity to insulin adjusted for the prevailing insulin level and was calculated by dividing the M-value by the mean insulin concentration during the same period of the clamp (261). The metabolic clearance rate for insulin (MCR) was also calculated at steady state (60-120 min) by dividing insulin infusion rate with the mean serum insulin level. All subjects had negligible C-peptide levels (< 0.2 nmol/l), and thus there was no need to correct for endogenous insulin production (262, 263). In patients on antihypertensive medication the treatment was withdrawn two weeks prior to the insulin clamp, as certain antihypertensives are known to affect insulin sensitivity.

Insulin action in vitro
In study II, isolated fat cells were used to assess cellular insulin sensitivity in an insulin target tissue. Needle biopsies of abdominal subcutaneous fat tissue were performed in the fasting state at 8.00 h and adipocytes were isolated by treatment with collagenase at 37°C for one hour. After cell isolation adipocytes were filtered through a nylon mesh and washed with fresh medium. During this procedure short-term effects of insulin, other hormones and humoral factors present in vivo should be eliminated (264).

Glucose uptake was measured in adipocytes that were preincubated in medium 199 containing 4% bovine serum albumin (BSA) without glucose for 15 min at 37°C in the absence or presence of different concentrations of insulin and subsequently ¹⁴C-U-D-glucose (0.86 µmol/L) was added. After 1 h glucose uptake was terminated and adipocytes were immediately separated by centrifugation through silicone oil. Cell-associated radioactivity was measured by scintillation counting (264), and cellular clearance of glucose from the medium was calculated. Under these conditions glucose uptake is mainly determined by the rate of transmembrane glucose transport (265).

To specifically assess cell-surface insulin binding, adipocytes were pretreated by 2 mM KCN treatment for 5 min in order to deplete the cells of ATP and stop receptor internalisation and recycling (266). Subsequently, cell surface binding of ¹²⁵I-insulin (0.2 ng/ml) was carried out for 60 min at 16°C. After the incubation period (1 h), cells and medium were separated by centrifugation and specific cell-associated ¹²⁵I-insulin binding was measured.

To determine insulin degradation in adipocytes, cells were incubated in medium 199 for 1 h at 37°C with ¹²⁵I-insulin (0.2 ng/ml). After separating medium from cells, intact ¹²⁵I-insulin in the cell-free medium was precipitated by 10% trichloroacetic acid. The radioactivity in the supernatant was considered to represent degraded insulin released from the adipocytes.

Lipolysis was measured in isolated adipocytes that were incubated at 37°C, for 1 h in medium 199 containing 5.6 mmol/L glucose, 4% BSA, insulin and 8-bromo-cAMP as indicated. Adipocytes were then rapidly separated from medium by centrifugation through silicone oil. Lipolysis was assessed by determining the glycerol content in the medium by a phosphorylation reaction using glycerokinase and [γ-³²P]ATP (267).
Registries, questionnaires and medical records
Since 1983, the registry of the Diabetes Incidence Study in Sweden (DISS), used in study III, has aimed to register all newly diagnosed cases of diabetes, except those with gestational diabetes, in the age group 15-34 years. The overall ascertainment rate has been found to be 80-85% (268, 269). This rate was calculated by comparing the incidence of diabetes according to the DISS with other independent data sources such as routine administrative systems of hospital clinics. In 1994, all patients of the 1987-88 DISS cohort were invited to participate in a follow-up study on diabetic complications and 582 patients accepted. A questionnaire was sent to their treating physicians. Between 1994 and 1999 the treating physician delivered clinical data, including height, weight, smoking habits, type of diabetes (clinical classification), current medication, present blood pressure, all HbA1c values and clinical information on long-term complications. The presence of long-term complications based on clinical evaluation were indicated as follows: nephropathy (micro- or macroalbuminuria, elevated serum creatinine or renal replacement therapy), retinopathy (degree and visual acuity), neuropathy (peripheral and autonomic), and coronary, cerebrovascular or peripheral arterial disease.

Since 1 July 1977, the Swedish Childhood Diabetes Registry, used in study IV, has included all newly diagnosed cases of childhood-onset diabetes in the age group 0-14 years attending any pediatric department in Sweden. The registry covers 96-99% of all incident childhood diabetes cases in Sweden (270).

In both study III and IV, the collection of data was done retrospectively. Medical records and laboratory reports were retrieved to evaluate, and confirm or dismiss the diagnosis of a chronic diabetic complication. The data in medical records may not always be complete and are heterogeneous between different departments. However, in both these studies, all data were obtained and registered in a uniform and manner to ascertain that the available information was registered standardised as far as possible.

Statistical methods
Statistical analyses were performed using Stat View4.5® statistics software (Abacus Concepts, Inc.) (study I, II) and SPSS 10.0 Macintosh version (The Software MacKiev Company, Cupertino, CA, USA) (study III, IV). Conventional statistical methods with appropriate post hoc testing were used to compare mean values between groups. Albumin excretion rates were logarithmically transformed before analysis due to skewed distribution (study I, II). Associations between variables were analysed with simple regression or stepwise multiple regression. The Kaplan Meier analysis was used to calculate cumulative incidences and differences between groups were tested by log-rank test. The Cox proportional hazard analysis was used to assess the influence of different variables with respect to microvascular complications (study III, IV). P-values less than 0.05 were considered as statistically significant.
SUMMARY OF RESULTS

Paper I
Albuminuria is considered to be associated with insulin resistance in patients with type 1 and type 2 diabetes as well as in non-diabetic subjects. It has also been reported that among patients with type 1 diabetes, those with albuminuria are slightly more insulin-resistant than those without. The aim of this study was to elucidate if there is a direct quantitative relationship between the degree of albumin excretion rate (AER) and insulin resistance. In order to study a group of patients with albuminuria without major interference from the metabolic syndrome, we investigated patients with type 1 diabetes and “mild” nephropathy, i.e. micro- or macroalbuminuria but normal GFR. We also addressed the possible relationship between AER and the rate of insulin clearance. Insulin sensitivity and insulin clearance were measured with the euglycemic hyperinsulinemic clamp technique.

The study participants with type 1 diabetes and micro- or macroalbuminuria (n=18) displayed a large range of insulin sensitivity. They were slightly insulin-resistant when compared with non-diabetic healthy controls with similar age and BMI. Simple regression analyses showed no correlation between AER and insulin sensitivity assessed as M-value or insulin sensitivity index (ISI). This was also true in a stepwise multiple regression model including age (or alternatively diabetes duration), gender, tobacco use, HbA1c, BMI (or alternatively fat mass) as potential confounding factors that might affect insulin sensitivity. Similarly, no correlation was found between AER and insulin clearance. Moreover, regression analyses showed no correlation between GFR and insulin sensitivity or insulin clearance in this group of patients that all had GFR within the normal range. Both tobacco use and high HbA1c were associated with the magnitude of AER.

Main conclusions: It is presently not clear whether there are mechanisms by which insulin resistance per se can lead to albuminuria or by which albuminuria can contribute to the development of insulin resistance. Our results, however, argue against a direct link between the degree of albuminuria and insulin resistance, and hence, a direct cause-effect relationship appears unlikely. This was shown in type 1 diabetics, but could possibly be applicable to other groups of subjects as well.

Paper II
Insulin sensitivity and insulin clearance are compromised in end-stage renal disease, but in this study we focused on earlier stages of DN, which have not been well characterised in this respect. We studied three groups of patients with type 1 diabetes; patients with no signs of renal involvement (n=10, group C) were compared to those with isolated albuminuria but normal GFR (n=11, group A) and those with slightly reduced GFR (43-73 ml/min/1.73m², n=8, group G). Group A and G, respectively, were considered to represent two consecutive phases in the progression of DN. Patients in group G had significantly lower M-values, i.e. they were more insulin-resistant than patients in groups C and A, whereas group C and group A did not differ. The reduction in insulin sensitivity among patients with reduced GFR persisted also after adjusting for potential confounding factors such as age, gender, BMI, smoking and present HbA1c using stepwise multiple regression. In addition, when using simple regression analysis, there was a strong positive correlation between GFR and insulin sensitivity in all patients taken together. Patients in group A displayed a somewhat higher insulin clearance compared to those in group G but not when compared to group C. Importantly, the group with reduced GFR did not differ in insulin clearance compared to the control group.
However, among patients with reduced GFR, but not in the two other groups, there was a near-significant correlation ($r=0.72$, $p=0.051$) between GFR and insulin clearance.

Significant differences in serum levels of potential insulin-antagonistic hormones and cytokines were found between groups. Parathyroid hormone (PTH) was significantly elevated in group G and in a simple regression analysis inversely correlated with GFR in all patients taken together. Morning fasting serum cortisol was significantly reduced in group G and it was correlated to GFR. Insulin-like growth factor-1 (IGF-1) was higher in group G compared with group A but not with group C, and it was near-significantly related to GFR ($r=-0.33$, $p=0.077$). Leptin displayed no differences between groups when split by gender (possibly due to few subjects) but it was inversely correlated to GFR. Tumour Necrosis Factor-α (TNF-α) was higher in group G compared to the two other groups and inversely related to GFR. Interleukin-6 (IL-6) was significantly higher in both groups G and A, as compared to C and tended to be inversely correlated with GFR. PTH and IGF-1 were both negatively correlated with insulin sensitivity.

In vitro, there were no significant differences between subcutaneous fat cells from the three different patient groups when comparing the basal unstimulated glucose uptake, the insulin-stimulated glucose uptake, the anti-lipolytic effect of insulin, cell-surface insulin binding and insulin degradation.

Main conclusions: We found that in patients with type 1 diabetes, the appearance of albuminuria does not seem to alter insulin sensitivity and clearance. However, when GFR becomes slightly reduced the patients clearly exhibit insulin resistance but no consistent impairment in insulin clearance. These alterations do not seem to be accompanied by general intrinsic defects in insulin target cells, e.g. adipocytes. Instead alterations in the levels of insulin-antagonistic hormones and cytokines may possibly be involved.

Paper III

The aim of this study was to estimate the early occurrence of renal involvement in young adults treated with modern diabetes treatment. Therefore we studied a cohort of patients from the DISS registry that were 15-34 years old when diagnosed with diabetes during 1987-1988. We also wanted to study whether the occurrence of incipient and overt nephropathy was affected by factors besides glycemic control such as type of diabetes, gender, smoking and blood pressure.

During the follow-up time, median 9 (range 6-12) years, 6.6% of the patients displayed signs of incipient nephropathy, i.e. microalbuminuria, (5.1%) or overt nephropathy, i.e. macroalbuminuria, (1.5%). Diabetes classification was done by a combination of clinical evaluation and islet antibody analysis. 5.6% of patients with type 1 diabetes and 16% of patients with type 2 diabetes displayed signs of renal involvement. This difference in the occurrence between diabetes forms was clearly significant ($p=0.016$). Incipient or overt nephropathy was diagnosed at median 8 (range 0-12) years following diabetes diagnosis. In a Cox regression analysis a high mean level of HbA1c during the follow-up period and a high blood pressure at follow-up were risk markers for renal involvement. Compared to patients with type 1 diabetes, those with type 2 tended to have an increased risk to develop incipient or overt nephropathy also after adjusting for gender, tobacco use, and blood pressure as potential confounders. Patients with type 2 diabetes also tended to have a more rapid progression of nephropathy compared to patients with type 1 diabetes.
Main conclusions: Despite modern diabetes management including intensive insulin treatment and self-monitoring of blood glucose there are some patients who develop incipient or overt nephropathy during the first ten years of diabetes. The main risk markers for early development of renal involvement were poor glycemic control and high blood pressure. Type 2 diabetes, in itself, was also a risk marker. Hence, a correct classification of diabetes is important to predict the risk and time-course for development of nephropathy as well as other long-term complications.

Paper IV
The aim of this study was to estimate the occurrence of incipient nephropathy and background retinopathy in a population-based cohort of patients with childhood-onset type 1 diabetes who were treated according to a standardised nationwide diabetes care program. We also wanted to study the impact of early versus late glycemic control as well as age at onset after adjustment for other potential risk factors such as disease duration, tobacco use, birth weight and gender.

During the follow-up period, mean 12 (range 5-19) years, 18% exhibited signs of incipient nephropathy, 48% of background retinopathy and 52% had either or both complications. A Cox proportional hazard regression, modelling duration to event of incipient nephropathy or retinopathy, showed that glycemic control, reflected by mean HbA1c, during follow-up was significantly associated with both complications, also when adjusting for gender, birth weight, age at onset and tobacco use as potential confounders. Mean HbA1c during the first five years of diabetes was a near-significant determinant for development of incipient nephropathy and it was significant for retinopathy. The age at onset of diabetes significantly influenced the risk of developing background retinopathy. Thus, in a Kaplan-Meier analysis onset of diabetes before the age of five, compared to the age groups 5-11 and >11 years, had a longer time to development of retinopathy. No clear tendencies were seen for incipient nephropathy in this respect, perhaps due to fewer cases and, hence, lower statistical power.

Main conclusions: More than 50% of patients with childhood-onset type 1 diabetes developed signs of early microvascular complications after ~12 years of diabetes, despite modern insulin treatment. Poor glycemic control, also during the first five years of diabetes, seems to increase the risk, whereas a young age at onset of childhood diabetes may delay development of microvascular complications.
DISCUSSION

Initiation and early development of diabetic nephropathy
In the development of microvascular diabetic complications, such as DN, hyperglycemia is necessary and the structural and morphological findings are specific to diabetes (8). Many pathways have been described, e.g. metabolic and hemodynamic alterations, by which hyperglycemia can potentially initiate and aggravate long-term dysfunction in various organs. However, hyperglycemia does not seem to be a sufficient factor for the development of diabetic renal disease. An individual, partly genetic, susceptibility and other contributing risk factors, such as low birth weight, short stature, male gender, puberty, dyslipidemia and smoking have been described in epidemiological studies (55, 84, 220, 271).

In study III and IV, we have shown that, despite generally established modern treatment guidelines for diabetes and widespread use of intensive insulin regimens, there are still patients who early-on, after ~10 years of diabetes duration, develop microvascular complications. Incipient nephropathy was found in patients with childhood-onset type 1 diabetes (~18%) as well as in young adults with diabetes, with both type 1 (~5%) and type 2 diabetes (~9%). In addition, some of these young adults had overt nephropathy (~1.5%, i.e. 1% for type 1 diabetes and 7% for type 2, respectively). The development of complications is significantly associated, as shown previously, with the level of both short- and long-term glycemic control (11, 272). However, some patients developed complications despite reasonably good glycemic control. We were able to show that the glycemic control, already during the first five years in childhood-onset diabetes, may be an important predictor of later development of both incipient DN and background retinopathy. This was found in a relatively small, retrospective and population-based study among patients treated according to a modern, nationwide diabetes care program. Early glomerular hyperfiltration has been shown to predict both overt and incipient DN (57, 58). Our findings may suggest an effect of hyperglycemia soon after onset of diabetes to promote glomerular hyperfiltration and later on, the development of DN. It has even been hypothesised that an increased glomerular filtration will start a self-perpetuating glomerular injury (56, 96). Hyperglycemia is also associated with the development of advanced glycation end products (AGE), which are believed to play an important role in the pathogenesis of both DN and retinopathy. Changes in the level of AGE are seen early, within two years of diabetes, and are correlated to the long-term glycemic levels as reflected by HbA1c (35).

In study III, 45% of the patients with incipient or overt nephropathy (38% in patients with type 1, 71% in type 2 diabetes) and ~77% in study IV had concomitant retinopathy indicating that these two microvascular complications not necessarily occur together. This is compatible with the hypothesis that diabetic microangiopathy in different organs is genetically heterogeneous (273). After a long diabetes duration, retinopathy is almost universal, but not all patients develop nephropathy (9). Neither do patients with nephropathy invariably have retinopathy (274). Probably, certain genetic polymorphisms exert selective impact on the development of DN as compared to retinopathy (27). The importance of other factors, such as glycemic control, cholesterol and diastolic blood pressure, also seem to be different between these two microvascular complications (275). In study III and IV there was a difference in the occurrence of concomitant retinopathy between patients with onset of type 1 diabetes in childhood and as young adults, respectively. This could be a true difference in propensity, but, on the other hand, the procedures for detection and identification of retinopathy differed between the two studies and may contribute to the observed difference. However, it should also
be emphasised that these findings are important from the clinical point of view, as a lack of retinopathy does not exclude DN.

In a recent study on retinopathy from the DISS registry (276), the prevalence of severe retinopathy was significantly higher in patients with type 2 diabetes as compared to type 1 diabetes. This is compatible with our study, where a greater proportion of patients with type 2 diabetes had overt nephropathy. In contrast to our finding, that signs of renal involvement were more prevalent among patients with type 2 diabetes, the overall prevalence of retinopathy was not significantly higher in this group of patients (276). This could possibly indicate that a genetic trait for type 2 diabetes and the metabolic syndrome might have a specific impact on the development of DN. However, these differences may also, to some extent, be the result of different thresholds of detection between two screening methods for microvascular damage, e.g. AER and retinal photographs.

The occurrence of diabetic complications, such as incipient DN (18%) and background retinopathy (48%), within ~12 years, in our study of patients with childhood-onset type 1 diabetes is in accordance with some previous studies in children and adolescents (11, 271). However, a previous Swedish study, the Linköping study, reported a lower prevalence of persistent microalbuminuria (7.8%) in patients with childhood-onset of type 1 diabetes between 1976-1980 after ~15 years. In accordance with study IV, none of these patients had overt DN (10). Besides the DISS, there are no other studies on early occurrence of diabetic complications in patients with diabetes onset as young adults (15-34 years). The occurrence of renal involvement in patients with type 1 diabetes in the DISS study is in comparison with the Linkoping study (10), however, in contrast, a few patients in study III had overt nephropathy. Among the patients with type 2 diabetes, our results indicate a slightly lower prevalence of renal impairment than in a Danish study in older patients (277).

In study IV, older age at childhood-onset of diabetes was a weak but statistically significant risk factor for background retinopathy, also after adjusting for metabolic control and duration. Accordingly, diagnosis of diabetes before the age of five significantly prolonged the time to occurrence of background retinopathy, when compared to the groups with diabetes onset at age 5-11 and >11 years, suggesting a protective effect of prepubertal vs postpubertal duration. This was not found for incipient nephropathy but the number of patients in our study was small and the statistical power low. This is in accordance with other studies indicating that differences in pubertal status at the onset of diabetes may have an impact on the development of diabetic complications (271). However, the results have not been consistent. Some studies have shown an impact of prepubertal diabetes duration on the development of complications (82, 278) and others have failed to do so (81, 279). However, the fact that early diabetic complications can be found in the prepubertal period (271, 280, 281) indicates that hyperglycemia before puberty is of importance. The finding that early glycemic control, during the first five years of diabetes, may be of importance for future development of diabetic complications also clearly points out the clinical dilemma for the pediatric diabetologist to use intensive insulin treatments to decrease hyperglycemia without increasing the frequency of severe hypoglycemic episodes. On the other hand, the observation that the youngest age group, who are most sensitive to hypoglycemia, may have a relative protection or a longer time to development of complications is supported by other studies (271). Perhaps in this age group a less tight glycemic control during the very first years might be compensated by a better glycemic control later on. The hypothesis of a “long-time glycemic memory” is nevertheless supported by our present findings and also by a follow-up study from the DCCT, where the reduction in risk of complications achieved by intensive insulin therapy persisted several years later despite in-
creasing hyperglycemia (88). This memory could be mediated via alterations in AGE levels that remain over several years (35). However, in most of the studies, including ours, the follow-up period is limited. It must also be acknowledged that microalbuminuria is a surrogate marker for DN.

We have also, to our knowledge, for the first time shown that type 2 diabetes in young adults may be a risk determinant for early-onset renal involvement. In addition, patients with type 2 diabetes in this age group also appear to have a more rapid progression of nephropathy as compared to patients with type 1 diabetes. This observation highlights the importance of a correct diabetes classification in this age group and as clinical diagnosis is difficult, measurement of islet antibodies may be recommended (282). This interesting new finding may be of further importance since the clustering of insulin resistance, dyslipidemia, hypertension, alterations in hemostatic function and abdominal obesity, i.e. components of the metabolic syndrome, is closely linked to type 2 diabetes and probably also to nephropathy. This may partly explain the cardiovascular morbidity among these patients. In addition, it may support a hypothesis of a common genetic predisposition in patients prone to develop DN and in patients with the metabolic syndrome (21, 22, 283). Accented metabolic abnormalities similar to those of the metabolic syndrome can also be found during the progression of DN and they are particularly prominent in patients with severe renal failure.

One of the most important factors for the progression of DN is the development of hypertension (54). In study III, we were able to confirm that high blood pressure is a risk marker for DN. However, we only had information on blood pressure at follow-up and it was measured with ongoing medication in patients with antihypertensive treatment. Therefore we can only speculate that hypertension contributed to the development of renal complications in our patient cohort. In addition, systemic hypertension is seldom seen before the stage of overt DN however it is of major importance in the progression of established DN. A recent study from the DCCT suggests that diabetic renal disease follows a more favourable course in patients who receive intensive treatment, i.e. intensive insulin treatment and ACE-inhibition (284).

**Insulin action in patients with albuminuria**

Microalbuminuria is a well-known predictor for the development and progression of DN and is used as a screening tool to identify patients at risk of developing DN. It may also be one of the first clinical signs of DN. In addition, microalbuminuria may be a marker of a general endothelial dysfunction and is considered to be a component in the metabolic syndrome according to the WHO definition (285). Microalbuminuria has been linked to insulin resistance both in patients with diabetes and in non-diabetics (105, 286), but other studies have not shown any direct relationship between microalbuminuria and insulin resistance (107, 287, 288).

Our studies in paper I and II were based on patients with type 1 diabetes in whom albuminuria presumably is primarily caused by chronic hyperglycemia and when established indicates that incipient or overt DN is present. In our studies, the albuminuric subjects with normal GFR did not differ significantly in insulin sensitivity compared to patients with type 1 diabetes without albuminuria. In addition, we did not find a direct quantitative association between the degree or the duration of albuminuria and insulin resistance, arguing against a cause-effect relationship. Conversely, insulin resistance did not seem to be a major factor that influences the degree of albuminuria in patients with type 1 diabetes. Instead, other underlying factors, such as glycemic control, tobacco consumption, hypertension, dyslipidemia and perhaps neuroendocrine dysregulation or endothelial factors as well as genetic background, may be more important in determining the degree of albuminuria and they could also lead to insu-
lin resistance. The mechanisms responsible for the previously reported association between albuminuria and insulin resistance are, however, still not clear and they are probably multifactorial. If albuminuria or the presumed underlying microvascular dysfunction in some way would lead to insulin resistance then a change in the degree of albuminuria would be expected to be accompanied by a parallel shift in insulin sensitivity and this was not seen. On the other hand, insulin resistance could indirectly increase albuminuria via other mechanisms such as dyslipidemia or hyperglycemia. However, a direct quantitative relationship between insulin resistance and AER would have been expected also if insulin resistance was a major cause of albuminuria. This hypothesis may, on the other hand, be supported by results with rosiglitazone, an “insulin sensitizer”, that ameliorates insulin resistance and was found to reduce AER in type 2 diabetes patients (289). In type 1 diabetes, an interesting observation is the association between microalbuminuria and autonomic nervous dysfunction (74, 290), as autonomic nervous dysfunction also may be associated with insulin resistance (170, 171, 291). Since many factors, such as blood pressure, insulin, glucose and lipid levels, hemostatic function interact with each other, it is difficult to selectively study the individual relationships. However, our present data may suggest that also in non-diabetic subjects, for example those with the metabolic syndrome, albuminuria and insulin sensitivity are not directly linked and a temporal and causal relationship between albuminuria and insulin resistance is not proven.

Insulin action in patients with reduced glomerular filtration rate (GFR)

Patients with diabetes who develop overt DN and exhibit declining GFR also have metabolic and endocrine perturbations that may be of importance for the progression of both the diabetic state and nephropathy and also for the clinical management of these conditions. Similar alterations are also seen in other diseases accompanied by renal impairment. Patients with severe renal failure and uremia display impairments in both insulin sensitivity and insulin clearance. An increased peripheral insulin resistance, mainly located in skeletal muscle tissue has been demonstrated when glomerular filtration rate is severely reduced, GFR <40 ml/min/1.73m$^2$ (118, 119). Besides the liver, the kidneys are important for insulin clearance (114). Insulin is filtered and then reabsorbed and degraded by renal tubular cells. A decline in GFR is compensated by increased uptake of insulin be from the peritubular capillaries by tubular cells. Until a GFR of 15-20 ml/min, no reduction in insulin clearance has been detected. For lower GFR values, insulin clearance in the kidneys, probably in parallel with hepatic clearance, is drastically reduced (116). In our study (paper II) we assessed the relationship for GFR versus insulin sensitivity and insulin clearance, respectively, by studying three groups of patients with type 1 diabetes and different degrees of renal involvement (none, isolated albuminuria, and slightly reduced GFR, i.e. ~40-70 ml/min/1.73m$^2$, respectively). In parallel we performed in vitro studies on subcutaneous abdominal fat cells from the patients, to address cellular insulin action on glucose uptake and lipolysis and also insulin degradation. A marked impairment in insulin sensitivity, but not in insulin clearance, in vivo was seen in patients with a slightly reduced GFR compared to the other groups. In vitro, no difference in cellular insulin sensitivity or insulin degradation was found between the groups. This finding is compatible with the hypothesis that perturbations in the in-vivo milieu surrounding the cells, e.g. humoral factors, may contribute to insulin resistance in these patients (261).

Endocrine perturbations

In parallel with the decrease in whole-body insulin sensitivity seen in the group with slightly reduced GFR, we found concomitant changes in some insulin-antagonistic hormones and cytokines, which potentially could contribute to insulin resistance. Renal clearance and degradation of peptide hormones such as insulin and PTH are not only dependent on glomerular filtration but also on uptake and degradation in tubular cells. Tubular dysfunction has been
demonstrated in both incipient and overt DN (292). This could possibly contribute to changes in renal clearance and degradation of hormones despite normal or only slightly reduced GFR. Accordingly, we found elevated levels of PTH in the group with slightly reduced GFR. However, an increase in production of PTH could also contribute, as this occurs already early in renal impairment in response to hypocalcemia. In our study, PTH was both negatively correlated with GFR and insulin sensitivity. The relevance of the observed association with insulin resistance is unclear since evidence for a direct interaction between PTH and insulin action is lacking. PTH has, however, previously been suggested to play a role in insulin resistance among patients with renal failure (148). The level of PTH is also closely associated with D-vitamin levels and the calcium/phosphate homeostasis and it may be a surrogate marker for alterations in these systems, which could potentially affect insulin sensitivity.

Patients with severe renal failure have reduced levels of urine cortisol but no change in the circulating levels of cortisol have been shown (120). Surprisingly, in our study we found a lower morning cortisol level in the group with reduced GFR. The cortisol level was correlated to GFR and near-significantly to insulin sensitivity. However this finding should be confirmed in larger studies and its relevance in relation to insulin resistance is unclear. Moreover cortisol participates in a complex network of hormones and cytokines that may interfere with insulin sensitivity and besides insulin they include for example leptin, TNF-α and IL-6.

Elevated levels of glucagon and growth hormone are detected in uremic subjects (120, 140) and a high GH level has been associated with insulin resistance (293). Despite the improvement in insulin sensitivity after introduction of dialysis treatment in uremia, the levels of these hormones are unchanged so they probably do not have major impact on insulin resistance in severe renal failure (184). The role of the IGF-1/GH system in an early stage of renal dysfunction has to be further elucidated and this includes the involvement in initiation and progression of DN. In our study, we found elevated levels of IGF-1 in the group with reduced GFR and this might mirror high levels of GH that could be involved in the appearance of insulin resistance. On the contrary, reduced levels of IGF-1 have been shown in both diabetic patients with poor glycemic control, and particularly in patients with microvascular complications and microalbuminuria (142). In moderate renal failure IGF-1 may be normal, while in end-stage renal disease (ESRD), IGF-1 levels are slightly decreased (140).

Previous studies have shown an association between TNF-α and insulin resistance (123). Elevated levels of cytokines such as IL-1 β, IL-6 and TNF-α have been found in patients with renal failure and they correlate with the serum creatinine level (179). In accordance with this, we found elevated levels of TNF-α in the group with reduced GFR and TNF-α was negatively associated with GFR. Both TNF-α and IL-6 could potentially contribute to insulin resistance, however, no significant association to insulin sensitivity was found in our study. IL-6 is also interesting because it may play a role in the proliferation of renal mesangial cells, which may involved in the progression of DN (294). On the other hand, these cytokines may be elevated due to preexisting vascular damage and inflammatory processes, and they are related to the degree of adiposity. So far there are no prospective follow-up studies that have assessed such a relationship over time.

Leptin is associated with plasma insulin levels and sympatho-adrenergic activation and may via different pathways also be associated with insulin resistance. This could be of relevance in nephropathy and may be another important factor since leptin levels become elevated with renal impairment (295). On the other hand, leptin levels could also become elevated as a consequence of insulin resistance (162). Autonomic neuropathy or dysfunction is a well-known
complication in diabetes but also in patients with renal failure (169). However, autonomic dysfunction may also be an even earlier defect associated with insulin resistance and development of type 2 diabetes (170, 171) and perhaps be of relevance in several stages of the disease (296). The renin-angiotensin system (RAS) should also be considered. It is important in the development of DN, but in addition there are some observations that the RAS may be related to insulin resistance, and treatment with both ACE-I (297) and ARB (298) may improve insulin sensitivity although the molecular mechanisms are not elucidated. A low activity of ACE, I/I polymorphism in the ACE gene, and also treatment with ACE-I have also been suggested to increased the risk of hypoglycemia, possibly via an enhanced insulin sensitivity (182, 183, 299).

Taken together, the results from our study and others suggest that the mechanisms of insulin resistance in renal failure probably are multifactorial. In addition to the endocrine alterations there is accumulation of uremic toxins, which probably is a contributing factor since insulin sensitivity is improved by initiation of dialysis treatment and kidney transplantation. There are several other abnormalities that may have an impact on insulin sensitivity, and they include impaired physical activity, altered body composition, metabolic acidosis, hyperosmolality and medication.

Insulin resistance in a phase with only minor renal impairment, as suggested by our present results (study II), has not previously been recognised. The mechanisms and the consequences are not fully understood at present. Uremic toxins seem unlikely to play a role in such early phases of renal dysfunction. Moreover, the role of glucose metabolism in the kidneys per se should be minor, since they account for only a few percent of insulin-mediated glucose turnover. Instead it is likely that the demonstrated endocrine perturbations contribute to whole-body insulin resistance. However, at present, it is not clear which endocrine factor(s) that may be most important for the described reduction in insulin sensitivity. Perhaps the hormones and cytokines targeted in our study could be critical, but other factors such as sympathoadrenergic activation and/or parasympathetic dysfunction, sex hormones or novel adipokines should be considered. This should be evaluated in future studies.

Another finding of interest was the elevated level of fasting triglycerides (TG) in the group with reduced GFR, and this may be a consequence of insulin resistance with respect to lipid turnover. Elevated TG level may be caused by a high hepatic production of VLDL and this could, in turn, be a consequence of high NEFA concentrations in plasma. NEFA may also directly induce insulin resistance in skeletal muscle and liver. However, our present results did not indicate a high level of NEFA, in the fasting state or during the clamps and, hence, does not support a role of NEFA in causing insulin resistance among these patients. Neither was the expected reduction of HDL cholesterol found in the group with reduced GFR. The lipid disorders seen in both early and severe renal failure resemble those seen in the metabolic syndrome and it is speculated that the relative insulin deficiency with accompanying glucose intolerance in non-diabetic uremic patients could lead to the observed dyslipidemia (217). Altered plasma lipoprotein levels are probably also important for the increase in cardiovascular disease among patients with DN.

Renal damage, itself, can not explain the impaired glucose handling in the group of patients with reduced GFR and the sites and mechanisms of insulin resistance in these patients remain unknown. It is likely however, that skeletal muscle, the main target for insulin-mediated glucose utilisation is one important site (122) and so is also the liver (118). In our study, insulin binding, action and degradation in adipocytes did not differ between the groups. Although the
adipose tissue is not the major site of insulin-regulated glucose turnover, it may reflect other insulin sensitive extrarenal tissues, e.g. skeletal muscle. Therefore it appears that there are no intrinsic defects in cellular insulin action, and that cellular insulin resistance occurs as a consequence of factors in the surrounding in vivo milieu, as suggested above.

From a clinicians point of view the disturbances of glucose metabolism and insulin action in non-diabetic patients with chronic renal failure usually are of moderate magnitude and may not alter the clinical management. Nevertheless, insulin resistance and slight glucose intolerance may contribute to the acceleration in atherosclerosis noted in non-diabetic uremic subjects. However, in patients with diabetes and chronic renal failure the situation is different. In these patients alterations in glucose and insulin kinetics must be considered in order to optimise therapy and outcome of treatment. Insulin resistance may also be an additional factor explaining the high prevalence of cardiovascular complications seen in patients with DN.

Some critical considerations

Since the investigations were extensive and tedious the number of patients was rather small in study I and II, and hence, the statistical power was relatively low. It should be acknowledged that our studies therefore may not have sufficient power to completely exclude a quantitative relationship between albuminuria and insulin sensitivity. However, despite this we were able to show a significant decrease in insulin sensitivity in patients with slightly reduced GFR. These finding should nevertheless be confirmed in larger studies. The impairment in insulin sensitivity was found in patients with type 1 diabetes and to be able to generalise this finding to other conditions it has to be investigated in patients with non-diabetic renal diseases. Furthermore, as study I and II were cross-sectional prospective, longitudinal studies should be preformed to elucidate the time-course and cause-effect relationships in the development of insulin resistance in early nephropathy. More studies are also needed to elucidate the suggested mechanisms involved in insulin resistance in early stages of and maybe later interventional studies could be performed. We are presently undertaking a few studies and planning others that hopefully will help to elucidate some of these issues.

Studies III and IV are retrospective cohort studies that are based on medical records. Therefore there is some lack in precision on data on diabetic complications since these are extracted from clinical evaluation and laboratory reports. Future longitudinal follow-up studies using a standardised screening for diabetic complications in these patient cohorts are underway and should be more accurate in determining the occurrence of diabetic complications. Hopefully, they will be able to answer the question whether there is a true decline in the incidence of diabetic complications. In study III, we may possibly have underestimated the occurrence of renal involvement and other diabetic complications since complete information only was received in ~60% patients from the initial DISS cohort. There are some indications that the participants probably had better outcome with respect to complications than the non-participants in the study. This will also be taken into consideration in the design of future studies.
SUMMARY

1. Poor glycemic control also during the first five years of type 1 diabetes, appear to be a major risk factor in the development of diabetic complications. A young age at onset of childhood type 1 diabetes may delay the development of microvascular complications.

2. Despite modern diabetes management there are still patients that early-on develop signs of diabetic complications, e.g. incipient nephropathy and retinopathy. In childhood-onset type 1 diabetes 18% developed incipient nephropathy and 48% background retinopathy within ~12 years. Among adults with diabetes onset at ages 15-34 years, 7% of the patients exhibited signs of renal involvement, incipient nephropathy (5%) or overt nephropathy (2%) after ~9 years. Patients with type 2 diabetes appear to have a more rapid and severe renal involvement than patients with type 1 diabetes. As type 2 diabetes seems to be a risk marker for the development of early diabetic complications a correct classification of diabetes is important.

3. The appearance of microalbuminuria in type 1 diabetes does not seem to alter insulin sensitivity and clearance. Neither is there any strong quantitative relationship between the degree of albuminuria and insulin resistance, and a cause-effect relationship thus seems unlikely. Other underlying mechanisms may explain the previously observed association between albuminuria and insulin resistance.

4. Patients with type 1 diabetes and nephropathy that have a slightly reduced GFR display a marked impairment in whole-body insulin sensitivity when compared with patients with isolated albuminuria or patients with no signs of renal involvement. No consistent impairment in insulin clearance was found between these patient groups. The decline in insulin sensitivity does not seem to be accompanied by intrinsic defects in insulin target cells. Instead, alterations in the levels of insulin-antagonistic hormones and cytokines may possibly be involved.
CONCLUDING REMARKS
Hyperglycemia is necessary for the development of DN, but other factors contribute. This study suggests that glycemic control, also early in disease, is important with respect to the development of DN. Onset of type 1 diabetes before five years of age may prolong the time period to development of complications, and this may suggest that early childhood is a period relatively free from other factors promoting microvascular complications. In addition to previously established risk factors, we suggest that insulin resistance may be an additional factor, as type 2 diabetes seems to be associated with rapid appearance of DN. Insulin resistance also occurs as a metabolic consequence of DN already at a stage with slightly reduced GFR. The primary factors responsible for insulin resistance in early stages of DN are not fully elucidated. Alterations in the levels of circulating hormones and cytokines and possibly neuroendocrine dysregulation may be of importance for evolution of insulin resistance and also for the progression of the renal disease. Both genetic and acquired diabetes-related factors are likely to be involved in the pathogenesis of DN. Genetic polymorphisms associated with the susceptibility for developing DN have been extensively investigated but their exact role still is unknown. However, there are indications of a common genetic trait between susceptibility for renal injury and the metabolic syndrome, and therefore the propensity for insulin resistance may be an important common denominator. Accordingly, young adults with an early onset of type 2 diabetes may have a combination of genes that also promote chronic vascular complications.

To understand insulin action and kinetics in diabetic renal disease is important from a clinical point of view in order to optimise insulin therapy. In the long-term perspective this could probably improve glycemic control and prevent or postpone further deterioration in renal function and, consequently, the need for renal replacement therapy.

A summary of possible pathophysiological pathways in the development of DN suggested in this thesis is depicted in Fig. 4.
Fig. 4
A hypothetical scheme of the interplay between different factors involved in the pathophysiology of DN.
POPULÄRVETENSKAPLIG SAMMANFATTNING


Vi fann dock inget direkt samband mellan mängden av albumin i urinen och känsligheten för insulin eller insulinomsättningen. Denna studie talar därför emot att det skulle finnas ett direkt koppling mellan mängden albumin i urinen och den minskad känsligheten för insulin, och att det skulle finnas ett orsakssamband mellan dessa två fenomen. Troligen finns det andra underliggande orsaker som hittills är okända och som kan förklara att man sett en koppling mellan ökad albumin i urinen och nedsatt känslighet för insulin.

I delarbete II gick vi vidare och studerade sambandet mellan känslighet för insulin och insulinomsättningen hos patienter med typ 1 diabetes och olika grad av njurpåverkan: ingen njurpåverkan, enbart albumin i urinen respektive lätt nedsatt njurfunktion (sk filtration). Vi använde oss även i denna studie av glukosclamp för att mäta känsligheten för insulin och insuli-
nomsättningen. Patienter med lätt nedsatt njurfunktion hade en klart nedsatt känslighet för insulin jämfört med de övriga patienterna i studien. Sammantaget fanns det även ett starkt samband mellan njurfunktion och känslighet för insulin hos alla patienter. Ett viktigt fynd var också att patienter med lätt nedsatt njurfunktion inte skilde sig i insulinomsättning jämfört med kontrollgruppen med normal njurfunktion.

När vi analyserade vissa hormoner, såsom biksköldkörtelhormon, tillväxthormon, stresshormon och fettvävshormoner fann vi skillnader i nivåerna av dessa mellan de tre olika grupperna av patienter. Parallellt studerade vi även känsligheten för insulin och insulinomsättningen i fettceller från underhuden hos patienterna, men i dessa undersökningar fann vi inga skillnader mellan grupperna.

Sammantaget fann vi en minskad känslighet för insulin redan vid en lätt nedsatt njurfunktion. Minskningen av känsligheten för insulin var beroende av graden av njurfunktionsnedsättning. I studierna av fettceller fann vi inget samband mellan njurfunktion och insulinkänslighet, vilket talar för att det är faktorer i cellernas omgivning i kroppen är av betydelse för förändringen i känsligheten för insulin. Vi kunde också påvisa förändringar i nivåerna av vissa hormoner som skulle kunna bidra till den minskade insulinkänsligheten. Inga betydelsefulla skillnader i insulinomsättning påvisades mellan grupperna. En klinisk konsekvens av dessa observationer skulle kunna vara ett ökat behov av insulinliförsörjning när njurfunktionen blir nedsatt hos patienter med diabetes.

I delarbete III studerade vi förekomsten av tidig (före 10 års diabetesduration) njurpåverkan hos unga vuxna, som insjuknat med diabetes (både typ 1 och 2) i åldern 15-34 år, under åren 1987-88 i Sverige. Vi undersökte också om förekomsten av njurpåverkan påverkades av andra faktorer än blodsockerkontrollen, t.ex. diabetestyp, kön, tobaksbruk och blodtryck. Klassifikationen av diabetestyp gjordes med hjälp av klinisk undersökning som kombinerades med analys av sk diabetesantikroppar.

Under uppföljningstiden, ~9 år, utvecklade 6.6% av patienter njurpåverkan trots modern diabetesbehandling. I de flesta fallen rörde det sig om en tidig njurpåverkan med enbart ökad albuminutsläppning i urinen. De viktigaste faktorerna för utveckling av njurpåverkan var bristande blodsockerkontroll och högt blodtryck. 5.6% av patienterna med typ 1 diabetes och hela 16% av patienterna med typ 2 diabetes utvecklade njurpåverkan. Patienter med typ 2 diabetes tenderade också att ha en snabbare utveckling och en svårare grad av njurpåverkan än patienter med typ 1 diabetes. Trots modern diabetesbehandling kan alltså patienter med diabetes utveckla njurpåverkan tidigt i diabetessjukdomen. Dock verkar graden av njurpåverkan hos dessa patienter på det hela taget vara av en lindrigare grad jämfört med tidigare studier. Viktigaste faktorerna för utveckling av njurpåverkan var dålig blodsockerkontroll och högt blodtryck. Eftersom patienter typ 2 diabetes hade en ökad risk för tidig njurpåverkan i denna åldersgrupp är korrekt klassifikation av diabetestypen viktig för att bättre kunna bedöma risken för diabeteskomplikationer.

I delarbete IV undersökte vi förekomsten av tidig njurpåverkan och tidiga ögonbottenförändringar hos patienter, som insjuknat med typ 1 diabetes i barndomen, vid 0-14 års ålder, under tiden 1981-1992 i Umeå. Vi undersökte även betydelsen av både tidig (fem första åren) respektive sen blodsockerkontroll och ålder vid diabetesdebuten.

Under uppföljningstiden, ca 12 år, då patienterna var i åldern 12-26 år, hade 18% tidiga tecken på njurpåverkan och 48% tidiga tecken på ögonbottenförändringar. Blodsockerkontroll-
len, även tidigt i sjukdomsförloppet, var viktig för utvecklingen av diabeteskomplikationer. Åldern vid diabetesdebuten påverkade också risken att utveckla ögonbottenförändringar, och de som fått sin diabetes före 5 års ålder hade en lägre risk att utveckla komplikationer än de som fått sin diabetesdiagnos senare under barndomen. Sammanfattningsvis så utvecklar ca 50% av patienter som debuterat med typ 1 diabetes som barn tecken till tidiga diabeteskomplikationer inom ca 12 år av diabetessjukdom. Detta sker trots modern insulinbehandling. Dålig blodsockerkontroll, även under de fem första åren, verkar öka risken. En tidig diabetesdebut, före fem års ålder, verkar i stället fördröja utveckling av diabeteskomplikationer.
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REFERENCES


62. Rudberg, S, Osterby, R, Bangstad, HJ, Dahlquist, G and Persson, B. Effect of angiotensin converting enzyme inhibitor or beta blocker on glomerular structural changes in young micro-


189. Dahl-Jorgensen, K, Hanssen, KF, Kierulf, P, Bjoro, T, Sandvik, L and Aagenaes, O. Reduc-
tion of urinary albumin excretion after 4 years of continuous subcutaneous insulin infusion in
goingen-dependent diabetes mellitus. The Oslo Study. Acta Endocrinol (Copenh), 1988, 117:
19-25.
190. Krolewski, AS, Laffel, LM, Krolewski, M, Quinn, M and Warram, JH. Glycosylated hem-
globin and the risk of microalbuminuria in patients with insulin-dependent diabetes mellitus
191. Reichard, P. Are there any glycemic thresholds for the serious microvascular diabetic compli-
192. The absence of a glycemic threshold for the development of long-term complications: the
193. Rave, K, Heise, T, Pfutzner, A, Heinemann, L and Sawicki, PT. Impact of diabetic nephropa-
thy on pharmacodynamic and Pharmacokinetic properties of insulin in type 1 diabetic patients.
194. Aisenpreis, U, Pfutzner, A, Giehl, M, Keller, F and Jehle, PM. Pharmacokinetics and pharma-
codynamics of insulin Lispro compared with regular insulin in haemodialysis patients with
195. Taylor, CA, 3rd, Abdel-Rahman, E, Zimmerman, SW and Johnson, CA. Clinical pharmacoki-
netics during continuous ambulatory peritoneal dialysis. Clin Pharmacokinet, 1996, 31: 293-
308.
196. Wikdahl, AM, Granbom, L and Stegmayr, BG. No increased risk for peritonitis by insulin
injected into peritoneal dialysis solution bag through Coloplast. Anna J, 1997, 24: 401-6; dis-
cussion 407-8.
197. Quellhorst, E. Insulin therapy during peritoneal dialysis: pros and cons of various forms of
198. Nevalainen, P, Lahtela, JT, Mustonen, J and Pasternack, A. The influence of peritoneal dialy-
sis and the use of subcutaneous and intraperitoneal insulin on glucose metabolism and serum
199. Nevalainen, PI, Kallio, T, Lahtela, JT, Mustonen, J and Pasternack, AI. High peritoneal per-
meability predisposes to hepatic steatosis in diabetic continuous ambulatory peritoneal dialysis
200. Rossing, P, Hommel, E, Smidt, UM and Parving, HH. Impact of arterial blood pressure and
albuminuria on the progression of diabetic nephropathy in IDDM patients. Diabetes, 1993, 42:
715-9.
201. Should all patients with type 1 diabetes mellitus and microalbuminuria receive angiotensin-
converting enzyme inhibitors? A meta-analysis of individual patient data. Ann Intern Med,
202. Mathiesen, ER, Hommel, E, Hansen, HP, Smidt, UM and Parving, HH. Randomised con-
trolled trial of long term efficacy of captopril on preservation of kidney function in normoten-
of angiotensin-converting enzyme inhibition on plasma creatinine and on proteinuria in norm-
204. Randomised placebo-controlled trial of lisinopril in normotensive patients with insulin-
dependent diabetes and normoalbuminuria or microalbuminuria. The EUCLID Study Group.
205. Andersen, S, Tarnow, L, Rossing, P, Hansen, BV and Parving, HH. Renoprotective effects of
angiotensin II receptor blocker blockade in type 1 diabetic patients with diabetic nephropathy. Kidney
effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabe-
effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to


