

## **Glycaemic control and growth hormone in diabetes mellitus**

Bloedsuikerregulatie en groeihormoon  
in diabetes mellitus

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# **Glycaemic control and growth hormone in diabetes mellitus**

**Bloedsuikerregulatie en groeihormoon  
in diabetes mellitus**

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# GLYCAEMIC CONTROL AND GROWTH HORMONE IN DIABETES MELLITUS

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# Chapter 1

## Introduction

- 1.1 Glycaemic control and the prevention of complications
  - 1.2 Diabetic nephropathy and growth hormone
  - 1.3 Pathophysiological mechanisms of renal hyperfiltration in diabetes mellitus
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## 1.1 Glycaemic control and the prevention of complications

Diabetes mellitus is an incurable disease. Since the discovery of insulin in 1923 by Banting and Best, life expectancy of patients with juvenile diabetes has increased, but the longer survival has been accompanied by the development of microvascular complications [1]. In patients with insulin-dependent diabetes mellitus (IDDM), diabetic retinopathy (DRP), of which the proliferative form can result in blindness, develops in more than 90% of the patients [2]; diabetic nephropathy (DNP) with an increased risk of early death develops in 30-40% [2-5]; and diabetic neuropathy (DNeuP) with increased risk of foot ulcers and amputation is found in approximately 60% of the patients [6].

These microvascular complications are common in both IDDM and NIDDM (non-insulin-dependent diabetes mellitus), which share chronic hyperglycaemia as the primary feature. Therefore, it has long been suspected that elevated glucose levels play a causal role in the pathogenesis of these complications. Many studies have been published suggesting a preventive effect of strict glycaemic control on the development of DRP [7,8], DNP [7,9,10] and DNeuP [11], but often these studies were too small or too short-term to offer a definite proof of the 'hyperglycaemia hypothesis'. Moreover, in some studies even a deterioration of DRP was seen during the first years of strict glycaemic control [9,11,12].

In 1993 a major breakthrough was published by the authors of the multicenter Diabetes Control and Complications Trial (DCCT) [13]. They achieved a 50% overall reduction in complications in a large group of IDDM patients after 6.5 years of intensive insulin treatment compared to conventional treatment. These hopeful results strongly support the hypothesis that (near) normoglycaemia can prevent complications.

However, it is questionable whether such strict glycaemic control can be achieved in routine diabetes care, since (1) the implementation of intensive treatment was uniquely well organized in the DCCT, (2) intensive treatment was associated with some adverse effects, and (3) the results may have limited applicability.

Firstly, in the DCCT, the participating patients were relatively young, highly selected and well-motivated. Patients on intensive treatment had to administer subcutaneous insulin by three injections or more per day, or by continuous infusion via a pump. The insulin dose had to be adjusted daily,

according to blood glucose values measured at least four times a day, dietary intake and anticipated exercise. Monthly visits to the study center with ample consultation time by both a physician and a specialized diabetes nurse, and weekly telephone contacts were needed to achieve and maintain a substantial decrease in HbA1c as a parameter of long-term glycaemic control, and the associated reduction in complications. For many patients these strenuous efforts may be unacceptable to reconcile with normal life.

Secondly, main side effects of strict glycaemic control were considerable weight gain and a threefold increased risk of serious hypoglycaemia [13]. To many patients, these side effects may be an unacceptable price for strict glycaemic control, because of both physical and emotional negative consequences.

Thirdly, it is unknown whether the results of the DCCT can be extrapolated to older patients with IDDM, to children, and to patients with NIDDM, the majority of patients with diabetes. Young children may be hampered in their normal development when too much stress is put to glycaemic control, and repeated hypoglycaemic attacks may be dangerous. Older patients may not live long enough to benefit from long-term preventive measures. In patients with NIDDM, prevention of macrovascular complications is of equal or greater importance in order to reduce morbidity and mortality. With respect to microvascular complications, the pathogenetic role of hyperglycaemia is probably similar in NIDDM as in IDDM, so intensive treatment is likely to be beneficial [8]. Macrovascular complications, the major cause of death in patients with NIDDM, appear to be influenced by long-term glycaemic control as well [14-16]. However, no conclusive study on the risks and benefits of intensive treatment in patients with NIDDM has been published yet.

At present, the prevalence of complications is still high [2-6]. In the St.Vincent declaration of the World Health Organization and the International Diabetes Federation, ambitious goals have been proposed to reduce the number of complications in the whole diabetic population [17]. To achieve this, it is necessary to investigate the current quality of glycaemic control and other aspects of diabetes care in routine daily practice. If glycaemic control needs to be improved, studies must be undertaken to investigate whether intensive treatment according to the criteria of the DCCT can be implemented in routine diabetes care. Presently, the cost-benefit ratio of a reduction in complications, against undesirable side-effects and tremendous efforts of both patients and physicians, is not known for the general, unselected diabetes population compared to highly selected study groups [18,19].

Apart from the above-mentioned caveats, there is another problem. Despite intensive treatment and strict glycaemic control, a number of patients still develop complications [13,20], while advanced DRP and manifest DNP do not seem to be influenced by blood glucose concentrations at all [12,21]. These data suggest that the development of diabetic complications is also dependent on other factors [22-25]. Among these contributing factors, sex hormones have been thought to play a role, since patients with IDDM generally do not develop microvascular complications before puberty [26,27]. Furthermore, hereditary factors were also suggested to influence the risk of developing diabetic complications, because of clustering of DNP in families [28-31]. Also dietary factors, especially protein intake [32-34], blood pressure [35-37], haemodynamic factors [38,39], endothelial factors [40,41] and growth factors [42-44] have all been investigated concerning their potential role in the development of diabetic complications.

Several of these contributing factors are targets for additional treatment, when complications are imminent despite maximal efforts to improve glycaemic control. Since growth factors seem to be involved especially in the early stages of the development of complications, they are attractive study objects for possible prevention. In the next part of this Introduction, the focus will be on growth hormone and the development of DNP, since this is the most life-threatening complication of diabetes mellitus, and therefore most urgent to be prevented.

## 1.2 Diabetic nephropathy and growth hormone

### 1.2.1 Hyperfiltration and the development of diabetic nephropathy

Declining renal function, hypertension and proteinuria, the hallmarks of diabetic nephropathy (DNP), ultimately result in end-stage renal failure (ESRF) and dependency on renal function replacement therapy. Clinical nephropathy has a high mortality rate of 49% at 7 years after the onset of proteinuria [3]. Overt proteinuria is a symptom of late DNP and can not be reversed. Therefore, understanding and diagnosis of the earlier abnormalities leading to DNP is necessary to prevent this complication.

Although late DNP is characterized by a subnormal clearance function (low glomerular filtration rate, GFR), it has been found already in the 1930s that patients with uncomplicated short-term diabetes often have supranormal renal function [45-49]. The reported prevalence of hyperfiltration varies from 30 to 80% of the patients with diabetes mellitus [45,46,48,50]. The mean clearance rate of newly diagnosed patients with IDDM is reported to be 12-35% higher than in normal subjects [49,50-53]. Hyperfiltration can be observed both in patients with type I and type II diabetes mellitus [50].

Mogensen described that IDDM patients with renal hyperfiltration were at risk for diabetic nephropathy [54,55], which was confirmed in a larger prospective study by others [56], although not by all [57]. Additionally, studies of shorter duration demonstrated that patients with IDDM and renal hyperfiltration were subject to a more rapid decline of renal function than patients with normofiltering kidneys [58,59].

Based on the GFR and on the urinary albumin excretion rate (UAE), the development of DNP can be divided into 5 stages [23,60-62] (Table 1). According to this hypothesis, hyperfiltration might be considered as a risk factor for the development of diabetic nephropathy, and ample investigations on renal function have been performed during the years. Renal hyperfiltration was initially studied in patients with short-term diabetes, and in patients with newly diagnosed diabetes before insulin treatment was started [51,63-65].

After the installation of insulin therapy with reduction of glucose concentrations, the GFR decreased towards normal values within one week [48,63-65]. This suggests a causal role of hyperglycaemia in the pathogenesis of renal hyperfiltration, although no relation was found between the level of hyperfiltration and the momentary blood glucose concentration at the time of

the investigation [47,48,52,66].

Whether complete normalization of glucose levels can restore normofiltration and thereby prevent the progression to ESRF, is not yet known. Six months of strict metabolic control with continuous subcutaneous insulin infusion (CSII) did lead to reduction of the GFR by 9%, while GFR rose by 2% in patients on unchanged conventional insulin therapy [7]. Insulin infusion without a concomittant decline in blood sugar levels did not lead to a decrease in GFR [67].

**Table 1. Stages in the development of diabetic nephropathy**

Stage	Onset	Functional abnormalities	Structural abnormalities	Progression to next stage
I: Early hypertrophy and hyperfunction	Present at time of diagnosis	↑GFR, ↑glomerular capillary pressure	↑kidney size, ↑glomerular volume, ↑capillary filtration surface area	100%
II: Renal lesions, no clinical signs	by 2-3 yr after diagnosis	↑GFR, ↑glomerular capillary pressure	↑thickness of glom. and tub. capill. BM, ↑mesangial volume, glomerulosclerosis	35-40%
III: Incipient nephropathy	7-15 yr after diagnosis	↑UAE (30-300 mg/day) GFR normal to slightly ↓, but beginning to ↓	Further glomerulosclerosis	80-100%
IV: Clinical diabetic nephropathy	10-30 yr after diagnosis	UAE > 300mg/day, GFR normal to slightly ↓, declines steadily	Widespread glomerulosclerosis	75-100%
V: End-stage renal failure	20-40 yr after diag	GFR < 10ml/min, serum creatinine ↑↑	Nephron closure	-

BM = basement membrane; UAE = urinary albumin excretion; GFR = glomerular filtration rate

### 1.2.2 Association between hyperfiltration and growth hormone

An elevated clearance function is found in a few other circumstances such as liver cirrhosis [68], pregnancy [69,70] and acromegaly [45]. In patients with acromegaly, increased kidney size was associated with the renal hyperfiltration [45,71-73]. It was suggested that hyperfunction of the growth hormone producing cells of the pituitary was responsible for the syndrome of hyperfiltration and large kidneys [74]. In dogs, administration of a growth

hormone preparation was able to stimulate renal function [75,76]. Later it was demonstrated in humans too, that substitution of growth hormone (GH) in patients with hypopituitarism leads to an increase in renal function [77,78]. In liver cirrhosis a state of GH overproduction [79] and increased renal function exist as well [68].

Because destruction of the pituitary had been shown to ameliorate the diabetic state in animals and humans [80-83], a relation between GH and diabetes was suspected. It was already known that GH overproduction could be the cause of diabetes mellitus in acromegalic patients [73,84]. Administration of an anterior pituitary extract called 'growth hormone', to hypophysectomised dogs could induce diabetes [85], and it was found that the growth promoting and diabetogenic factor of the anterior pituitary were the same [86]. Later it was demonstrated that administration of GH to hypophysectomised diabetic patients induced a deterioration of the diabetic state with elevated blood sugar levels and ketonuria [87].

The suggestion that diabetes itself causes GH overproduction was made in 1970, when it was shown that patients with IDDM have more frequent and higher peaks of GH pulses [42,88]. The elevated plasma GH concentrations are the consequence of the diabetic state, since treatment of hyperglycaemia resulted in lower GH concentrations in newly-diagnosed and poorly-controlled diabetic patients [89-91]. Plasma GH could not always be reduced to normal [92,93]. It was suggested that elevated GH concentrations were responsible for the renal hyperfiltration, since the decrease in GH after treatment of the hyperglycaemia coincided with a decrease in GFR [51,63].

Hypophysectomy, performed for the treatment of severe diabetic retinopathy (DRP) in the 1950s [82], also led to decreased renal function [94,95] and decreased GH values [95]. Reversely, when GH was administered for 1 week to patients with well-controlled IDDM, their previously normal GH concentrations were raised to the levels usually found in patients with poor metabolic control, and their GFR increased concomitantly [96]. Administration of GH for 7 days to healthy persons produced the same effect [97]. The fact that administration of GH for 2 hours did not increase GFR [98], does not refute the hypothesis that GH has a stimulatory effect on renal hyperfiltration, because the effects of GH are thought to be mediated by insulin-like growth factor-I (IGF-I) [99-101], which is GH dependently synthesized in the liver and other tissues.

In conclusion, these observations in animals and humans indicate that GH is related to hyperglycaemia and hyperfiltration, and may therefore play a role in the development of DNP in diabetes mellitus.



### 1.2.3 The growth hormone mediator: IGF-I

Insulin-like growth factor (IGF)-I is a small protein, synthesized by the liver in a GH-dependent fashion. It is found in plasma, mainly bound to IGF binding proteins, that have a modulating effect on its actions. IGF-I is also found in other tissues, where it can act in an endocrine way, or in an autocrine or paracrine way, since it can be produced locally [100]. IGF-I has insulin-like activity, but also growth-promoting actions, and hence it is called the mediator of GH [100].

It has been demonstrated that administration of GH induces increased plasma IGF-I concentrations within 24 hours, which is accompanied by a rise in GFR [101-103]. Infusion of IGF-I also increases GFR [104,105]. However, diabetic patients usually have low plasma IGF-I concentrations compared to normal controls [106,107]. This suggests that systemic IGF-I concentrations are not directly related to kidney function. Therefore it is hypothesized that high plasma GH concentrations, a consequence of untreated hyperglycaemia in diabetes mellitus, induce local accumulation of IGF-I [108], which could lead to damage at organ level.

Results from animal studies have supported this hypothesis. Early kidney growth in diabetic rats has been demonstrated to be dependent on IGF-I accumulation in the kidneys [109-111]. Non-diabetic transgenic mice overproducing GH develop glomerular lesions comparable to human diabetic glomerulosclerosis [112,113]. Remarkably, transgenic mice overproducing IGF-I do not develop renal lesions, although plasma IGF-I levels were higher than in GH-transgenic mice [114]. The fact that high systemic IGF-I levels alone are not sufficient to produce kidney growth and glomerular lesions, supports the hypothesis that local IGF-I production, induced by diabetes mellitus or high circulating GH levels, is required.

Interestingly, acromegalic patients do not develop glomerulosclerosis, despite high plasma GH levels and several years of renal hyperfiltration [73,115]. Kidney IGF-I is probably elevated in patients with acromegaly, since rats implanted with GH tumors had increased kidney IGF-I mRNA levels, suggesting local production [108]. Increased kidney IGF-I content without hyperglycaemia apparently does not produce glomerulosclerosis in man.

In summary, growth and hyperfiltration are the earliest abnormalities of the diabetic kidney. These early diabetes-induced changes might be mediated by GH or IGF-I. The determinants of kidney function and the possible role of hyperglycaemia, GH and IGF-I will be discussed in more detail in §1.3.

### 1.3 Pathophysiological mechanisms of renal hyperfiltration in diabetes mellitus

The total glomerular filtration rate (GFR) is an entity representing the sum of all single nephron glomerular filtration rates (SNGFRs) in both kidneys, which can not be measured separately in human beings. Therefore, specific data on the determinants of total GFR and SNGFR result mostly from studies in animals. As described in §1.1 and §1.2, hyperglycaemia and changes in GH/IGF-I are important abnormalities in the diabetic state and may be related to renal hyperfunction. In this paragraph, the mechanisms leading to glomerular hyperfiltration will be discussed in more detail.

From micropuncture studies in rats, it has been discovered that the GFR depends on four main determinants (Table 2), that will be discussed in §1.3.1 to §1.3.4, respectively: the renal blood or plasma flow (RPF), the glomerular ultrafiltration coefficient ( $K_f$ ), the transcapillary glomerular hydraulic pressure gradient ( $\Delta P$ ), and the oncotic pressure gradient ( $\Delta\pi$ ) opposing ultrafiltration [116,117]. The RPF is mainly dependent on kidney volume, renal vascular resistance and systemic blood pressure.  $K_f$  is the product of the glomerular filtration surface area and the glomerular water permeability.  $\Delta P$  is the difference between the hydraulic pressures in the glomerular capillary and in Bowman's space. The oncotic pressure  $\Delta\pi$ , opposing filtration, is exerted by plasma proteins and other colloids.

**Table 2. Determinants of glomerular filtration**

Main determinants		Contributing factors	
Renal plasma flow (RPF)	kidney size -glucose load -growth hormone	renal resistance -extracellular volume -IGF-I	blood pressure plasma glucagon protein intake
Ultrafiltration coefficient ( $K_f$ )	surface area -mesangial volume	water permeability	protein concentration
Transcapillary glomerular hydraulic pressure ( $\Delta P$ )	blood pressure	efferent resistance -Angiotensin-II -Kinins, prostaglandins -Nitric oxide	afferent resistance -IGF-I
Transcapillary oncotic pressure ( $\Delta\pi$ )	plasma protein concentration		

### 1.3.1 Renal plasma flow

Renal plasma flow is thought to be the most important determinant of glomerular filtration [118]. Total RPF can be measured in humans. In the early studies on diabetes and renal hyperfunction, elevated renal plasma flow was found by some authors [51,65], but not by all [52,119]. The different results might be due to differences in investigational techniques. The increased flow was thought to be due to the increased kidney size, since kidneys of diabetic patients were found to be enlarged at autopsy [120]. When measurements of renal size could be performed *in vivo*, with intravenous pyelography or sonography, it was confirmed that the kidneys were enlarged in patients with diabetes [64,65,121-124]. Enlarged kidneys were also found in patients with acromegaly [73], in whom hypophysectomy leads to reduction of kidney volume and GFR [125]. Increased kidney size in the presence of elevated GH concentrations could thus well be the anatomical substrate for kidney hyperperfusion and hyperfunction.

Some authors suggested the enlarged kidneys in diabetic patients to be the sole determinants of hyperfiltration, because kidney size and GFR decreased simultaneously after the institution of good metabolic control [64,126]. However, other factors also have to be involved, because hyperfiltration can be reduced within 8 days, while kidney size does not decrease during this time period [127,128], but only after a few months [64]. Sometimes no reduction can be seen even after good glycaemic control for 1 year [129]. Reversely, in a study of diabetic patients with microalbuminuria, strict metabolic control by CSII for one year decreased kidney size but not GFR [130]. Therefore, hyperfiltration can not be explained completely by increased flow through enlarged kidneys alone.

Since nephropathy develops only in subjects with hyperglycaemia, elevated blood glucose might be a major causal factor for hyperfiltration. Controversial data exist on whether acute elevation of blood glucose can [131-133] or can not [134] elevate GFR in healthy or diabetic persons. Long-term elevated glucose and GH concentrations increase extracellular volume (ECV) and subsequently renal blood flow by osmotic pressure and water and salt retention [72,135,136]. However, GFR and RPF decreased after hypophysectomy in man, without a reduction in ECV [135]. Administration of GH to heart-lung-kidney preparations of spinal cats reduced water and salt excretion but not GFR or RPF [137]. These findings, and the finding that diabetic patients had elevated GFR with normal ECV compared to controls

[138] suggest that hyperperfusion and -filtration do not depend only on glucose-induced expansion of the ECV in diabetes mellitus.

Elevated plasma glucagon might also play a causal role in diabetic hyperfiltration. Infusion of glucagon to patients with short-term IDDM induced a dose-dependent increase in GFR and RPF [118]. GFR and RPF were found to be correlated with glucagon levels in patients with IDDM [139]. It has been demonstrated that a protein-rich meal and amino-acid infusion induce increased glucagon concentrations, as well as increased GFR and RPF [140-142]. Apparently, protein, glucagon and renal function are linked to each other. Experimentally, this is supported by the fact that diet modifications influence the development of renal lesions in diabetic rats: a low-protein diet prevented elevation of intraglomerular pressure and albuminuria after the induction of diabetes [143,144]. In humans, the progression of DNP can also be halted by protein restriction [32-34]. However, glucagon does not explain the elevated GFR in well-controlled patients with IDDM, since glucagon levels in these patients are only slightly elevated, while GFR is markedly higher than in normal controls [118]. Other investigators also could not confirm that glomerular hyperfiltration was associated with plasma glucagon levels [145].

Probably more convincing is the association between GH and diabetic kidney function, as has been outlined in §1.2. It is thought that the growth and flow stimulating effects of GH on the kidney are mediated by IGF-I. Experimental studies have shown that two days after the induction of insulin-deficient diabetes in mice and rats, kidney growth is apparent [109,146,147], which is correlated with the severity of hyperglycaemia [110]. Accumulation of IGF-I in the kidney precedes the renal growth [109,147], and the amount of kidney IGF-I is correlated with blood glucose levels [110]. Both insulin therapy [110] and GH suppression by somatostatin (SMS) [148,149] can prevent kidney IGF-I accumulation and the associated kidney growth. The effects of SMS treatment support the hypothesis that GH and IGF-I have a causal role in the initial growth of the kidney. Dwarf rats deficient in GH and IGF-I have less kidney growth and kidney IGF-I accumulation than normal rats after the induction of diabetes [150,151]. These attenuated kidney changes can be stimulated by injection of GH [150].

The effects of IGF-I on the kidney are not only anatomical, but also haemodynamic. In rats, SNGFR, glomerular plasma flow and total kidney GFR are elevated after the induction of moderately severe diabetes [152], probably due to a reduction in renal resistance [153]. Administration of GH or IGF-I to GH-deficient rats normalized the low GFR and RPF, by reducing arteriolar resistance and increasing glomerular ultrafiltration coefficient and kidney

weight [154]. In healthy humans, renal vascular resistance decreased as well after subcutaneous IGF-I injections [155]. Already 6 hours after the first injection, RPF and GFR were higher than at baseline, indicating that renal hypertrophy is not necessary for the increase in renal haemodynamics [155].

The mechanism by which IGF-I reduces renal arteriolar resistance is not exactly known. There is evidence for a role of prostaglandins [156-158], kinins [159] and nitric oxide [160-162] as vasodilating factors that are induced after the onset of experimental diabetes, or after the administration of GH or IGF-I.

In summary, increased renal plasma flow through enlarged kidneys is a main determinant of the diabetes-associated hyperfiltration. This high RPF might be caused by hyperglycaemia-induced expansion of the ECV, elevated glucagon levels, or reduced renal resistance. The latter finding is associated with GH-induced accumulation of IGF-I in the diabetic kidney.

### 1.3.2 Glomerular ultrafiltration coefficient

The glomerular ultrafiltration coefficient  $K_f$  equals the water permeability of the capillary wall times the glomerular surface area available for filtration [116]. Data on water permeability are not available. Enlargement of the glomerulus can be seen already at the onset of diabetes [163]; therefore, an increased filtration surface area was suggested as a contributing factor for the increased GFR [163-165]. Especially mesangial volume was found to be correlated with GFR [166]. The increase in glomerular volume may be dependent on GH [73,112-114]. In studies with rats, however,  $K_f$  in hyperglycaemic animals was not significantly higher than normal and could not account for the increased SNGFR [116].

The protein concentration in afferent arterioles seems to affect the ultrafiltration coefficient [116], but altered plasma protein concentrations have not been described in patients with diabetes [167,168]. Moreover, increased protein concentrations would also increase the oncotic pressure, and thus oppose ultrafiltration and GFR [116].

Summarizing, an increased filtration surface area in the diabetic glomerulus, maybe due to elevated GH concentrations, might contribute to renal hyperfiltration by elevation of the glomerular ultrafiltration coefficient, one of the determinants of the GFR.

### 1.3.3 Transcapillary glomerular hydraulic pressure

A relationship between diabetic complications and local capillary blood pressure has been suggested in the case of diabetic retinopathy (DRP) [169-171]. Higher intraocular pressure, opposing retinal blood flow, was found to protect eyes from DRP [170]. Not the systemic blood pressure, but the pressure in the artery providing blood supply was important in the development of retinal complications. Asymmetric DRP was found to occur when a diastolic difference of at least 15% was found between the two eyes, with lesser DRP always in the eye with lower retinal artery pressure [169]. Likewise, unilateral renal artery stenosis resulting in lower renal perfusion pressure, protects the homolateral kidney from glomerulosclerosis [172]. Hence, glomerular hypertension is thought to be an important factor inducing glomerulosclerosis [167]. Efferent arteriosclerosis as a cause for elevated capillary pressure has been described already in 1941 [173].

This theory is supported by experimental data in rats, in whom moderately severe experimental diabetes was associated with normal efferent and low afferent arteriolar resistance, resulting in higher transcapillary glomerular pressures ( $\Delta P$ ) than in control rats [152]. Again a role for GH is suggested, since IGF-I is thought to be the cause for this selective afferent vasodilation [155,156], increased  $\Delta P$  [174] and thus hyperfiltration.

An increased  $\Delta P$  has been associated with albuminuria and glomerulosclerosis in diabetic rats fed high-protein diets [143]. Reduction of the  $\Delta P$  in diabetic rats by administration of an angiotensin-converting-enzyme (ACE) inhibitor prevented albuminuria [174]. In humans, ACE inhibition is also known to prevent the progression of diabetic nephropathy [175]. Efferent vasodilation by suppression of angiotensin-II is supposed to be the effective mechanism. This does not reduce glomerular flow, but glomerular pressure is reduced by ACE inhibition.

In summary, the glomerular hypertension underlying renal hyperfiltration appears to be a main factor responsible for diabetic nephropathy. Local IGF-I, induced by elevated plasma GH in untreated hyperglycaemia, is thought to cause selective afferent vasodilation, resulting in elevated transcapillary glomerular pressure. If this abnormality persists, glomerulosclerosis will develop, ultimately leading to a decline in renal function.

#### 1.3.4 Transcapillary oncotic pressure

The plasma protein concentration rises as blood flows through the glomerular network, since proteins do not appear in the ultrafiltrate. The increase in the oncotic pressure opposes the hydraulic pressure in the glomerular capillary, hence a decline in protein concentration could theoretically lead to an increase in GFR [116]. However, no altered protein concentrations have been described in patients with diabetes mellitus [167,168].

## 1.4 Summary

Diabetic nephropathy (DNP) is one of the most serious long-term complications of diabetes mellitus. The pathogenetic role of persistent hyperglycaemia in the development of this and other microvascular complications has been accepted. Additionally, an increasing body of evidence has implicated a role for GH in diabetic end organ damage:

1. Poor glycaemic control is associated with elevated plasma GH concentrations.
2. Loss of the pituitary function (and thus of GH) reduces hyperglycaemia and reverses or slows the progression of DRP and DNP.
3. GH-deficient dwarf animals are protected from diabetic complications.
4. Transgenic mice overproducing GH have increased formation of glomerulosclerosis, which resembles human diabetic nephropathy.

Glomerular hyperfiltration is the earliest abnormality in the diabetic kidney and probably a risk factor for the development of DNP. The main determinants of the glomerular filtration rate are renal plasma flow, the glomerular ultrafiltration coefficient, and the transglomerular pressure difference. GH and its local mediator IGF-I can affect these components, because it is hypothesized that high plasma GH concentrations can induce increased production of IGF-I in the kidney. IGF-I is thought to have the following effects:

1. Kidney growth early after the onset of hyperglycaemia, with associated enhanced renal plasma flow.
2. Increased glomerular filtration surface area by mesangium proliferation.
3. Reduced renal resistance by selective afferent arteriolar vasodilation, which leads to increased transcapillary glomerular pressure.

These factors are thought to be responsible for hyperfiltration and ultimately glomerulosclerosis. Some questions, however, need to be resolved.

1. What is the quality of glycaemic control in routine diabetes care?
2. Is plasma GH also elevated in patients with moderate or good glycaemic control?
3. What is the mutual relationship between plasma GH and renal IGF-I in diabetes mellitus?
4. Can GH be suppressed, in order to reduce the glomerular filtration rate and thereby the risk of DNP?



## 1.5 Scope of the thesis

Manifest diabetic nephropathy (DNP) develops in approximately one-third of the patients with diabetes mellitus (DM), resulting in serious morbidity and mortality. Because advanced DNP is largely irreversible, prevention of the development and progression of earlier stages is the only treatment option. Elevated blood glucose concentrations are associated with the development of DNP and other diabetic complications, although the pathogenetic mechanism is not elucidated. Reduction of hyperglycaemia has been proven to halt or reverse the progression of imminent DNP to end-stage renal failure, when started in an early stage. Thus, strict glycaemic control is essential for the prevention of DNP.

In this thesis we investigated the state of glycaemic control in routine diabetes care settings in general practice and outpatient clinics. These studies demonstrate that at present many diabetic patients are not in good glycaemic control with the current ways of treatment (§2.2.1, §2.2.2, §2.3.1). Only the strict application of a standardized treatment protocol results in a significant reduction of glucose levels in patients with non-insulin-dependent DM (NIDDM) in a general practice (§2.3.2). These data suggest that many diabetic patients are in need of intensified glucose control or other forms of treatment to prevent the development of DNP.

To further clarify the pathogenesis of DNP, and to find other ways of treatment, we focussed on the role of growth hormone (GH) in the development of this complication. In patients with insulin-dependent DM (IDDM) and moderate glycaemic control we find inappropriate GH secretion, which is related to abnormal renal clearance function (§3.2). In a mouse model of DM, GH overproduction results in enhanced renal growth and renal IGF-I accumulation, while the absence of GH is protective of these early changes in the diabetic kidney (§3.3.1, §3.3.2). These data support the hypothesis that increased GH secretion, together with hyperglycaemia, are important causal factors in the early development of DNP. The possible therapeutic effect of GH suppression on early renal changes in patients with IDDM was investigated in a pilot study (§3.4).

In Chapter 4 the results of the previous chapters are discussed.

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## **Chapter 2**

### **Glycaemic control in routine diabetes care**

2.1 Introduction

2.2 Evaluation of glycaemic control in current routine diabetes care

2.3 Can glycaemic control be improved?

2.4 Conclusions

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## 2.1 Introduction

Restoration of normoglycaemia is the best way to prevent diabetic complications. Also renal hyperfiltration can be reversed after the institution of intensive treatment. In the Netherlands, many modern tools are available for optimal treatment of diabetic patients, like insulin pens, self-control of blood glucose concentrations, diabetes nurses and internists with a special interest in diabetes mellitus. Most studies describe carefully selected patients who were willing to participate on strictly controlled conditions. In these settings good metabolic control has been achieved, at least for the time of the study duration, although trends to worsening glycaemic control are visible after several study years.

In §2.2 we describe patients investigated in ordinary care settings. We investigated whether screening of adult persons in a single general practice is feasible (§2.2.1), in order to estimate the number of patients with unknown NIDDM, and to investigate the level of metabolic control in known and newly-diagnosed patients. Risk factors for cardiovascular disease were scored, since this is the most common cause of death in patients with NIDDM. In §2.2.2 we describe the effect of obesity, insulin dose and number of daily insulin injections, on glycaemic control in insulin-using patients, treated in several hospitals within the region of Rotterdam.

In §2.3 we investigated if insulin is superior to oral treatment in achieving glycaemic control in patients with NIDDM, treated by internists in the same region of Rotterdam (§2.3.1). In general practice we investigated whether strict application of a standard protocol for the treatment of NIDDM (NHG-standard) would lead to better metabolic control compared to using this protocol merely as a guideline (§2.3.2).



## 2.2 Evaluation of glycaemic control in current routine diabetes care

### 2.2.1 Screening for NIDDM in a general practice

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Submitted

## Summary

**Background.** Screening studies have demonstrated that approximately one-half of the people with type II diabetes is undiagnosed. Non-insulin-dependent diabetes mellitus (NIDDM) is a strong risk factor for the development of cardiovascular complications. Therefore, we screened for NIDDM in a general practice, and we studied the risk factors for cardiovascular disease in patients with newly detected NIDDM compared to patients with known NIDDM.

**Methods.** Nine-hundred and fourteen patients above 20 years of age participated in a screening test for NIDDM. A random capillary blood glucose concentration was measured. A random blood glucose level of 11.1 mmol/l or higher was considered as indicative for diabetes. When glucose was between 7.8 and 11.1 mmol/l, a 75 g oral glucose tolerance test was performed to diagnose diabetes according to WHO criteria.

**Results.** Twelve patients had newly diagnosed NIDDM. No patient was younger than 40 years. All NIDDM patients had at least one additional risk factor for the development of cardiovascular complications.

**Conclusions.** Screening for NIDDM leads to only 1.3% new cases. NIDDM is clustered with risk factors for cardiovascular complications; therefore, screening in older patients with (central) obesity, hypertension and hypercholesterolaemia will increase the *a priori* chance of finding undetected diabetes mellitus.



## **Introduction**

Morbidity and mortality in patients with non-insulin-dependent diabetes mellitus (NIDDM) are high, mostly due to an increased risk of cardiovascular disease [1-7]. The prevalence of risk factors like hypertension, dyslipidaemia and obesity is high, both in patients with known and with newly-diagnosed NIDDM [1,3,6]. Screening studies have shown that approximately half of the subjects with persistent hyperglycaemia lack specific symptoms of diabetes mellitus, especially older people [8-10].

The aim of screening is to find asymptomatic individuals that probably have a disease. Screening is justified only when certain prerequisites are fulfilled [11], which might be the case for NIDDM. Firstly, the disease must be serious and have a high prevalence, which is true for NIDDM [9]. Secondly, the disease must have a pre-clinical phase in which it can be detected, which holds true for NIDDM as well [12]. Thirdly, early detection and treatment have to improve prognosis. Although this issue has not yet been elucidated for NIDDM, convincing evidence is available from studies on patients with insulin-dependent diabetes (IDDM) and chronic hyperglycaemia [13]. Furthermore, there is growing evidence about the relation between glycaemic control and the risk of complications in patients with NIDDM [14]. Besides, even though no direct benefit of early detection and treatment has yet been proven, prevention of blindness, renal failure, and amputation, and emphasis on the treatment of cardiovascular risk factors will be additional advantages of early detection [11], since awareness of the diagnosis will increase medical attention, e.g. by vaccination programs and foot care.

Several screening tests can be used to detect diabetes [15-19]. In the present study, we chose the random capillary blood glucose test, since it is a fast and simple method to assess the blood glucose concentration. The aim of our study was to investigate the feasibility of screening for NIDDM in a normal general practice. Furthermore, in order to assess the overall risk of macrovascular complications in the newly diagnosed patients, cardiovascular risk factors were investigated and compared with those in patients with known NIDDM.

## **Methods**

### *Testmethods*

The screening was done in a general practice in the centre of Rotterdam, The Netherlands, comprising approximately 2500 patients. From the

~1000 subjects over 20 years of age who were asked to participate, 914 (90%) gave permission to perform a random capillary blood glucose test with the Accutrend glucose meter (Boehringer-Mannheim, Almere, The Netherlands; correlation with capillary blood test in reference laboratory 0.99). As described by Bourn et al. [20], a random blood glucose level below 7.8 mmol/l was defined as normal, in order to minimize the number of false-positive results. A random blood glucose level between 7.8 and 11.1 mmol/l was defined as borderline, which was further investigated with a 75 g OGTT. The WHO criteria for fasting and 2h post-glucose levels were used for the diagnosis of impaired glucose tolerance (IGT) and NIDDM [21]. When random blood glucose was 11.1 mmol/l or higher, a second blood test was done on another day to confirm the diagnosis of diabetes mellitus [21].

All known and newly diagnosed patients with NIDDM were invited to participate in an examination to assess the presence of cardiovascular risk factors, with emphasis on smoking habits, alcohol use, physical activity, and the use of medicaments. Physical examination comprised the measurement of blood pressure, body mass index (BMI) and waist-to-hip girth ratio (WHR [22]). Laboratory investigations included HbA1c (HPLC, reference range 4.0-7.0%), urinary albumin excretion (Micral-Test, Boehringer-Mannheim, Almere, The Netherlands), serum creatinine (reference range 55-120  $\mu$ mol/l) and blood cholesterol (Accutrend GC, Boehringer-Mannheim, measuring range 3.88-7.75 mmol/l; correlation with blood cholesterol in reference laboratory 0.98). For albuminuria or cholesterol values above the test strip range, a confirmative measurement was done in the laboratory by reference methods. Fundoscopy of the eyes was done by a specialized ophtalmologist.

### Definitions

Hypertension was defined as regular use of antihypertensive medication and/or systolic blood pressure above 140 mm Hg, and/or diastolic blood pressure above 90 mm Hg. Hypercholesterolaemia was defined as a blood cholesterol level above 5.2 mmol/l; unsatisfactory glycaemic control as an HbA1c level above 7.0%. Obesity was defined as a BMI above 25 kg/m<sup>2</sup>. These definitions were chosen according to the 'strict' guidelines of the Desktop Guide for the Management of NIDDM [23]. Abdominal (central) obesity was defined as a WHR > 0.85 for women and a WHR > 0.90 for men.

Nephropathy was defined as albuminuria > 20 mg/l and/or a serum creatinine above 140  $\mu$ mol/l. Retinopathy was defined as background or proliferative retinal abnormalities. Macroangiopathic complications (myocardial infarction, cerebrovascular accident, amputation of lower limb, angina pectoris

or claudicatio intermittens) were scored from the patient record. Smoking was scored as yes or no. Physical activity was scored as absent when no sportive or household activities were performed.

### Statistical analysis

Data are presented as means  $\pm$  SD. Bivariate correlations were calculated with Spearman's rank correlation test. Because the groups were small, differences between continuous variables were calculated with Wilcoxon's Mann-Whitney-U test. Correction for age and sex was done with linear regression analysis. Differences in proportions were tested by Chi-square. Differences were considered significant when  $p < 0.05$ .

### Results

Blood glucose concentrations grouped by age are shown in Table 1. No patients with NIDDM were younger than 40. Random glucose concentration increased significantly with age ( $r = 0.21$ ;  $p < 0.001$ ).

**Table 1. Mean random blood glucose concentrations by age groups**

Age cohorts	no diabetes	newly diagnosed patients	patients with known NIDDM
20-40 years	4.6 $\pm$ 1.0 (n=419)	-	-
40-60 years	4.9 $\pm$ 1.1 (n=280)	10.2 $\pm$ 2.3 (n=9)	8.9 $\pm$ 2.9 (n=14)
>60 years	5.0 $\pm$ 1.4 (n=174)	14.9 $\pm$ 8.3 (n=3)	9.7 $\pm$ 2.8 (n=15)
Total: n=914	873	12	29

Data are mean  $\pm$  SD

Normal glucose tolerance was found in 860 subjects (glucose  $< 7.8$  mmol/l), borderline glucose tolerance was found in 20 persons (glucose between 7.8 and 11.0 mmol/l), and 5 individuals had a random blood glucose level  $\geq 11.1$  mmol/l (range 12.0-24.3 mmol/l). Sixteen of the 20 patients with borderline glucose tolerance underwent the 75 g OGTT. Five of them had normal glucose tolerance, 4 had IGT, and 7 patients had diabetes mellitus. Hence, the total number of newly diagnosed NIDDM patients was 12 (1.3% of the tested population; 95% confidence interval 0.6-2.1%). All newly diagnosed patients had a repeat glucose measurement above 11.1 mmol/l.

Twenty-nine patients had known NIDDM. Five subjects were treated with insulin, 21 with oral hypoglycaemic agents and 3 with a diet. Their mean random blood glucose was  $9.3 \pm 2.8$  mmol/l.

Demographic, physical and laboratory data of the diabetic patients are shown in Table 2. Corrected for age and sex, no significant differences in BMI, WHR or blood pressure were found between patients with known and newly diagnosed diabetes. After correction for age and sex, blood cholesterol was significantly higher in patients with newly diagnosed compared to known diabetes. Both patients with known and with newly diagnosed NIDDM had elevated HbA1c ( $8.3 \pm 1.8\%$  and  $8.5 \pm 2.5\%$ , respectively).

**Table 2. Demographic, physical and laboratory data of patients with known and newly diagnosed NIDDM**

	Known NIDDM (n = 29)	Newly diagnosed NIDDM (n = 12)	Age and sex corrected difference <sup>a</sup>
sex (M/F)	11/18	6/6	-
age (years)	$62 \pm 11$	$55 \pm 10^*$	-
known duration of NIDDM (years)	$8 \pm 7$	-	-
ethnicity (% Dutch)	35	67	-
BMI (kg/m <sup>2</sup> )	$29.0 \pm 5.1$	$30.8 \pm 4.0$	0.9
WHR	$0.94 \pm 0.08^b$	$0.91 \pm 0.07^c$	-0.04
blood pressure (mm Hg)			
HbA1c (%)	$8.3 \pm 1.8$	$8.5 \pm 2.5$	0.2
cholesterol (mmol/l)	$5.6 \pm 0.9$	$6.2 \pm 1.0$	$0.7^*$
creatinine ( $\mu$ mol/l)	$73 \pm 8$	$87 \pm 19^*$	$14^{**}$
microalbuminuria (mg/l)	$15 \pm 12$	$17 \pm 20$	2

Data are means  $\pm$  SD, or percentages when indicated

<sup>a</sup> Correction for age and sex by linear regression analysis; <sup>b</sup> n = 12; <sup>c</sup> n = 10

\*p < 0.05; \*\*p < 0.005 patients with known versus newly diagnosed NIDDM

BMI: body mass index; WHR: waist-to-hip girth ratio

The prevalence of long-term complications was not significantly different between patients with known and newly diagnosed NIDDM. Diabetic retinopathy was found in 4 of 22 examined known patients, against 0 of 8 examined newly diagnosed patients. Nephropathy was found in 3/29 known

patients, and in 1/12 newly diagnosed patients. Macroangiopathy was the most frequent complication, and existed in 7/29 (24%) of the patients with known NIDDM and in 5/12 (42%) newly diagnosed patients.

Both known and newly diagnosed diabetic patients had an obvious cardiovascular risk profile, as shown by the high frequency of hypertension, hypercholesterolaemia and obesity (Table 3). Even when obesity was defined as a BMI > 27 kg/m<sup>2</sup>, still 65% of the known, and 75% of the newly diagnosed patients scored positive. Approximately three quarters of the patients had central adiposity. All patients had at least 1 additional cardiovascular risk factor (apart from diabetes mellitus itself), when smoking, hypertension, obesity and hypercholesterolaemia were taken into consideration.

**Table 3. Cardiovascular risk factors in patients with known and newly diagnosed NIDDM**

Risk factor	Patients with known NIDDM (n = 29)	Patients with newly diagnosed NIDDM (n = 12)
smoking	6 (21%)	5 (42%)
no physical activity	3 (10%)	1 (8%)
hypertension	21 (72%)	7 (58%)
hypercholesterolaemia	19 (65%)	8 (67%)
obesity	22 (76%)	12 (100%)
central obesity	10 (83%) <sup>a</sup>	7 (70%) <sup>b</sup>
insufficient glycaemic control	15 (52%)	7 (58%)
number of risk factors apart from NIDDM (1/2/>2)	4/12/13 (14%/41%/45%)	1/3/8 (8%/25%/67%)

Data are numbers of patients with percentages in parentheses.

Definitions of risk factors: see Methods (section *Definitions*).

<sup>a</sup> n = 12; <sup>b</sup> n = 10

## Discussion

Our study shows that a random capillary blood glucose test is easy to use for screening for NIDDM in a general practice, but yields only a low number of positive results. This may be due, firstly, to the large number of young participants, since the prevalence of NIDDM in people younger than 40

years of age is low [9], and in our study 0%. Secondly, we used a cut-off value with relatively high specificity but low sensitivity, so this will have reduced the number of positive patients. Thirdly, we can not exclude some selection bias, because non-participants may be less health conscious and therefore more at risk to have a disease, compared to participants [11].

The positive predictive value of a screening test depends on its sensitivity, specificity and the prevalence of the disease in the screening population [11]. Hence, screening in a high-risk population increases the positive predictive value of any test, because it increases the *a priori* chance. A positive family history, ethnicity, obesity, high age and prior gestational diabetes mellitus are currently considered as risk factors for the development of NIDDM [16]. In addition, cardiovascular disease, hypertension, renal disease, eye disease, hyperlipidaemia, neuropathy and periodontitis may all be indications of underlying diabetes mellitus [16].

Although the 75 g OGTT is the gold standard for the diagnosis of NIDDM [17,21], no agreement exists on what test should be used for screening. Fasting plasma glucose is probably the most widely used test [17,19,24,25], but fasting can not be ascertained and the test is neither very specific nor sensitive [19]. Personal convenience and practical feasibility may therefore influence the choice of the screening method [18]. The random blood glucose test with a cut-off value at 7.8 mmol/l that we used, is comparable to the method described by others [20,26]. It has been suggested that age-specific cut-off values should be used, which was confirmed in our study by the increasing glucose concentration with age. Also, adjustment of the random capillary blood glucose measurements for the duration of the postprandial period is supposed to be useful [27].

There has been a lot of debate on whether to screen or not to screen for NIDDM [15-17,20]. Non-medical arguments are often used against screening [16]. Getting a diagnosis of diabetes may have important consequences at social level (insurance, job facilities), psychological level (fear, depression), and physical level (diet, physical exercise, medications). Being in a stressful screening situation may elevate blood glucose concentrations by increased adrenaline production [28], and subjects with false-positive test results might be harmed when medication is started. This may justify a less sensitive and more specific test for screening in an asymptomatic population. On the other hand, unjustified reassurance after false-negative screening results is unsafe as well, but this can be avoided when repeated testing is performed e.g. on a yearly basis, depending on co-existing risk factors.

Positive arguments in favour of screening for hyperglycaemia are more convincing from a medical point of view [15,16]: awareness of impaired glucose tolerance will theoretically increase medical attention in order to prevent conversion to NIDDM, by efforts to lose weight, dietary advice and physical activity [29,30]. When overt NIDDM is diagnosed, early treatment may prevent the development of long-term complications [14]. Whether treatment of hyperglycaemia itself is necessary to reduce micro- and macrovascular complications has not yet been proven. However, extrapolation of the findings in patients with IDDM [13], where intensive treatment could reduce the development of microvascular complications with 50%, is supportive evidence for similar effects in NIDDM [14]. Comparably, the incidence of macrovascular complications seems to have a relation with blood glucose concentrations as well [31,32]. Furthermore, knowledge of the diagnosis of NIDDM will stimulate a more vigorous approach towards the other risk factors for cardiovascular disease. Instructions on life style, reduction of treatable risk factors, and adequate preventive measures like influenza vaccination and foot care are prerequisites in the treatment of all diabetic patients, in order to reduce morbidity and mortality [33]. Additionally, subjects with untreated hyperglycaemia may improve well-being after diagnosis of NIDDM and adequate treatment, even when overt symptoms are not present [34].

In our opinion, the general practitioner should be aware of the presence of patients with undetected NIDDM and their increased risk for macrovascular complications, because the benefits of diagnosing such a serious disease far outweigh the non-medical arguments against screening. Most conspicuous in the present study, was the presence of a high frequency of cardiovascular symptoms in newly diagnosed asymptomatic diabetic patients, which was already shown in other studies [3,6]. Although we did not check the completeness of the registration, and no definite evidence like ECGs or exercise tests was used, more than 40% of the newly diagnosed patients was known to have macrovascular abnormalities (myocardial infarction, cerebrovascular accident, lower limb amputation, angina pectoris or claudicatio intermittens). Yet, these complications had not prompted testing for diabetes, which may have resulted in less adequate treatment and prevention of progression.

In conclusion: it is feasible to use the random capillary blood glucose test as a screening test for hyperglycaemia in general practice. However, clustering of NIDDM with other cardiovascular risk factors implies that screening will have the highest predictive value in subjects over 40 years of

age, with hypertension, hypercholesterolaemia, (central) obesity and evidence of macrovascular complications.

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### 2.2.2 Effect of BMI, insulin dose and number of injections on glycaemic control in insulin-using patients

Jacobs ML, Elte JWF, Van Ouwerkerk BM, Janssens ENW, Schop C, Knoop JA, Hoogma RPLM, Groenendijk R, Weber RFA and the Studygroup Diabetes Rijnmond.

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## Summary

**Background.** Strict glucose control is essential to the prevention of diabetic complications. The level of glycaemic control in insulin-treated patients with diabetes mellitus (DM) in a routine clinical setting is not known.

**Methods.** In a cross-sectional survey comprising 8 hospitals in the Rijnmond area, The Netherlands, age, body mass index (BMI), insulin dose, number of injections, and HbA1c were scored in 712 patients with insulin-dependent DM (IDDM) and 462 patients with non-insulin-dependent DM (NIDDM).

**Results.** In IDDM and NIDDM patients, respectively, age (mean  $\pm$  SD) was  $40 \pm 17$  and  $65 \pm 12$  years, BMI was  $24.1 \pm 3.5$  and  $27.3 \pm 4.1$  kg/m<sup>2</sup>, daily insulin dose was  $49 \pm 18$  and  $44 \pm 18$  U ( $p < 0.001$ ). Intensive therapy ( $\geq 4$  injections or continuous subcutaneous insulin infusion) was used in 59% of IDDM and 13% of NIDDM patients. HbA1c below the upper normal limit was achieved in 11% of the patients, and within 20% above the upper normal limit in 37%. Obesity was positively associated with HbA1c in NIDDM patients ( $p < 0.01$ ). A higher insulin dose was associated with higher HbA1c in both IDDM and NIDDM patients ( $p < 0.01$ ).

**Conclusions.** Good glycaemic control is established in 37% of our patients. Intensive insulin treatment and higher insulin dose did not improve glucose regulation. Obesity is a risk factor for poor glycaemic control.

## Introduction

Diabetes mellitus is a risk factor for several major invalidating complications, due to micro- and macrovascular damage. The burden on personal and community welfare and costs is augmenting, because of increasing prevalence of diabetes mellitus (DM) in the population [1], and the increased wish for better regulation.

As a result of recent investigations, the importance of strict glycaemic control for the prevention of long-term diabetic complications has been proven for patients with insulin-dependent DM (IDDM) [2]. It remains to be established whether these results can be extrapolated to patients with non-insulin-dependent DM (NIDDM). However, similar observations were made in a prospective study of the effects of long-term glycaemic control on the development of microvascular complications in patients with NIDDM [3]. Cardiovascular disease, the most important complication of NIDDM, is supposed to be more dependent on factors part of the insulin resistance syndrome, such as obesity, dyslipidaemia and hypertension [4], than on glycaemic control. However, some investigators have found an independent relation between glucose level and the risk of macrovascular complications in non-diabetic [5] as well as in diabetic adults [6-8].

In the Netherlands, patients with NIDDM treated by diet and oral hypoglycaemic agents (OHA) are usually controlled by their general practitioners according to the standard 'Diabetes mellitus type II' of the Netherlands College of General Practitioners (NHG-standard) [9]. When this standard is strictly applied, good glycaemic control is possible for the majority of patients [10]. Due to secondary failure of OHA, however, approximately 50% of NIDDM patients will need insulin therapy after a mean period of 10 years after diagnosis [11], but it has not been elucidated what type of insulin treatment is most effective. In a regional study we investigated glycaemic control in patients with NIDDM during 6 months of OHA treatment versus 6 months of insulin therapy. We did not find a significant improvement in glycaemic control in either treatment group [Jacobs, submitted]. Ongoing studies investigate the effects of treatment strategy and strict glycaemic control on long-term complications in patients with NIDDM [12]. At present, little information is available regarding the level of glycaemic control in routine outpatient hospital care. A variety of treatment facilities is available for these patients and their doctors, including specialized diabetes nurses, insulin pen systems for multiple injection regimens, and self-control. The aim of the present study was to investigate the level of glycated haemoglobin (HbA1c)

as a parameter of long-term glycaemic control, in insulin-using patients with both IDDM and NIDDM, living in the Rijnmond area, The Netherlands.

## Subjects and methods

Patients with IDDM and NIDDM were recruited from the outpatient clinics of 8 participating hospitals in the Rijnmond area of Rotterdam, The Netherlands. A total number of approximately 8000 diabetic patients attend these outpatients clinics, of whom one third is treated by internists with special interest in diabetes mellitus. These internists recorded every subsequent insulin-using patient during a period of 1-4 months. Type of diabetes, age, body weight, height, daily insulin dose, number of insulin injections and HbA1c concentrations were assessed. HbA1c was analyzed in the separate hospitals, using various techniques including electrophoresis and different variants of high performance liquid chromatography. HbA1c values of one hospital are not included, since the method and reference range of the HbA1c assay changed during the registration period. In the other hospitals, the upper level of normal HbA1c varied from 5.5 to 7.0%.

Differentiation between IDDM and NIDDM was made on the basis of age, body weight and presence of ketoacidosis at presentation, and on the necessity of insulin therapy during the first year after diagnosis. Conventional therapy was defined as 1 to 3 injections per day, while  $\geq 4$  injections or continuous subcutaneous insulin infusion (CSII) were defined as intensive insulin therapy. Obesity was defined as a body mass index (BMI) above 25 kg/m<sup>2</sup>. Strict glycaemic control was defined as an HbA1c concentration below the upper level of normal in each hospital. Good glycaemic control was defined as an HbA1c concentration within 20% above the upper limit of normal of each hospital, in reference to the DCCT [2].

Data are given as means  $\pm$  SD. Differences between groups were tested by Mann-Whitney-U test or Chi-square test if appropriate. Correlation coefficients were calculated using Pearson's correlation test. Multiple regression analysis was used to estimate the influence of confounding factors on HbA1c concentration. The level of statistical significance was set at  $p < 0.05$ . Since not all data were available for all patients, different numbers of patients are given in the Tables.

## Results

A total of 1174 patients were recorded, of whom 712 patients with

IDDM and 462 patients with NIDDM. The overall HbA1c was  $8.2 \pm 1.8\%$ . Strict glycaemic control (HbA1c in the normal range for each respective hospital) was reached in 11% of the patients, while 37% of the patients had an HbA1c concentration lower than 20% above the upper limit of normal ('good control'). When 25% above the upper limit of normal was chosen as cut-off value for 'good control', 44% of the patients reached this qualification. As shown in Table 1, the number of patients in good glycaemic control was not significantly different between patients with IDDM and NIDDM. Age and BMI were significantly higher in patients with NIDDM compared to patients with IDDM ( $p < 0.0001$ ). Total insulin dose was significantly lower in NIDDM than in IDDM patients, as was the dose per kg bodyweight ( $p < 0.0001$ ). The majority of IDDM patients (59%) was treated with intensive therapy, against only 13% of NIDDM patients.

**Table 1. Differences between patients with IDDM and NIDDM**

	IDDM n = 712	NIDDM n = 462
age (years)	$40 \pm 17$	$65 \pm 12^*$
BMI ( $\text{kg}/\text{m}^2$ )	$24.1 \pm 3.5$	$27.3 \pm 4.1^*$
conventional/intensive therapy (%) <sup>1</sup>	41/59	87/13 <sup>*</sup>
insulin dose (IU/day)	$49 \pm 18$	$44 \pm 18^*$
insulin dose per kg bodyweight (IU/kg)	$0.69 \pm 0.24$	$0.59 \pm 0.22^*$
good/poor control (%) <sup>2,3</sup>	37/63	37/63

Values are means  $\pm$  SD, or % if stated otherwise

\*  $p < 0.0001$  compared to patients with IDDM

<sup>1</sup> conventional therapy: 1-3 insulin injections per day

intensive therapy:  $\geq 4$  injections or continuous insulin infusion

<sup>2</sup> n = 477 IDDM patients, n = 339 NIDDM patients

<sup>3</sup> good control: HbA1c  $\leq 20\%$  above upper limit of normal

poor control: HbA1c  $> 20\%$  above upper limit of normal

Table 2 shows that patients treated with intensive therapy were significantly younger than patients treated with conventional therapy ( $p < 0.0001$ ). In IDDM patients treated with intensive therapy, daily insulin dose and dose per kg bodyweight were significantly higher compared to patients on conventional therapy ( $p < 0.0001$ ), although BMI was significantly lower ( $p < 0.0001$ ). In NIDDM patients the same trend was seen, but the

difference in BMI was not statistically significant. The level of glycaemic control was not significantly different in patients treated with intensive compared to conventional therapy.

**Table 2. Differences between patients treated with conventional and intensive insulin therapy**

		conventional therapy (1-3 insulin injections/day)	intensive therapy (≥4 insulin inj./day or CSII)
IDDM	number of patients	283	413
	age (years)	45 ± 19	36 ± 14 <sup>*</sup>
	BMI (kg/m <sup>2</sup> )	24.8 ± 3.7	23.8 ± 3.2 <sup>*</sup>
	insulin dose (IU/day)	44 ± 17	53 ± 18 <sup>*</sup>
	insulin dose per kg bodyweight (IU/kg)	0.62 ± 0.22	0.74 ± 0.24 <sup>*</sup>
	good/poor control (%) <sup>1,2</sup>	38/62	36/64
NIDDM	number of patients	367	56
	age (years)	67 ± 11	56 ± 12 <sup>*</sup>
	BMI (kg/m <sup>2</sup> )	27.4 ± 4.2	26.7 ± 3.8
	insulin dose (IU/day)	42 ± 16	56 ± 22 <sup>*</sup>
	insulin dose per kg bodyweight (IU/kg)	0.56 ± 0.21	0.73 ± 0.27 <sup>*</sup>
	good/poor control (%) <sup>1,3</sup>	39/61	33/67

Values are means ± SD, or % if stated otherwise. CSII: continuous subcutaneous insulin infusion

<sup>\*</sup> p<0.0001 compared to conventional therapy

<sup>1</sup> good control: HbA1c ≤ 20% above upper limit of normal

poor control: HbA1c > 20% above upper limit of normal

<sup>2</sup> n = 199 patients with conventional therapy, n = 263 patients with intensive therapy

<sup>3</sup> n = 270 patients with conventional therapy, n = 33 patients with intensive therapy

In Table 3 the differences between obese and nonobese patients are shown. Obese IDDM patients used more insulin per day than nonobese patients (p<0.001); however, dose per kg bodyweight was significantly lower (p<0.05). NIDDM patients with obesity also received higher total insulin dose per day compared to nonobese NIDDM patients (p<0.001); their dose per kg bodyweight was not significantly different. Fewer obese (33%) than nonobese patients (43%) had good glycaemic control, but the differences were not statistically significant. With the cut-off value at 25%



above the upper limit of normal, significantly fewer obese than nonobese NIDDM patients had good glycaemic control (39% vs. 57%;  $p < 0.01$ ).

As shown in Table 4, 37% of the patients were in good glycaemic control, i.e. with an HbA1c concentration lower than 20% above the upper limit of normal. The proportion of patients treated by intensive insulin therapy was not different between the patients in good and poor control. IDDM patients in good glycaemic control used significantly less insulin per day and per kg body weight ( $p < 0.05$ ). In the group of poor control, significantly more adolescents (patients of 20 years or younger) were found compared to the group of good control (7.9% vs. 4.2%;  $p < 0.05$ ). NIDDM patients in good glycaemic control were older and less obese than NIDDM patients in poor control ( $p < 0.05$ ).

**Table 3. Differences between obese and nonobese diabetic patients**

		obese (BMI $> 25$ kg/m <sup>2</sup> )	nonobese (BMI $\leq 25$ kg/m <sup>2</sup> )
IDDM	number of patients	222	452
	age (years)	44 $\pm$ 17	36 $\pm$ 16 <sup>*</sup>
	BMI (kg/m <sup>2</sup> )	28.0 $\pm$ 2.9	22.3 $\pm$ 1.8 <sup>*</sup>
	insulin dose (IU/day)	54 $\pm$ 20	47 $\pm$ 16 <sup>*</sup>
	insulin dose per kg body weight (IU/kg)	0.67 $\pm$ 0.24	0.71 $\pm$ 0.24 <sup>*</sup>
	good/poor control (%) <sup>1,2</sup>	30/70	38/62
NIDDM	number of patients	285	136
	age (years)	65 $\pm$ 12	63 $\pm$ 13
	BMI (kg/m <sup>2</sup> )	29.3 $\pm$ 3.4	23.1 $\pm$ 1.7 <sup>*</sup>
	insulin dose (IU/day)	47 $\pm$ 19	38 $\pm$ 15 <sup>*</sup>
	insulin dose per kg body weight (IU/kg)	0.59 $\pm$ 0.23	0.58 $\pm$ 0.22
	good/poor control (%) <sup>1,3</sup>	33/67	43/57

Values are means  $\pm$  SD, or % if stated otherwise

<sup>\*</sup>  $p < 0.05$ , <sup>\*</sup>  $p < 0.001$  compared to obese patients

<sup>1</sup> good control: HbA1c  $\leq 20\%$  above upper limit of normal

poor control: HbA1c  $> 20\%$  above upper limit of normal

<sup>2</sup>  $n = 141$  obese patients,  $n = 300$  non-obese patients

<sup>3</sup>  $n = 207$  obese patients,  $n = 94$  non-obese patients

**Table 4. Differences between diabetic patients in good and poor glycaemic control**

		good control (HbA1c ≤ 20% above upper normal limit)	poor control (HbA1c > 20% above upper normal limit)
IDDM	number of patients	177	300
	age (years)	43 ± 17	39 ± 16 <sup>*</sup>
	BMI (kg/m <sup>2</sup> )	23.9 ± 3.0	24.5 ± 3.3
	insulin dose (IU/day)	47 ± 18	51 ± 17 <sup>*</sup>
	insulin dose per kg body weight (IU/kg)	0.66 ± 0.25	0.72 ± 0.23 <sup>*</sup>
	conventional/intensive treatment (%) <sup>1</sup>	44/56	42/58
NIDDM	number of patients	126	213
	age (years)	67 ± 11	64 ± 13 <sup>*</sup>
	BMI (kg/m <sup>2</sup> )	26.7 ± 3.2	28.0 ± 4.4 <sup>*</sup>
	insulin dose (IU/day)	43 ± 16	47 ± 19
	insulin dose per kg body weight (IU/kg)	0.58 ± 0.22	0.61 ± 0.24
	conventional/intensive treatment (%) <sup>1</sup>	90/10	88/12

Values are means ± SD, or % of patients if stated otherwise

<sup>\*</sup> p < 0.05

<sup>1</sup> conventional treatment: 1-3 insulin injections per day  
intensive treatment: ≥4 insulin injections per day or CSII

The different assay-techniques used in this study caused wide inter-hospital variations in HbA1c concentrations. Therefore, comparison of the HbA1c data between the hospitals was not possible, and relations between HbA1c and other factors had to be corrected with 'hospital' as a confounding factor. Multiple regression analysis with HbA1c concentration as the dependent variable and hospital, number of injections, insulin dose, BMI and age as possible confounding factors, demonstrated significant influence on HbA1c concentration of the various hospitals where patients were treated. Furthermore, in patients with IDDM a higher insulin dose was significantly associated with a higher HbA1c. In patients with NIDDM a higher insulin dose and higher BMI were significantly associated with a higher HbA1c concentration.

## Discussion

In this study we show that strict glycaemic control (a normal HbA1c) is being achieved in 11% of diabetic patients treated with insulin by internists with special interest in diabetes mellitus. Good glycaemic control, defined as an HbA1c concentration not higher than 20% above the upper limit of normal, was reached in 37% of the patients. Intensive insulin treatment, defined as multiple injection technique (basal/prandial) or CSII, is related to increased daily insulin usage but not to improved glycaemic control. Furthermore we demonstrate that poor glycaemic control is associated with a higher BMI, especially in patients with NIDDM.

The finding that diabetic patients treated with intensive therapy did not achieve a lower HbA1c compared to patients treated with conventional therapy, is surprising. Indeed, other studies in patients with IDDM [2] and NIDDM [13,14] did report substantial decreases in HbA1c after the introduction of intensive treatment. However, since our study is cross-sectional, we cannot rule out the possibility that glycaemic control would be worse when intensive treatment would be replaced by conventional treatment. Nevertheless, our data show that even when a patient is treated intensively in a routine setting, normalization of glycaemic control is not being achieved. 'Intensive treatment' apparently requires more than just frequent insulin injections, but also specialized education and frequent appointments with the attending physician and diabetes nurse, as described in the DCCT [2].

The positive relation between insulin dose and HbA1c is also surprising. This relation may have two causal directions. Firstly, it might indicate that patients receiving a high dose of insulin have large glucose excursions with many hypoglycaemic events, leading to reactive hyperglycaemia. However, with many hypoglycaemic events one could expect a lower HbA1c as well. Secondly, it might indicate that patients in poor glycaemic control tend to receive more and more insulin in a vain effort to lower HbA1c. Our results indicate that patients in poor glycaemic control are more obese and younger than well-regulated patients, but the differences were small and probably clinically not relevant. We did not score other possible factors like psychological stability, frequency of self-monitoring of blood glucose and concomittant illnesses.

The largest difference in HbA1c was seen between obese and nonobese patients with NIDDM. We noticed that obesity led to increased insulin usage per day, and that insulin dose was positively correlated with HbA1c concentration. This last finding suggests that lower insulin doses

might be beneficial in obese patients with NIDDM. However, theoretically this seems unlikely, since obesity has an additive effect on the insulin resistance of NIDDM [15-17]. The higher insulin dose was not proportional to the increased BMI, because insulin dose per kg body weight was equal in obese and nonobese patients. Therefore, our results might also indicate that obese patients with NIDDM, on the opposite, received too little insulin and needed larger doses, to overcome insulin resistance. This hypothesis is supported by other studies in which large insulin doses were given to obese patients, which led to a decrease in HbA1c [13,14,18,19]. We did not study patients treated by combination therapy with both insulin and oral hypoglycaemic agents. Other authors, however, did achieve good glycaemic control with this treatment modality [20,21(meta-analysis)]. Maybe this treatment option is especially suited for obese patients with NIDDM, because of the possible reduction of insulin resistance by some oral agents [22,23].

Large inter-hospital differences in HbA1c reference values made direct comparison of glycaemic control between the participating hospitals impossible. In one hospital a shift in assay method and reference range occurred. The HbA1c results of this hospital were therefore excluded from the analysis. Awareness of these technical problems has grown in the past years, and attempts to standardize have been made since.

In conclusion, only 11% of our insulin-treated diabetic patients had a normal HbA1c, and 37% of our patients had an HbA1c concentration less than 20% above the upper limit of normal. HbA1c results differed widely between the various hospitals. Further investigations on how to improve glycaemic control are needed; institution of multiple injection regimens is not by itself correlated with a lower HbA1c. Obesity in patients with NIDDM is a risk factor for poor glycaemic control.

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## Chapter 2

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## 2.3 Can glycaemic control be improved?

### 2.3.1 Failure to achieve optimal glycaemic control in patients with NIDDM with routine diabetes care

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*Submitted*

## Summary

The aim of the present study was to investigate whether improvement of glycaemic control in patients with NIDDM can be achieved in a routine outpatient setting. Seventy-nine patients with NIDDM and a history of oral treatment for at least 1 year were treated for 12 months in an open prospective crossover trial. Patients were randomly divided into two treatment groups: one group continued with oral treatment, the other group started with insulin; after 6 months they crossed-over. Baseline HbA1c was  $8.4 \pm 1.3\%$  (mean  $\pm$  SD). HbA1c after insulin treatment was on average 0.1% lower (95% CI: -0.1 to 0.3%) than after oral treatment, without significant improvement in metabolic control. Mean insulin dose was  $35 \pm 19$  units per day. After the first period of 6 months, mean body weight was 1.7 kg higher (95% CI: 0.3 to 3.2 kg) after insulin than after oral therapy ( $p=0.02$ ). Hypoglycaemic events were significantly more common during insulin therapy than during oral therapy ( $p<0.001$ ). In conclusion, with routine outpatient diabetes care, insulin therapy is not superior to oral treatment in patients with NIDDM.



## **Introduction**

The Diabetes Control and Complications Trial has unequivocally proven that intensive treatment of patients with insulin-dependent diabetes mellitus (IDDM) reduces microvascular complications with approximately 50% [1]. Persistent hyperglycaemia is probably of similar importance as a causal factor for microangiopathic complications in patients with non-insulin-dependent diabetes (NIDDM) [2]. Increasing evidence is accumulating that macroangiopathy, the main cause of morbidity and mortality in NIDDM, is also related to chronic hyperglycaemia [3,4]. Therefore, one might expect intensive treatment to be equally effective in patients with NIDDM as in patients with IDDM. This has not yet been investigated in a sufficiently large, long-term trial.

It is an important question whether the positive results of these strictly controlled studies can also be achieved in routine practice, where the majority of patients is treated during the main part of their diabetes life. Because we estimated that many of our patients do not achieve the goals of the DCCT, we performed a multicenter prospective randomized open crossover trial to investigate glycaemic control, body weight, arterial bloodpressure and lipid profile in orally treated patients with NIDDM after conversion to insulin therapy, treated by the usual diabetes care team of each participating hospital.

## **Patients and methods**

### *Study population*

Fourteen hospitals in the region of Rotterdam, The Netherlands, participated in the study. Specialists in internal medicine, all members of the Studygroup Diabetes Rijnmond (SDR, names listed below), with special interest in diabetes, recruited 1-17 patients each. Inclusion criteria were: NIDDM treated with oral hypoglycaemic agents (OHA) for at least 1 year, age < 80 years, Body Mass Index (BMI) < 35 kg/m<sup>2</sup>, and last HbA1c < 12%. Exclusion criteria were: serum creatinine > 120 µmol/l, macroproteinuria (positive dipstick or > 300 mg protein/24h), pregnancy, major operation less than 6 months before the study, myocardial infarction less than 3 months before the study, unstable angina pectoris, or other serious illnesses. According to our sample size calculation, 34 patients had to be enrolled in the study to demonstrate a treatment effect of 1% HbA1c with a power of 90%. The medical ethical committees of the participating hospitals approved the

study and all patients gave written informed consent.

### *Protocol*

A blocked randomization per center was carried out after a 4-week run-in period. Patients were instructed by their internist on the allocated treatment order (continuation of OHA and conversion to insulin therapy after 6 months, or start with insulin and return to OHA after 6 months). Treatment goals with both therapies were (near-)normal blood glucose values between 4 and 8 mmol/l, and HbA1c < 7%. OHA were either continued as before, or increased if necessary, according to the results of self-monitoring of blood glucose (SMBG). Maximal oral treatment was defined as maximal dosages of a sulfonylurea preparation (gliclazide 240 mg or glibenclamide 15 mg daily) in combination with 1500 or 1700 mg metformin daily. Depending on individual characteristics such as age, lifestyle and motivation, insulin therapy was given either as 2 injections Mixtard 30/70 (Novo Nordisk, Denmark) or as a basal/prandial scheme with Insulatard (Novo Nordisk) at bedtime and 3 injections Actrapid (Novo Nordisk) before meals. Initial dose was assessed by the specialist and individual doses were adjusted according to the results of subsequent SMBG. By the time scheduled to change to insulin treatment, patients received appropriate instructions from a specialized nurse and from a dietician.

The patients were investigated by their specialist 2 weeks after conversion to insulin, thereafter and during OHA treatment at 4-10 week intervals. Body weight, arterial bloodpressure, hypoglycaemic events, complaints, and changes in (co)medication were recorded, and the blood glucose values of the previous weeks were copied in the patient record form.

### *Biochemical measurements*

Patients not used to SMBG received an Accutrend glucose meter (Boehringer-Mannheim, Mannheim, Germany) and appropriate instructions. Patients were asked to perform a 7-point blood glucose profile once every week during the first month after conversion to insulin, and a 4-point blood glucose profile every two weeks for the rest of their insulin treatment period. Patients on OHA were asked to measure a fasting and postprandial blood glucose every two weeks. At weeks -4, 0, 16, 26, 42 and 52, HbA1c was measured in one central laboratory (University Hospital Dijkzigt) with HPLC (reference range 5.0-6.3%). Additionally, in a subgroup of 31 patients blood was drawn at baseline, at 6 months (crossover) and at 12 months (end of the study) for lipid measurements. This subgroup of patients did not differ from

the other patients with respect to clinical characteristics and treatment modality.

### *Statistical analysis*

Data are presented as mean  $\pm$  SD, or as median (range) when not approximately normally distributed. HbA1c, bloodglucose values, body weight and hypoglycaemic events were analysed according to the standard procedure as described by Armitage [5], as follows. First, results were checked for a significant carry-over effect. If not, then the treatment effect, corrected for a possible period effect, was estimated and tested by Student's t-test. Means between subgroups were tested with Mann-Whitney-U's rank sum test or one-way ANOVA. Pearson correlation coefficients were calculated between individual variables. Possible modifiers (baseline HbA1c and BMI) of treatment effect were tested by multiple regression analysis. A p-value  $< 0.05$  was considered to be statistically significant.

## **Results**

### *Description of the patients*

Of the 87 patients that initially consented to participating in the study, 8 patients (4 men and 4 women, age  $63 \pm 12$  years (mean  $\pm$  SD)) dropped out during the run-in period because of haemoglobinopathy ( $n=2$ ) or personal objections ( $n=6$ ). Eleven of the remaining patients (6 men and 5 women, age  $61 \pm 11$  years) dropped out during the study because they were unwilling to return to OHA after 6 months of insulin therapy ( $n=8$ ), retinal hemorrhage with blindness ( $n=1$ ), non-Hodgkin lymphoma ( $n=1$ ) and early need for insulin therapy ( $n=1$ ). In the next part, the results for the 68 patients with complete data are given.

Thirty-five patients were randomized to continue with oral treatment for the first 6 months and then switched to insulin therapy; 33 patients started with insulin therapy and returned to OHA after six months. Baseline characteristics were not significantly different between the two randomization groups, except for the proportion of patients treated with maximal oral treatment at baseline (Table 1). Twenty-two percent of the study population smoked. According to the 'strict' guidelines of the European NIDDM Policy Group,[6] 74% of our patients had hypertension, 80% was in poor metabolic control, 65% had obesity and 47% had dyslipidemia.

Eighteen of the 68 patients (27%) were being treated with maximal oral medication. Comedication was used by 46 patients (64%) and consisted

of antihypertensives (34%), lipid-lowering agents (12%), anticoagulant agents (14%), and various other medications (40%; mainly hypalgesics and hypnotics).

**Table 1. Initial characteristics of the 68 patients who completed the study**

	first OHA, then insulin	first insulin, then OHA
sex (M/F)	17/18	14/19
age (years)	63 ± 10	59 ± 11
duration of diabetes (years)	11 ± 8	10 ± 7
age at diagnosis (years)	53 ± 11	49 ± 12
BMI (kg/m <sup>2</sup> )	27.1 ± 3.8	26.4 ± 3.1
systolic blood pressure (mm Hg)	156 ± 20	153 ± 21
diastolic blood pressure (mm Hg)	88 ± 8	85 ± 10
neuropathy	7 (21%)	4 (12%)
nephropathy (microalbuminuria)	7 (20%)	11 (34%)
retinopathy		
no retinopathy	24 (75%)	30 (97%)
background	6 (19%)	0
proliferative	2 (6%)	1 (3%)
maximal oral treatment	13 (37%)	5 (15%)
HbA1c (%)	8.6 ± 1.4	8.2 ± 1.2
cholesterol (mmol/l)	5.5 ± 1.1	5.4 ± 1.0
triglycerides (mmol/l)	2.48 ± 1.33	2.15 ± 1.08
HDL-cholesterol (mmol/l)	1.10 ± 0.30	1.09 ± 0.39
smoking		
non-smokers	18 (51%)	12 (38%)
ex-smokers	12 (34%)	10 (31%)
current smokers	5 (14%)	10 (31%)

Values are mean ± SD, or number (%)

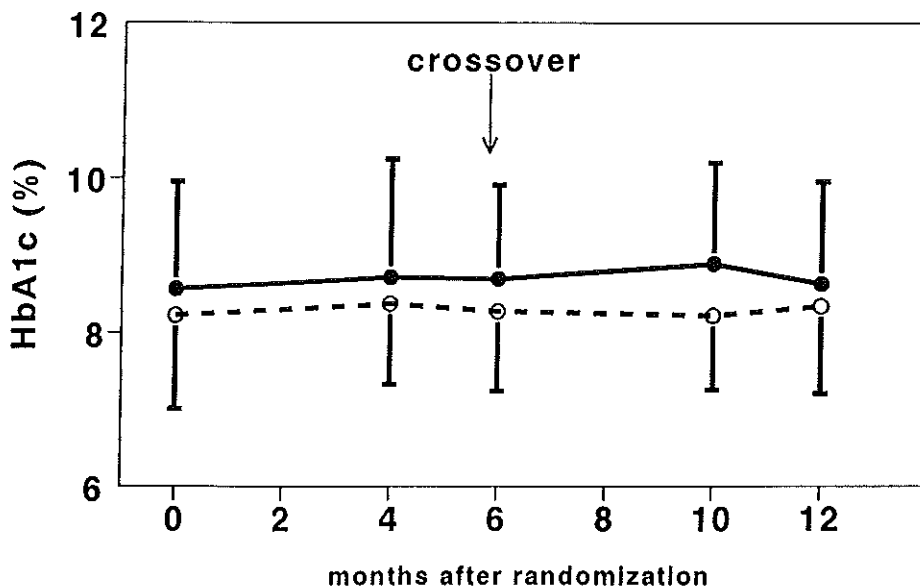
OHA: oral hypoglycaemic agents

### *Treatment efficacy*

During insulin treatment, mean dosage was 35 ± 19 U/day (range 12 to 112 U/day), i.e. 0.44 ± 0.20 U/kg body weight (range 0.14 to 1.29 U/kg). Two patients were treated with one injection of mixed insulin per day, 51

patients were treated with 2 injections of mixed insulin per day, one patient with 3 injections of mixed insulin and 14 patients with the 4 injections regimen of short-acting insulin before meals and intermediate acting insulin at bedtime.

Follow-up HbA1c values of the two randomization groups are shown in Figure 1. There was no significant carry-over effect. The insulin treatment effect was a 0.1% decrease in HbA1c (95% CI: -0.1 to 0.3%; NS), without a clinically relevant improvement in metabolic control. Total daily insulin dose was positively correlated with HbA1c ( $r=0.28$ ;  $p=0.04$ ). There was no independent effect of insulin dose on HbA1c corrected for obesity, as estimated by multiple regression analysis.



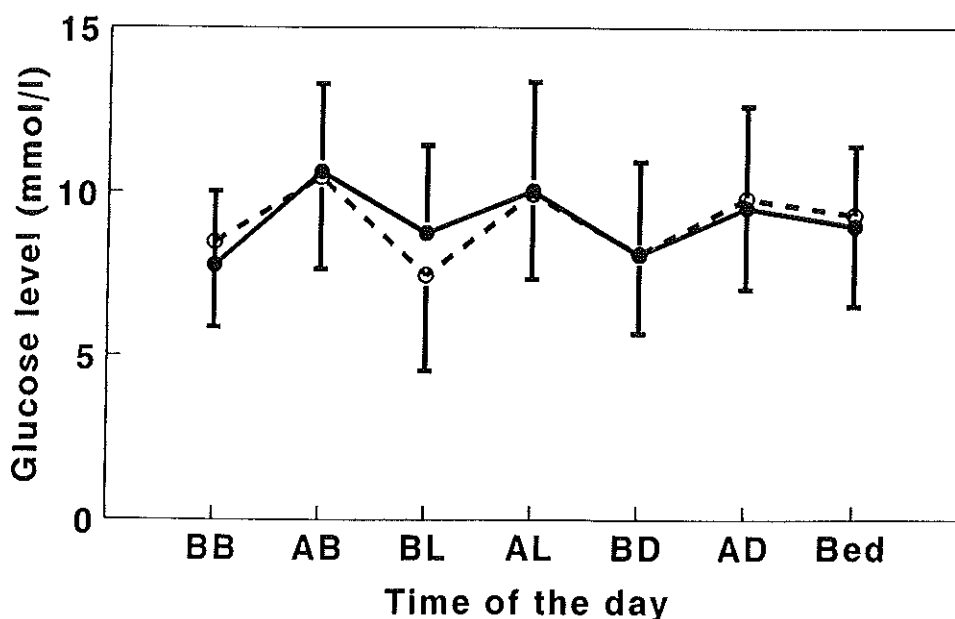
**Figure 1. HbA1c concentrations in the two randomization groups**

Values are mean  $\pm$  SD. Continuous line: patients who continued OHA treatment first and switched to insulin after 6 months ( $n=35$ ). Dotted line: patients who used insulin for the first 6 months and OHA in the last 6 months ( $n=33$ ).

A small beneficial effect of insulin was seen in lean patients ( $BMI \leq 25$  kg/m<sup>2</sup>) with high HbA1c at baseline, while insulin had a detrimental effect in obese patients with initially good metabolic control. However, after multiple regression analysis with the difference in HbA1c after insulin therapy vs. OHA

therapy as the dependent variable, no independent effect of baseline HbA1c and BMI, and their interaction, was found. Obese patients tended to receive more insulin per day than lean patients:  $38 \pm 23$  U/day vs.  $29 \pm 6$  U/day, but the insulin dose per kg body weight was not significantly different:  $0.45 \pm 0.25$  U/kg vs.  $0.44 \pm 0.11$  U/kg. Lean patients using less than 0.45 U/kg per day had significantly lower HbA1c compared to lean patients using more than 0.45 U/kg per day ( $7.6 \pm 0.9\%$  vs.  $8.4 \pm 0.6\%$ ;  $p=0.04$ ). In obese patients HbA1c did not differ between the two dose groups.

Results of home blood glucose measurements were not significantly different between insulin and OHA treatment (Figure 2). Although treatment goals were set to achieve (near) normal glycaemia, especially fasting blood glucose values remained elevated both during insulin and during oral treatment ( $8.5 \pm 2.6$  mmol/l and  $7.8 \pm 2.3$  mmol/l, resp.; NS).



**Figure 2. Results of home blood glucose measurements during insulin and oral treatment.**

Values are mean  $\pm$  SD.

BB: before breakfast; AB: after breakfast; BL: before lunch; AL: after lunch; BD: before dinner; AD: after dinner; Bed: bedtime.

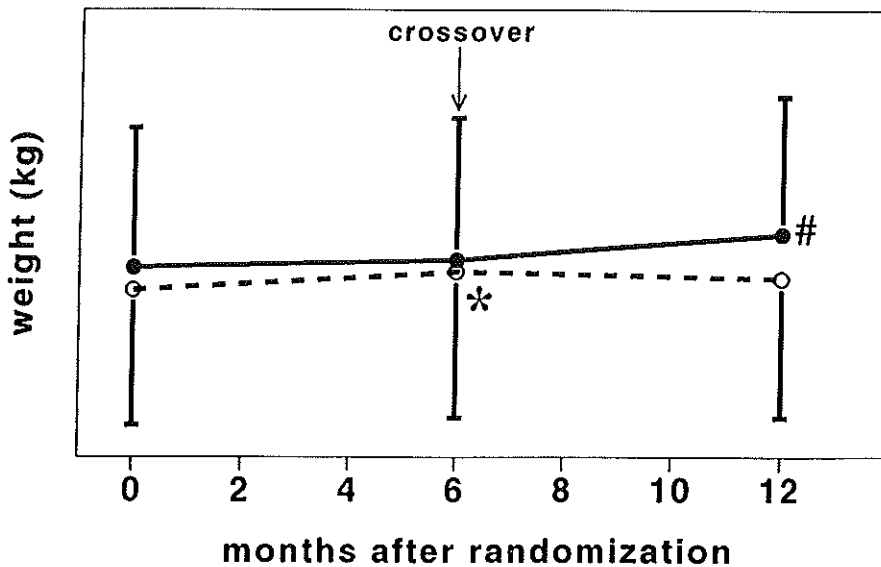
Continuous line: measurements during OHA treatment in all the 68 patients.

Dotted line: measurements during insulin therapy in all the 68 patients.

Lipid analysis in 31 patients showed small changes in lipid concentrations after insulin vs. oral therapy: median HDL-cholesterol improved from 0.92 (range 0.44 to 1.45) mmol/l after OHA treatment to 1.01 (range 0.71 to 1.45) mmol/l after insulin treatment. However, with the standard procedure for testing results in a crossover design, the difference in HDL-cholesterol was not significant ( $p=0.1$ ). Triglyceride and total cholesterol levels were also not significantly different after insulin treatment compared to OHA treatment.

#### Adverse effects

As shown in Figure 3, insulin treatment resulted in a small but significant weight gain. Mean body weight was 1.7 kg higher after 6 months of insulin therapy than after 6 months of oral therapy (95% CI: 0.3 to 3.2 kg;  $p=0.02$ ). There was no correlation between the change in body weight and insulin dose or change in HbA1c.



**Figure 3. Body weight in the two randomization groups**

Values are mean  $\pm$  SD.

Continuous line: patients who continued OHA treatment first and switched to insulin after 6 months ( $n=35$ ). Dotted line: patients who used insulin for the first 6 months and OHA in the last 6 months ( $n=33$ ).

\*  $p=0.001$  vs. baseline, #  $p=0.01$  vs. 6 months.

Hypoglycaemic events (defined as symptoms of low blood glucose) were more frequent during insulin than during oral treatment. Seven of 66 patients (11%) reported a total of 8 hypoglycaemic events during the 6 months of OHA treatment, while 36 of 66 patients (45%) reported 57 hypoglycaemic events during the insulin treatment period ( $p < 0.001$ ). None of the events was serious or required assistance of other persons.

Bloodpressure was not different during insulin treatment compared with oral treatment.

### *Dropouts*

In the 8 patients who did not crossover to oral treatment after 6 months of insulin therapy, HbA1c decreased from  $9.3 \pm 1.3\%$  to  $8.6 \pm 1.2\%$  ( $p = 0.052$ ). They received insulin in a mean dose of  $49 \pm 20$  U/day, or 0.60 U/kg per day (range 0.39 to 0.89 U/kg). Their initial BMI was  $26.9 \pm 4.4$  kg/m<sup>2</sup>. Mean weight in this group increased 3.5 kg: from  $77.1 \pm 11.1$  kg at baseline to  $80.6 \pm 10.5$  kg after 6 months of insulin therapy (NS).

When all 77 patients who completed the first period were included in the analysis (general linear mixed model analysis with SAS Proc MIXED, which allows for missing values), HbA1c after insulin treatment was again on average 0.1% lower than after oral treatment (95% CI: -0.3 to 0.1%). Thus, even after inclusion of those patients with successful insulin therapy, in the overall group still no beneficial effect of insulin over OHA was seen.

### *Final treatment*

After the last visit, 45 of the 79 randomized patients continued treatment with insulin, while 27 patients continued treatment with OHA, and 4 patients started with a combination of insulin and OHA. Data are missing in 3 patients who had dropped out.

## **Discussion**

In this crossover study, performed in a routine diabetes outpatient setting, insulin treatment was not superior to treatment with OHA for glucose control in patients with NIDDM. Only our poorly controlled nonobese patients experienced a small decrease in HbA1c after insulin therapy compared to OHA treatment. In the whole group, HbA1c did not change from baseline, neither after 6 months of insulin therapy, nor after 6 months of OHA treatment.

Our results are in contrast with other studies that demonstrated significant reductions in HbA1c after conversion to insulin therapy [6-9]. Since



we tried to incorporate the study patients in routine diabetes care, there are several explanations for our failure to achieve better metabolic control. Firstly, it could be due to the less intensive supervision of the patients compared to other studies. This can also be concluded from the lack of any 'study effect' in our patients, since not even a slight decrease in HbA1c after the start of the study was seen. The fact that, during the study, HbA1c values were not known at the time patients had an appointment, limited the feedback of the physicians to evaluate their treatment efficacy. However, results of SMBG were available at all times, so apparently, in routine practice, doctors either lack sufficient time to supervise their patients more intensively, or they do not aim at normal blood glucose values in this elderly and obese population. The latter is supported by the fact that only a quarter of our patients received maximal oral treatment at baseline, although HbA1c and fasting blood glucose values were elevated above the desired targets [6].

Secondly, the cooperating internists treated their patients with a median insulin dose of 35 units per day, or only 0.45 units per kg body weight, which is far less than any other controlled study [7,9-11]. Fear of an increased frequency of hypoglycaemic events [12], weight gain [1,8-12] and hyperinsulinaemia [13,14] might explain the reluctance to increasing insulin dosage. Higher insulin doses were not associated with better metabolic control in the present study, and HbA1c was even higher in patients receiving more insulin per day. This is remarkable, because especially obese patients need higher insulin doses to overcome the extra insulin resistance [9,11,15,16]. Insulin treatment augmented the difference in HbA1c between our obese and lean patients. Although we did not measure C-peptide levels, we assume that the selective beneficial effect of insulin therapy in nonobese patients with NIDDM might be explained by their lower endogenous insulin concentrations compared to obese patients [11]. Combination therapy of insulin and OHA might be of special interest for obese patients with NIDDM, in order to restrict excessive weight gain and hyperinsulinaemia [10].

The small increase of 10% in HDL-cholesterol can be considered as a beneficial effect of insulin treatment, and has been demonstrated before [8,17]. Other positive elements of insulin therapy in patients with NIDDM might be improvement in well-being and sense of self-control, even without a significant decrease in HbA1c, since 60% of our patients preferred further treatment with insulin after the study was completed.

Formation of interdisciplinary teams and implementation of systematic guidelines as used in Staged Diabetes Management [18] have been successful means of achieving intensive treatment in routine diabetes care,

that could not be achieved with 'ordinary' care [19]. Frequent consultation of team members, structured treatment steps and more frequent selfcontrol are the necessary tools to achieve this level of diabetes management [1,18].

We conclude that insulin is not superior to OHA with respect to metabolic control and thus the prevention of complications in patients with NIDDM, when diabetes care is applied as a routine.

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2.3.2 Better diabetes control by strict observance of the standard 'Diabetes mellitus type II' of the Netherlands College of General Practitioners.

Jacobs ML, Akkerhuis KM, van Dijk MJ, Kuis FBM, Veldkamp RTM, Weber RFA.

Published in *Ned Tijdschr Geneesk* 1995;139:1241-1245.

## Summary

*Objective.* Comparison of diabetes regulation by strict or less strict observance of the relevant standard protocol of the Netherlands College of General Practitioners (NHG standard).

*Setting.* Two general practices in the region 'Nieuwe Waterweg Noord', near Rotterdam, the Netherlands.

*Design.* Cross-sectional comparative descriptive study.

*Method.* Type II diabetic patients from two general practices were investigated for diabetic symptoms, type of medication, metabolic abnormalities and (risk factors for) complications in relation to their HbA1c as a measure of mean blood glucose regulation. One GP strictly observed the NHG standard as a protocol, the other GP used it as a guideline.

*Results.* Metabolic control was significantly better in the 'strictly observing' general practice than in the 'guideline' practice: HbA1c  $7.3 \pm 1.7\%$  versus  $8.1 \pm 1.5\%$  ( $p < 0.01$ ). Systolic blood pressure was also significantly lower in the 'strict' practice:  $141 \pm 23$  mmHg versus  $155 \pm 21$  mmHg ( $p < 0.01$ ), but mean age was lower and mean duration of diabetes was significantly shorter. The prevalence of macrovascular complications, diabetic retinopathy and nephropathy did not differ significantly in these practices; symptoms of polyneuropathy, however, were found more often in the patients of the 'strict' practice (41 versus 20%;  $p < 0.05$ ).

*Conclusion.* Good metabolic control in patients with type II diabetes mellitus is made possible by strict application of the NHG standard for general practitioners.

## **Betere diabetesregulatie door strikt volgen van de standaard 'Diabetes mellitus type II' van het Nederlands Huisartsen Genootschap.**

### **Samenvatting**

*Doel.* Vergelijking van diabetesregulatie bij het strikt en minder strikt toepassen van de standaard 'Diabetes mellitus type II' van het Nederlands Huisartsen Genootschap (NHG).

*Plaats.* Twee huisartsenpraktijken in de regio Nieuwe Waterweg Noord, Rotterdam.

*Opzet.* Transversaal descriptief vergelijkend onderzoek.

*Methode.* De type II-diabetespatiënten van twee huisartsenpraktijken werden onderzocht op klachten, medicatiegebruik, metabole afwijkingen en aanwezigheid van (risicofactoren voor) complicaties in relatie tot het HbA1c-percentage als maat voor de gemiddelde bloedglucosewaarde. Eén praktijk paste de NHG-standaard als strikt (geautomatiseerd) protocol toe, de andere praktijk alleen als algemene leidraad.

*Resultaten.* De diabetesregulatie in de geprotocolleerde praktijk was significant beter dan in de praktijk die de NHG-standaard alleen als leidraad gebruikte: HbA1c respectievelijk  $7,3 \pm 1,7\%$  en  $8,1 \pm 1,5\%$  ( $p < 0,01$ ). De systolische bloeddruk was eveneens significant lager in de geprotocolleerde praktijk:  $141 \pm 23$  mmHg versus  $155 \pm 21$  mmHg ( $p < 0,01$ ), doch de gemiddelde leeftijd was lager en de diabetesduur significant korter. De prevalentie van macrovasculaire complicaties, diabetische retinopathie en nefropathie was in beide praktijken niet verschillend; tekenen van polyneuropathie kwamen vaker voor in de geprotocolleerde praktijk (41% versus 20%;  $p < 0,05$ ).

*Conclusie.* Bij het strikt toepassen van de NHG-standaard is het bereiken van een scherpe diabetesregulatie mogelijk.

## Inleiding

De meeste patiënten met suikerziekte hebben de type II-vorm (niet van insuline afhankelijke diabetes mellitus, NIDDM) en worden in Nederland meestal door de huisarts behandeld. Het Nederlands Huisartsen Genootschap (NHG) heeft in 1989 een leidraad opgesteld voor de behandeling van type II-diabetes: de NHG-standaard 'Diabetes Mellitus Type II' [1]. Deze standaard is expliciet bedoeld als richtlijn en laat ruimte aan de huisarts om specifieke factoren van de individuele patiënt bij het beleid te betrekken.

De behandeling van type II-diabetes bestaat enerzijds uit preventie en behandeling ten aanzien van risicofactoren voor hart- en vaatziekten (die ziekten zijn frequente complicaties), anderzijds uit normalisering van de bloedglucosewaarden. Bij patiënten met type I-diabetes staat sinds de 'Diabetes control and complications trial' onomstotelijk vast, dat scherpe bloedglucoseregulatie het ontstaan van microvasculaire lange-termijncomplicaties voorkomt [2], hetgeen ervoor pleit ook bij patiënten met type II-diabetes naar normoglycaemie te streven, aangezien het aannemelijk is dat de pathogenese van microvasculaire complicaties bij beide vormen van diabetes hetzelfde is.

De hoge incidentie van complicaties geeft aan dat de huidige behandelwijze tekortschiet. Het is onbekend of dit te wijten is aan onvoldoende effectiviteit van de bekende therapieën dan wel aan een inadequate toepassing ervan.

In dit onderzoek gingen wij na in hoeverre strenge, geprotocolleerde toepassing van de NHG-standaard leidt tot een betere bloedglucoseregulatie dan toepassing van de standaard als richtlijn alleen. Als maat voor de effectiviteit van behandelen werd gekozen voor de fractie geglycosyleerd hemoglobine (HbA1c), die de gemiddelde glucosespiegel weerspiegelt [3], hoewel deze graadmeter niet in de NHG-standaard is opgenomen. Voor controle van de lange-termijnregulatie is HbA1c echter een stabielere maatstaf dan de momentopname van een nuchtere bloedglucosewaarde [4,5], vooral voor patiënten die op insulinetherapie zijn ingesteld [6].

Het tweede aandachtspunt van ons onderzoek was de prevalentie van complicaties.

## Methode

### *Praktijken*

Twee huisartsenpraktijken in de regio Nieuwe Waterweg Noord



waren bereid tot deelname aan het onderzoek. In praktijk A werd sinds eind 1991 voor de diabeteszorg een voor deze regio specifiek protocol toegepast [7]. Dit is een op de NHG-standaard gebaseerd behandelprotocol op basis van een stroomdiagram, dat in een regionaal softwareprogramma (ELIAS) in het Huisartsen Informatie Systeem is geïnstalleerd. Door gebruik te maken van de geautomatiseerde tekstbestanden bij het volgen van het protocol zal de huisarts eerder bedacht zijn op acties die hij moet ondernemen en zal hij adequaat reageren op de bevindingen en laboratoriumuitslagen met betrekking tot de patiënt. Er bestaan strakke richtlijnen voor verwijzing en terugverwijzing van huisarts naar internist en omgekeerd: de huisarts behoudt in principe de zorg voor patiënten die door de internist zijn ingesteld op insuline. In het protocol wordt in tegenstelling tot in de NHG-standaard een grote betekenis toegekend aan het HbA1c als maat voor de instelling van de diabetes, en aan de lipidenregulatie.

Praktijk B fungeerde als controlepraktijk. Hier werd de NHG-standaard eveneens als richtlijn gehanteerd, echter zonder protocollaire toepassing.

In beide praktijken werd de diagnose 'diabetes mellitus type II' gesteld op grond van criteria in de NHG-standaard (nuchtere bloedglucosewaarde  $\geq 6,7$  mmol/l of 2h na koolhydraatbelasting  $\geq 11,1$  mmol/l; bij asymptomatische patiënten dient men de meting 1 maal te herhalen) [1].

### *Onderzoeksmethoden*

Tussen januari en mei 1994 werden de patiënten op afspraak in hun eigen praktijk geïnterviewd en onderzocht door één onderzoeker. Tevoren werd informatie verkregen uit de medische status. Er werd een vragenlijst afgewerkt over de medische voorgeschiedenis, het medicatiegebruik, het leefpatroon en eventuele klachten en symptomen bij de diabetes.

Met eenvoudig lichamelijk onderzoek werd gezocht naar diabetische complicaties en risicofactoren voor hart- en vaatziekten; het onderzoek omvatte meting van bloeddruk en hartfrequentie in zittende houding, gewicht- en lengtemeting, meting van de middel- en heupomtrek [8], testen van achilles- en kniepeesreflex (APR en KPR) en van vibratiezin, en palpatie van de A. dorsalis pedis.

Door middel van een vingerprik werd bloed afgenomen voor de bepaling van (niet-nuchtere) bloedglucose- en cholesterolwaarden, die met een Accutrend-GC<sup>R</sup>-methode (Boehringer Mannheim, Mannheim, Duitsland) werden gemeten (bereik glucose: 1,1-33,3 mmol/l; bereik cholesterol: 3,88-7,75 mmol/l). Ochtendurine werd onderzocht op albumine met de Micral-test<sup>R</sup>

## Chapter 2

(Boehringer Mannheim, Mannheim, Duitsland), een semi-kwantitatieve immunologische bepaling via een teststrip. Serumcreatinine- (normaal: 50-115  $\mu\text{mol/l}$ ) en HbA1c-concentraties (normaal: tot 6,0%) werden bepaald in het door beide praktijken gebruikte lokale ziekenhuislaboratorium.

Indien de patiënt langer dan een jaar geleden door een oogarts was onderzocht, werd om een nieuw onderzoek gevraagd.

### Definities

Hypertensie werd aanwezig geacht bij één of meer van de volgende bevindingen: systolische bloeddruk  $\geq 160$  mmHg, diastolische bloeddruk  $\geq 95$  mmHg [9], gebruik van antihypertensiva en eerdere diagnose 'hypertensie'. Van 'overgewicht' werd gesproken bij een 'body mass index' (BMI)  $> 25$   $\text{kg/m}^2$  [1,10,11] en van 'centrale adipositas' bij een taille-heupomvangratio ('waist-hip-ratio', WHR)  $> 0,90$  (voor mannen) of  $> 0,85$  (voor vrouwen) [8]. 'Hypercholesterolemie' werd gedefinieerd als een cholesterolconcentratie  $> 6,5$  mmol/l, gebruik van antilipaemica en/of eerdere diagnose 'dyslipidemie'. Nefropathie werd aanwezig geacht bij albuminurie  $> 20$  mg/l en/of een creatinineconcentratie  $> 115$   $\mu\text{mol/l}$ . Van 'symptomatische neuropathie' werd gesproken bij paresthesieën in combinatie met spontane pijnsensaties of met bilateraal afwezige KPR of APR; bij gestoorde vibratiezin met bilateraal afwezige KPR of APR; bij bilaterale afwezigheid van zowel KPR als APR. Macroangiopathie werd gedefinieerd als de aanwezigheid van één of meer van de volgende aandoeningen: doorgemaakt myocardinfarct, cerebrovasculair accident (CVA) of 'transient ischaemic attack' (TIA), claudicatio intermittens, angina pectoris, afwezige pulsaties van de A. dorsalis pedis [1].

### Statistische analyse

Voor de bewerking van continue numerieke gegevens werd gebruik gemaakt van de rangtekentoets van Wilcoxon (U-toets van Mann en Whitney); categoriale variabelen werden met een  $\chi^2$ -toets geanalyseerd. Onderlinge relaties tussen de variabelen werden berekend met multiële lineaire en logistische regressieanalyse. Verschillen werden als significant beschouwd bij  $p < 0,05$ .

## Resultaten

### Patiënten

Praktijk A bestond uit 2178 patiënten, van wie 36 personen bekend waren met diabetes mellitus type II (prevalentie 1,65%); 32 patiënten parti-

cipeerden in het onderzoek. Praktijk B, geleid door 2 deeltijdhuisartsen, omvatte 3030 patiënten, van wie er 54 bekend waren wegens type II-diabetes (prevalentie 1,78%); 46 patiënten namen deel aan het onderzoek. Redenen om niet aan het onderzoek mee te doen waren: verblijf in het buitenland, ernstige invaliditeit of onvoldoende motivatie.

### *Praktijkverschillen*

De mediane leeftijd en de diabetesduur waren in praktijk A significant lager dan in praktijk B ( $p < 0,05$ ; Tabel 1). Er waren geen significante verschillen in de prevalentie van klachten als polydipsie (in praktijk A en B respectievelijk 28 en 17%), polyurie (19 en 13%), hypoglycaemische verschijnselen (41 en 48%), ontstekingen van de huid (in beide praktijken 9%), jeuk (16 en 24%) of recidiverende urineweginfecties (16 en 7%).

Bij lichamelijk onderzoek was de systolische bloeddruk significant verschillend; bij correctie voor HbA1c-waarde, diabetesduur en geslacht bleek de leeftijd dit verschil tweeweg te brengen. Hypertensie kwam daarmee significant minder voor in praktijk A dan in praktijk B: respectievelijk 53 en 78% ( $p < 0,05$ ). De prevalentie van overgewicht was niet verschillend (72 en 76%), hoewel de BMI van de vrouwelijke patiënten in praktijk A significant hoger was. De prevalentie van centrale adipositas was in beide praktijken ongeveer even hoog (respectievelijk 77 en 69%). Ook de medicatie verschilde niet.

### *Laboratoriumonderzoek*

Tabel 2 toont de resultaten van het laboratoriumonderzoek: in praktijk A waren meer patiënten goed gereguleerd voor hun diabetes dan in praktijk B: een normaal HbA1c-gehalte ( $\leq 6,0\%$ ) werd bij 29,0 en 2,3% van de patiënten gevonden ( $p < 0,001$ ), terwijl bij 58,1 en 25,0% een HbA1c-waarde  $\leq 7,0\%$  werd gevonden ( $p < 0,01$ ). Het aandeel slecht ingestelde patiënten ( $\text{HbA1c} \geq 8,5\%$ ) was in praktijk A kleiner dan in praktijk B: respectievelijk 19,4 en 34,1% ( $p < 0,01$ ). Ook na correctie voor geslacht, leeftijd, diabetesduur, BMI en waist-hip-ratio bleef het verschil in HbA1c tussen beide praktijken significant ( $p < 0,02$ ). Als men de patiënten buiten beschouwing laat die met insuline werden behandeld, was het verschil in de gemiddelde HbA1c- en glucosewaarden tussen de beide praktijken nog groter. De HbA1c-waarde was significant gecorreleerd met de niet-nuchtere glucosewaarde ( $r = 0,61$ ;  $p < 0,001$ ). De prevalentie van hypercholesterolemie verschilde niet significant in praktijk A ten opzichte van praktijk B (respectievelijk 25 en 35%), maar bleek bij correctie voor leeftijd, diabetesduur, geslacht en BMI significant

gecorrleerd met de HbA1c-uitslag. De prevalentie van nefropathie bedroeg respectievelijk 38 en 30%, en was ook na correctie voor leeftijd, diabetes-duur, geslacht en systolische bloeddruk niet significant verschillend en niet gecorrleerd met de HbA1c-waarde.

**Tabel 1. Demografische gegevens en medicatiegegevens van 78 patiënten met diabetes mellitus type II in twee huisartsenpraktijken**

	praktijk A* n = 32	praktijk B* n = 46	p-waarde
<b>DEMOGRAFISCHE GEGEVENS</b>			
aantal mannen/vrouwen	15/17	13/33	NS
leeftijd; mediaan in jaren (uitersten)	57,5 (38-89)	68,0 (43-87)	0,04
diabetesduur; mediaan in jaren (uitersten)	3,3 (0,2-30)	6,0 (0,2-30)	0,03
diabetes mellitus in familie	18 (56%)	26 (57%)	NS
aantal rokers	4 (13%)	12 (26%)	NS
systolische bloeddruk in mmHg $\pm$ SD	141 $\pm$ 23	155 $\pm$ 21	0,01
diastolische bloeddruk in mmHg $\pm$ SD	88 $\pm$ 9	86 $\pm$ 10	NS
hartfrequentie in slagen/min $\pm$ SD	75 $\pm$ 11	76 $\pm$ 9	NS
'body mass index' in kg/m <sup>2</sup> $\pm$ SD			
mannen	27,3 $\pm$ 5,3	26,0 $\pm$ 2,0	NS
vrouwen	32,2 $\pm$ 7,5	28,0 $\pm$ 3,8	0,04
taille-heupomvangratio in cm/cm $\pm$ SD			
mannen	0,93 $\pm$ 0,05	0,95 $\pm$ 0,06	NS
vrouwen	0,89 $\pm$ 0,06	0,87 $\pm$ 0,05	NS
<b>MEDICATIE (aantal patiënten (%))</b>			
diabetestherapie			
geen	4 (13%)	8 (17%)	NS
dieet	6 (19%)	5 (11%)	NS
oraal	14 (44%)	24 (52%)	NS
insuline	8 (25%)	9 (20%)	NS
antihypertensiva	10 (31%)	19 (41%)	NS
cholesterolverlagers	2 (6,3%)	3 (6,5%)	NS

\* In praktijk A werd de standaard 'Diabetes mellitus type II' van het Nederlands Huisartsen Genootschap strikt gevolgd, in praktijk B werd deze alleen als richtsnoer gebruikt.

**Tabel 2. Resultaten van laboratoriumonderzoek bij 78 patiënten met diabetes mellitus type II in twee huisartspraktijken**

		praktijk A* n = 32	praktijk B* n = 46	p-waarde
HbA1c in % ± SD	alle patiënten	7,3 ± 1,7	8,1 ± 1,5	0,008
	non-insuline-gebruikers	6,5 ± 1,1	8,2 ± 1,4	0,0001
glucose in mmol/l ± SD	alle patiënten	7,2 ± 3,7	8,6 ± 4,5	0,042
	non-insuline-gebruikers	5,9 ± 2,4	8,3 ± 3,4	0,002
cholesterol in mmol/l ± SD		5,4 ± 1,1	5,8 ± 1,1	NS
creatinine in µmol/l ± SD		86 ± 22	85 ± 20	NS
albuminurie	0 - 20 in mg/l	21 (68%)	33 (72%)	NS
	20 - 100 in mg/l	9 (30%)	11 (24%)	NS
	> 100 in mg/l	1 (3%)	2 (4%)	NS

\* In praktijk A werd de standaard 'Diabetes mellitus type II' van het Nederlands Huisartsen Genootschap strikt gevolgd, in praktijk B werd deze alleen als richtsnoer gebruikt.

### Complicaties

In Tabel 3 staat de prevalentie van oogafwijkingen: niet alle patiënten gingen na hun verwijzing naar de oogarts. De totale prevalentie van retinopathie was in praktijk A 11,1% en in praktijk B 12,2% (NS). Gecorrigeerd voor leeftijd en geslacht was er een significante correlatie van retinopathie met de diabetesduur ( $p < 0,01$ ), maar niet met de HbA1c-waarde.

In Tabel 3 staat ook het aantal afwijkingen bij neurologisch onderzoek. In praktijk A werd bij 41% en in praktijk B bij 20% van de patiënten symptomatische diabetische neuropathie geconstateerd ( $p < 0,05$ ). Gecorrigeerd voor dit praktijkverschil, het geslacht en de diabetesduur was de aanwezigheid van neuropathie significant gecorreleerd met zowel de HbA1c-waarde als de leeftijd (respectievelijk  $p < 0,01$  en  $p < 0,05$ ).

De prevalentie van symptomen passend bij macrovasculaire afwijkingen verschilde niet significant tussen beide praktijken (zie Tabel 3); de totale prevalentie (één of meer van de in Tabel 3 genoemde afwijkingen bij een patiënt) was 31% in praktijk A en 41% in praktijk B. Gecorrigeerd voor leeftijd en geslacht was de HbA1c-uitslag significant gecorreleerd met de prevalentie van macrovasculaire afwijkingen ( $p < 0,03$ ).

**Tabel 3. Retinopathie, symptomen passend bij neuropathie, en aanwijzingen voor macrovasculaire afwijkingen bij 78 patiënten met diabetes mellitus type II in twee huisartsenpraktijken**

COMPLICATIE	praktijk A* n = 27/32	praktijk B* n = 41/46	p-waarde
<b>RETINOPATHIE †</b>			
geen DRP	24 (88,9%)	36 (87,8%)	NS
background DRP	1 (3,7%)	2 (4,9%)	NS
proliferatieve DRP + laser	2 (7,4%)	3 (7,3%)	NS
<b>NEUROPATHIE</b>			
afwezige vibratiezin	10 (31%)	10 (22%)	NS
paraesthesieën	9 (28%)	8 (18%)	NS
afwezige KPR	7 (22%)	5 (11%)	NS
afwezige APR	14 (44%)	17 (39%)	NS
spontane pijnsensaties	14 (44%)	7 (16%)	0,02
<b>AANWIJZINGEN VOOR MACROVASCULAIRE AFWIJKINGEN</b>			
doorgemaakt myocardinfarct	7 (22%)	9 (20%)	NS
claudicatio intermittens	2 (6%)	4 (9%)	NS
doorgemaakt CVA of TIA	1 (3%)	3 (7%)	NS
afwezige pulsatie a. dors. pedis	4 (13%)	8 (17%)	NS
angina pectoris	5 (16%)	8 (17%)	NS

CVA = cerebrovasculair accident; TIA = 'transient ischaemic attack'.

† Percentages betrokken op het totale aantal patiënten dat de oogarts bezocht: in praktijk A 27, in praktijk B 41 patiënten

\* In praktijk A werd de standaard 'Diabetes mellitus type II' van het Nederlands Huisartsen Genootschap strikt gevolgd, in praktijk B werd deze alleen als richtsnoer gebruikt.

### *Medicatieverschillen*

De regulatie van de diabetes hing samen met de medicatie: in praktijk A waren de insulinegebruikers significant slechter ingesteld dan de niet-insulinegebruikers: de HbA1c-waarde bedroeg respectievelijk  $9,4 \pm 1,1\%$  en  $6,5 \pm 1,1\%$  ( $p < 0,001$ ) en de niet-nuchtere bloedglucosewaarde was hoger (respectievelijk  $10,8 \pm 4,7$  mmol/l en  $5,9 \pm 2,4$  mmol/l;  $p < 0,01$ ). De met insuline behandelde patiënten in praktijk A hadden een langere diabetesduur dan de niet-insulinegebruikers, respectievelijk  $10,9 \pm 8,5$  jr en  $3,4 \pm 5,4$  jr ( $p < 0,001$ ), en zij hadden meer macroangiopathie (63 en 21%;  $p < 0,05$ ). In praktijk B was er geen verschil in regulatie tussen de medicatiegroepen (HbA1c-waarde bij insulinegebruikers:  $8,0 \pm 2,0\%$  en voor de andere

medicatiegroepen  $8,2 \pm 1,4\%$  (NS)); wel kwamen in praktijk B in de insulinegroep significant meer retinopathie (33 versus 5%) en polyneuropathie (44 versus 14%) voor. Ook in praktijk B leek insulinetherapie samen te hangen met de diabetesduur ( $13,1 \pm 9,2$  jr bij de insulinepatiënten versus  $7,7 \pm 7,5$  jr in de niet met insuline behandelde groep), maar dit verschil was niet significant. De HbA1c-waarde van de insulinepatiënten in praktijk A verschilde overigens niet significant van die in praktijk B.

## **Beschouwing**

Dit dwarsdoorsnedeonderzoek in twee huisartsenpraktijken toont aan dat het strikt en protocollair toepassen van de NHG-standaard voor NIDDM tot een significant betere diabetesregulatie leidde dan wanneer deze standaard slechts als richtlijn werd gehanteerd. Een goede instelling (HbA1c  $\leq 7,0\%$ ) [12,13] werd bij 58% van de patiënten in de protocollair werkende praktijk gehaald; bij 29% van de patiënten werd een strikt normaal HbA1c ( $\leq 6,0\%$ ) gevonden.

Het streven naar lage bloedsuikerwaarden vindt zijn motivatie in de literatuur die over type I-diabetes bestaat [2,12]. Langlopende prospectieve onderzoeken bij type II-diabetespatiënten zullen moeten aantonen of ook voor deze (oudere) populatie de verwachte winst in levensduur en -kwaliteit door naleving van normoglycaemie voldoende opweegt tegen de inspanning van artsen en patiënten, en of macrovasculaire complicaties eveneens te voorkomen zijn door het streven naar normoglycaemie [13].

In praktijk A werd nog slechts sinds 2½ jaar met het beschreven protocol gewerkt, hetgeen mogelijk verklaart dat de prevalentie van diabetische complicaties niet duidelijk lager was dan bij een mindere diabetesregulatie, zelfs ondanks de significant lagere HbA1c-waarde, de gemiddeld kortere diabetesduur en de jongere leeftijd van de patiënten. Voor een gunstig effect op de preventie van complicaties zal een veel langere behandeling op deze wijze noodzakelijk zijn. Bovendien waren de uitgangssituaties in beide praktijken niet bekend en betrof het hier geen prospectief onderzoek.

De door ons gebruikte methode van onderzoek naar de prevalentie van complicaties behoeft enige kanttekeningen. De gegevens zijn met anamnestic onderzoek en met eenvoudige hulpmiddelen verzameld; er werd geen ECG of elektromyogram (EMG) gemaakt om respectievelijk cardiale en neuropathische complicaties objectief vast te stellen. Om deze redenen is het moeilijk om betrouwbare uitspraken te doen over de ware prevalentie van sommige complicaties. Andere onderzoeken naar lange-termijncomplicaties in

Nederlandse huisartsenpraktijken laten iets hogere totale prevalenties zien, mogelijk omdat iets andere definities zijn toegepast [14,15]; de prevalenties van afzonderlijke symptomen en de bevindingen zijn echter vergelijkbaar met die in het door ons uitgevoerde onderzoek.

Reeds eerder is beschreven dat een speciale interesse van de behandelaar en een op diabetes toegespitste uitrusting van de praktijk de grootste bijdrage leveren aan een goede diabetesregulatie [16]. Aangezien de onderliggende behandelingsdoelen in de beide onderzochte praktijken gelijk waren, kan men de vorm waarin de diabeteszorg werd aangeboden dan wel de moeite die de huisarts zich getroostte om deze doelen te realiseren, als belangrijkste verschil tussen de praktijken aanmerken. Gebruikmaking van additionele middelen die niet in de NHG-standaard zijn opgenomen (HbA1c-waarde voor terugkoppeling op de ingestelde therapie, een regionaal toepasbaar geautomatiseerd protocol voor uniformiteit van behandelstrategieën), kan eveneens tot de vorm van de diabeteszorg worden gerekend. Omgekeerd zou bij gebruik van een dergelijk standaardprotocol ook de attitude van de huisarts kunnen worden verbeterd.

Het percentage op insuline ingestelde patiënten was met name in de protocollair werkende praktijk relatief hoog (25%), hoewel de diabetesduur vrij kort was. Een verklaring hiervoor kan zijn dat de huisarts in deze praktijk zijn patiënten bij falende orale therapie relatief snel doorverwees voor insulinetherapie. Opmerkelijk was het relatief hoge HbA1c-percentage van deze patiëntengroep (resultaten niet weergegeven). Dat er behoefte is aan insulinetherapie betekent niet dat regulatie van de diabetes in principe minder goed mogelijk is [17]. Het merendeel van de insulinepatiënten in praktijk A was reeds op insuline ingesteld in de tijd voordat het protocol werd toegepast en was niet terugverwezen naar de huisarts; waarschijnlijk was dus de attitude van de internist bepalend voor de metabole instelling met vrij hoge HbA1c-waarden. Deze gedachte wordt ondersteund door het gelijke HbA1c-niveau van de insuline- en niet-insulinegebruikende patiënten in de controlepraktijk B, waarin huisartsen en internisten een vergelijkbare attitude hadden (geen speciale protocollen voor de diabeteszorg).

### *Conclusie*

In ons onderzoek werd aangetoond dat in de huisartsenpraktijk, waar het merendeel der type II-diabetespatiënten behandeld wordt, het bereiken van (bijna-) normoglycaemie in een substantieel deel van de diabetespopulatie haalbaar is. Het strikt en enthousiast nastreven van de doelen die in de NHG-standaard zijn aangegeven, eventueel aangevuld met de bepaling van het



HbA1c-percentage, lijkt hiervoor een belangrijk hulpmiddel.

Met dank aan Novo Nordisk voor het mogelijk maken van dit onderzoek, en aan Boehringer-Mannheim voor het beschikbaar stellen van de teststrips en de Accutrend-GC.

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## 2.4 Conclusions

As has been shown in the previous paragraphs, strict metabolic control is not so easy to achieve in every day diabetes care. Long-term normoglycaemia has thus far only been achieved in strictly controlled study-settings, and it is questionable whether it is a real possibility for ordinary outpatient care, even with the help of multiple injection techniques and internists with a specific interest in diabetes. Strict appliance of a protocol for treatment of patients with NIDDM, used by general physicians, may be a useful instrument of achieving this goal, but this requires a special attitude of the treating physician. In the hospitals, where most of the insulin-using diabetic patients are being treated, this appears to be a difficult task.

Therefore, an additional approach seems necessary to prevent the development of diabetic complications. This will be described in Chapter 3.



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## **Chapter 3**

### **Growth hormone as the focus for additional treatment**

3.1 Introduction

3.2 Growth hormone in patients with insulin-dependent diabetes mellitus

3.3 Growth hormone in experimental diabetes

3.4 Suppression of growth hormone in patients with diabetes mellitus

3.5 Conclusions

---



### 3.1 Introduction

As described in Chapter 1, growth hormone might be an important co-factor in the induction of diabetic complications, especially in the early stages of the development of these complications. For diabetic retinopathy, reduction of GH has proven its beneficial effects, but the same remains to be proven for diabetic nephropathy. Moreover, it has not yet been elucidated whether the systemic GH concentration is of main importance for the induction of complications, or the systemic IGF-I concentration, or mainly local concentrations of these peptides.

The following paragraphs describe some studies in mice and men, discussing the associations between diabetes, growth hormone and the kidney. The main questions to resolve are: are GH concentrations elevated in patients with moderately controlled IDDM? Do elevated GH concentrations in transgenic mice induce increased IGF-I concentrations in the kidney after the induction of diabetes? Is GH deficiency able to prevent these early kidney changes of the diabetic state? Is somatostatin therapy able to reduce GFR in IDDM patients with hyperfiltration of the kidney?





### 3.2 Growth hormone in patients with insulin-dependent diabetes mellitus

Growth hormone responses to growth hormone-releasing hormone and clonidine in patients with type I diabetes and in normal controls: effect of age, body mass index and sex.

Jacobs ML, Nathoe HMW, Blankestijn PJ, Stijnen Th, Weber RFA.

Published in *Clin Endocrinol* 1996;44:547-553.

## Summary

**Objective.** Increased plasma concentrations of GH and increased GH responses to provocative stimuli are reported in patients with poorly controlled type I diabetes and are suggested to be related to complications. Our aim was to investigate GH concentrations in moderately controlled patients.

**Patients and measurements.** We have investigated IGF-I concentrations and fasting GH concentrations and the response to 1  $\mu\text{g/kg}$  body weight GH-releasing hormone (GHRH) intravenously and/or to 150  $\mu\text{g}$  clonidine intravenously in 77 moderately controlled patients with type I diabetes and in 46 healthy controls.

**Results.** Median HbA1c in the patients was 8.5% (upper level of normal 6.3%). Fasting GH and GH concentrations after the administration of GHRH were not significantly different in patients with type I diabetes compared with normal controls. Fasting and stimulated GH concentrations after the administration of clonidine were significantly higher in the patients, but this could be explained by their lower age and body mass index compared with controls. In controls but not in patients there was a negative correlation between GH and glucose concentrations. IGF-I was significantly lower in patients with diabetes than in controls, even after correction for age, body mass index and sex.

**Conclusions.** Patients with moderately controlled type I diabetes mellitus have normal baseline and stimulated GH concentrations after the administration of GHRH or clonidine compared with healthy controls, when corrected for age, body mass index and sex. However, these 'normal' GH concentrations must be considered inappropriately high in view of the hyperglycaemia in these patients. The low plasma IGF-I concentrations might be responsible for the GH overproduction.

## Introduction

It is well established that in adults circulating GH concentrations are low or even undetectable between episodic secretional events [1,2]. The pattern of GH secretion is, however, sex and age dependent. Animal studies have provided evidence that GH secretion peaks are the results of a subtle interplay between GH-releasing hormone (GHRH) and somatostatin released from the hypothalamus, acting on the pituitary gland [3]. Administration of glucose is able to suppress GH secretion [4,5]. Nevertheless, raised concentrations of basal GH and/or an exaggerated GH response to provocative stimuli have been found in patients with insulin dependent diabetes mellitus (IDDM) with poor metabolic control [1,6-10], together with low or normal concentrations of IGF-I. Others have found similar GH responses after i.v. administration of GHRH or clonidine in patients with diabetes and controls, respectively [11,12]. The differences in GH secretion can be attributed to differences in metabolic control [13], test methods, time of the day [14,15], age [16,17] and body weight [12,18]. As a consequence, these factors should be taken into account when testing GH responses. This was done only to a limited extent (or not at all) in previous studies.

Therefore we studied basal and stimulated GH concentrations in a large, clinically well described group of patients with IDDM and in healthy controls to analyse the severity of GH abnormalities. We used GHRH and clonidine intravenously as two different stimulative tests for GH secretion. Both tests have been extensively used for this purpose [4-12,14-20].

## Subjects and Methods

### *Patients*

The study group comprised 77 consecutive patients; inclusion criteria were IDDM (age at diagnosis below 40 years and insulin dependency within 6 months of diagnosis), no other medication than insulin and a stable medical condition. Forty-six controls without any evidence of disease were recruited from the normal urban population.

Tests were performed after an overnight fast and in patients the morning insulin was omitted. Participants were requested to refrain from smoking after midnight. A catheter was inserted into an antecubital vein for the collection of blood before (-15, 0 minutes) and after (15, 30, 45, 60 and 90 minutes) the administration of the drug. Either GHRH (somatostatin acetate hydrate (Somatobiss), Pharma Bissendorf Peptide GmbH, Germany) 1 µg/kg,

or clonidine (clonidinehydrochloride (Catapresan), Boehringer Ingelheim KG, Germany) 150  $\mu$ g was injected intravenously. Clonidine was diluted with 10 ml of NaCl 0.9% and injected slowly. All persons remained in supine or sitting position during the tests. When a person was willing to participate in both tests, an interval of at least one week was taken between the tests. Blood samples were taken for the analysis of glucose, GH and IGF-I concentrations. Patients with diabetes were also screened for HbA1c and serum creatinine; diabetic retinopathy (DRP) was established by direct funduscopy as judged by an experienced ophtalmologist, and was graded as no DRP, background DRP or proliferative DRP; urinary albumin excretion was measured as a mean of 3 24-hour urine collections, excluding the day of the test.

The study was approved by the Ethics Review Committee of the Erasmus University Hospital Dijkzigt and all participants gave informed consent.

### Assays

Plasma GH was measured by immunoradiometric assay (Medgenix, Fleurus, Belgium). The detection limit of GH was 0.4 mU/l; normal fasting values are <10 mU/l. The intra- and interassay coefficients of variation were 7.7 and 6.5%, respectively. IGF-I was removed from its binding proteins by the acid-ethanol extraction method according to Daughaday *et al.* [21], and was measured by RIA (Medgenix, Fleurus, Belgium). Normal values are 8.6 to 63.5 nmol/l, depending on age and sex. The intra- and interassay coefficients of variation were 6.7 and 9.9%, respectively. HbA1c was measured by HPLC (normal values 5.0 to 6.3%). Blood glucose concentrations were measured in venous whole blood by an automatic hexokinase method. Urinary albumin excretion was measured by radial immunodiffusion on agarose gel, with a lower limit of detection of 2 mg/l and an interassay variability of 8%.

### Calculations and statistical analysis

Results are presented as median (range) because of very skewed distributions. The changes in GH and glucose during the 90-minute test period are expressed as peak values and as integrated area under the curve (AUC) corrected for baseline values. When a person had been submitted to both tests, we used the mean of basal GH concentrations in both tests for calculation of basal GH concentrations. Wilcoxon's rank test (Mann-Whitney-U) and Student's t-test after logarithmic transformation were used for comparing the groups. Multiple regression analysis on log-transformed data was done to correct for confounding variables. To assess correlations

between two variables Spearman's rank test was used. P-values <0.05 were considered to be significant.

## Results

Stimulation with clonidine was performed in 72 of the 77 patients and in 29 of the 46 controls; GHRH stimulation was performed in 59 patients and in 28 controls. Side-effects were mild and subsided within one to several hours (GHRH, flushing; clonidine, sleepiness).

The clinical characteristics of the patients with diabetes are shown in Table 1. No significant differences were observed in age, body mass index (BMI), duration of diabetes, insulin dosage per kg body weight, blood pressure and HbA1c in male and female subjects with diabetes. Fasting GH concentrations, however, were significantly higher in women than in men, both in patients (Table 1) and in controls (women 12 vs. men 0.5 mU/l;  $p < 0.001$ ).

**Table 1. Clinical characteristics of the patients with diabetes**

	IDDM males n = 44	IDDM females n = 33	p- value <sup>1</sup>
age (years)	33.5 (17-64)	32.2 (18-62)	0.95
body mass index (kg/m <sup>2</sup> )	23.9 (17.0-30.9)	23.5 (19.1-32.9)	0.96
age at diagnosis (years)	16.0 (2-40)	16.5 (3-36)	0.99
diabetes duration (years)	15.5 (2-35)	15.5 (4-43)	0.93
total insulin dosage per day (IU/day)	57.0 (26-96)	46.0 (21-72)	0.002
insulin dosage per kg body weight (IU/kg)	0.73 (0.40-1.26)	0.66 (0.32-1.38)	0.33
blood pressure (mm Hg)	systolic 119 (103-150)	116 (94-151)	0.30
	diastolic 72 (55-90)	73 (44-95)	0.80
serum creatinine (μmol/l)	89 (57-131)	77 (53-116)	0.012
HbA1c (%)	8.6 (6.0-11.2)	8.4 (5.7-12.9)	0.78
daily urinary albumin excretion (mg/day)	13 (3-632)	11 (2-595)	0.12
retinopathy (%; no/background/proliferative)	58/21/21	47/31/22	0.74
fasting blood glucose (mmol/l)	11.5 (3.8-22.2)	10.2 (4.2-24.8)	0.63
fasting growth hormone (mU/l)	4.4 (0.2-180.8)	9.6 (1.2-98.0)	0.028
IGF-I (nmol/l)	16.0 (8.0-31.0)	17.5 (7.0-31.0)	0.18

Values are the median and (range); retinopathy is expressed as percentage of patients

<sup>1</sup> Mann-Whitney-U test, except for retinopathy (Chi-square test)

Anthropometric characteristics and baseline glucose, GH and IGF-I concentrations of patients and controls are shown in Table 2. Fasting GH concentrations tended to be higher in patients with diabetes than in controls, but the difference did not reach statistical significance. Inter-individual variations, however, were very large in both groups (Table 2).

**Table 2. Baseline variables of the study population**

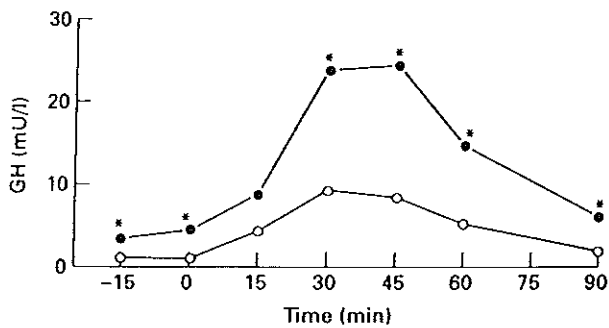
	IDDM patients n = 77	Controls n = 46	p-value
sex (male/female)	44/33	21/25	0.21 <sup>1</sup>
age (years)	32.2 (17-64)	35.0 (19-64)	0.46
body mass index (kg/m <sup>2</sup> )	23.9 (17.0-32.9)	24.9 (19.0-32.9)	0.11
fasting blood glucose (mmol/l)	10.9 (3.8-24.8)	4.5 (3.7-6.4)	<0.0001
fasting growth hormone (mU/l)	8.0 (0.2-180.8)	3.0 (0.2-53.4)	0.07
IGF-I (nmol/l)	17.0 (7.0-31.0)	24.6 (12.1-51.3)	<0.0001

Values are the median and (range)

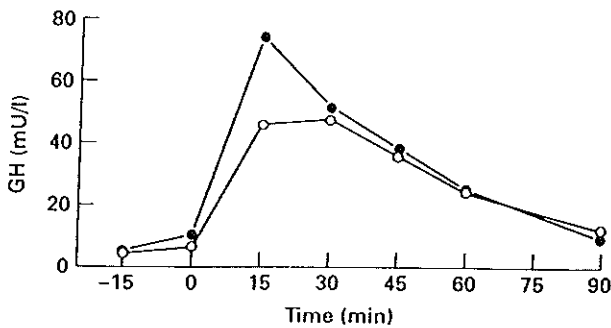
<sup>1</sup> From Chi-square test; other p-values are from Mann-Whitney-U test

Median basal and stimulated GH concentrations during the clonidine and GHRH tests are shown in Figures 1 and 2, respectively. Patients with diabetes had significantly higher basal ( $p < 0.05$ ) and clonidine stimulated GH concentrations than controls ( $p < 0.01$ , except  $t = 15$  min after injection). The differences in GH concentrations between patients and controls did not reach statistical significance when GHRH was administered, although peak concentrations of GH were much higher after stimulation by GHRH compared with stimulation by clonidine.

As age, BMI and sex are related to GH concentrations, we corrected for the effect of these potential confounding variables by multiple linear regression analysis in diabetics and controls. As shown in Table 3, after correction for these variables all differences in basal and stimulated GH concentrations between patients and controls disappeared. Thus, in the clonidine group, the slightly but non-significantly lower age and BMI in patients compared with controls explained entirely their higher basal and stimulated values displayed in Figure 1.



**Fig. 1** Growth hormone responses after the administration of clonidine. Values are the median. \*Statistical significance ( $P < 0.05$ ) between ●, patients and ○, controls.



**Fig. 2** Growth hormone responses after the administration of GHRH. Values are the median. ●, Patients; ○, controls.

We confirmed that basal and stimulated GH concentrations were negatively correlated with glucose concentrations in controls. In patients with diabetes these correlations were absent or positive (Table 4). Compared with controls, patients with diabetes had significantly lower concentrations of IGF-I, also after correction for age, BMI, sex and glucose concentration. We found a significantly negative correlation between IGF-I and glucose concentrations in controls but not in patients (Table 4).

**Table 3. Differences in growth hormone response between patients with diabetes and controls**

	uncorrected difference between pa- tients and controls <sup>1</sup>	corrected difference between pa- tients and controls <sup>2</sup>	effect of BMI <sup>3</sup>	effect of age <sup>3</sup>	effect of sex <sup>3</sup>
<b>CLONIDINE TEST</b>					
log (basal GH)	0.83 (0.03)	-0.63 (0.08)	-0.08 (0.10)	-0.03 (0.006)	1.49 (0.001)
log (peak GH)	0.64 (0.02)	-0.28 (0.23)	-0.07 (0.03)	-0.04 (0.001)	0.14 (0.49)
log (AUC GH)	0.93 (0.004)	-0.40 (0.16)	-0.13 (0.002)	-0.05 (0.001)	-0.10 (0.71)
<b>GHRH TEST</b>					
log (basal GH)	0.39 (0.37)	-0.51 (0.17)	-0.08 (0.19)	-0.06 (0.001)	1.21 (0.001)
log (peak GH)	0.21 (0.30)	-0.14 (0.49)	-0.09 (0.02)	-0.03 (0.001)	0.10 (0.61)
log (AUC GH)	0.15 (0.52)	-0.10 (0.62)	-0.09 (0.02)	-0.03 (0.001)	0.07 (0.71)

Data are expressed as the natural logarithmic values of basal GH, peak GH levels and area under the curve (AUC) of GH, after the administration of clonidine and GHRH, respectively.

<sup>1</sup> Statistics by t-test after log-transformation of data; values are differences between patients and controls (p-values in parentheses).

<sup>2</sup> Corrected for age, body mass index (BMI) and sex by multiple regression analysis.

<sup>3</sup> Multiple regression coefficients of the dependent variables (p-values in parentheses).

**Table 4. Spearman rank correlation coefficients between growth hormone and glucose levels in patients with diabetes and healthy controls.**

	patients	controls	p-value
fasting glucose with fasting GH	0.12 (0.3)	-0.37 (0.01)	<0.05
peak glucose with peak GH after GHRH	0.50 (0.001)	-0.47 (0.04)	<0.05
peak glucose with peak GH after clonidine	0.07 (0.6)	-0.16 (0.5)	NS
fasting glucose with fasting IGF-I	-0.14 (0.3)	-0.41 (0.004)	NS

Values are Spearman rank correlation coefficients; p-values in parentheses



Correlations between several clinical characteristics of the patients and IGF-I and peak GH concentrations are shown in Table 5. Urinary creatinine clearance was positively correlated and degree of retinopathy was negatively correlated with peak GH concentrations.

**Table 5. Spearman rank correlation coefficients between peak growth hormone, IGF-I and clinical variables in patients with type I diabetes.**

	peak GH after GHRH	peak GH after clonidine	IGF-I
age	-0.52 <sup>c</sup>	-0.56 <sup>c</sup>	-0.41 <sup>b</sup>
age at diagnosis	-0.45 <sup>c</sup>	-0.45 <sup>c</sup>	-0.27
body mass index	-0.48 <sup>c</sup>	-0.28 <sup>a</sup>	-0.04
HbA1c	0.21	0.15	-0.13
urinary creatinine clearance	0.23	0.39 <sup>c</sup>	0.30 <sup>a</sup>
albumin excretion per day	-0.07	-0.21	-0.09
serum creatinine	0.11	-0.04	-0.07
systolic bloodpressure	0.16	-0.10	-0.02
retinopathy	-0.39 <sup>b</sup>	-0.29 <sup>a</sup>	-0.16

<sup>a</sup>  $p < 0.05$ , <sup>b</sup>  $p < 0.01$ , <sup>c</sup>  $p < 0.001$

Glucose concentrations did not change significantly during the tests neither in patients nor in controls (maximal increment in patients 0.4, in controls 0.7 mmol/l).

## Discussion

In this study of patients with moderately controlled type I diabetes and healthy controls we demonstrated that age, BMI and sex are serious confounders in interpreting fasting and stimulated GH concentrations. Even slight, non-significant differences between mean age, BMI or sex of the study groups can cause misinterpretation of GH results. The exaggerated GH concentrations reported by others [1,6-10] might have been found because these confounders were not taken into account.

It is well known that increasing age is associated with lower basal and stimulated GH concentrations [16,17]. With respect to BMI, it has been

shown that obesity is often accompanied by reduced insulin sensitivity and concomitant hyperinsulinaemia. In rats, plasma insulin can directly inhibit GH secretion [22]. Also, altered interaction between endogenous GHRH and somatostatin might explain obesity related GH decrease [12]. Reports about the influence of sex or sex hormones on GH secretion are not conclusive [1,23,24].

We used a GHRH and a clonidine test to assess different pathways of GH secretion [19,20], although a GHRH mediated effect of clonidine can not be excluded [24,25]. Compared to the clonidine test, GH-responses after the administration of GHRH were much higher in both patients and controls but after correction for age, BMI and sex no significant differences in GH concentrations between patients and controls could be found with either test.

However, we argue that 'normal' GH concentrations during the tests must be considered inappropriately high for these patients with moderate diabetes regulation, since their hyperglycaemia was unable to suppress GH secretion adequately. This is in agreement with studies in patients with poor metabolic control, who have raised concentrations of basal GH and/or an exaggerated GH response to provocative stimuli [1,6-10]. In healthy persons administration of glucose is able to suppress GH secretion [4,5]. We also found that GH and glucose were negatively correlated in healthy controls. The absence of a negative correlation between glucose and GH concentrations in patients with diabetes in our and other studies [26], suggests a defective feedback mechanism by glucose on GH secretion in patients with IDDM.

Reduced negative feedback on GH secretion by decreased IGF-I availability may offer another explanation for the enhanced GH levels. The low plasma IGF-I concentrations that we and others [27,28] have found might be the result of defective hepatic IGF-I production, probably because of cellular starvation as is seen in other diseases with elevated GH and low IGF-I, e.g. malnutrition [29], anorexia nervosa [30,31] and liver cirrhosis [32]. Also, differences in IGF-binding proteins (IGFBPs) might be responsible for variations in bioavailability of plasma IGF-I [33,34]. It is known that plasma insulin has an inverse relation with IGFBP-1. Elevated IGFBP-1 has been demonstrated in the plasma of patients with IDDM [35], which may hinder the IGF-I bioactivity and hence its feedback action on GH secretion.

There is general agreement on the causal role of elevated GH concentrations for the development of diabetic long-term complications, especially retinopathy [36-39] and nephropathy [40,41]. Because we found that GH is secreted inappropriately, and elevated GH concentrations during periods of lower blood glucose levels cannot be excluded, we examined the

possible relation between GH and long-term complications of diabetes mellitus. We found a negative correlation between the degree of retinopathy and peak GH concentrations, which is in agreement with some [8] but not all authors [36,38,42]. In our patients, only urinary creatinine clearance showed a positive correlation with GH and IGF-I concentrations. Although urinary creatinine clearance is not a very reliable method to assess renal function, we and others suggest a causal relation between high GH concentrations and renal hyperfiltration [43-45].

Summarizing, we found that age, BMI and sex are confounders in determining GH secretion. We demonstrated low IGF-I and normal GH concentrations during stimulation tests in patients with moderately controlled diabetes compared with healthy controls, after correction for these confounders. However, we conclude that these 'normal' GH concentrations must be considered inappropriately high, in view of the elevated glucose concentrations in these patients.

#### **Acknowledgements**

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### 3.3 Growth hormone in experimental diabetes

#### 3.3.1 Early effects of streptozotocin-induced diabetes on IGF-I in the kidney of GH-transgenic and dwarf mice.

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*Exp Nephrol (in press)*

## Summary

**Background.** The renal growth and hyperfiltration observed in humans and animals with early diabetes might be dependent on growth hormone (GH) and insulin-like growth factor (IGF)-I. The aim of this study was to investigate the early changes in kidney IGF-I in experimental diabetes in mice transgenic for bovine GH and in genetically GH-deficient Ames-dwarf mice.

**Methods.** At 2, 4 and 8 days after a single intraperitoneal injection with streptozotocin, animals were weighed, bled and killed; plasma was analyzed for glucose and IGF-I. IGF-I levels were determined in tissue from snap frozen kidney and liver.

**Results.** Body weight decreased significantly after the induction of diabetes. Kidney weight increased significantly in GH- transgenic, but not in normal or dwarf mice. Plasma IGF-I was significantly decreased at day 2 in transgenic and normal mice, while liver IGF-I was increased at day 4 in all mice. Kidney IGF-I increased significantly in normal and GH-transgenic mice and was increased more than 3-fold at day 4 in GH-transgenic mice. In dwarf mice, no kidney IGF-I was detectable.

**Conclusion.** The diabetes-induced increase in renal IGF-I is dependent on the presence of GH. GH deficiency may protect diabetic animals from early changes in the kidney.



## **Introduction**

There is increasing evidence that growth hormone (GH) and its mediator insulin-like growth factor-I (IGF-I) contribute to some of the long-term complications of insulin-dependent diabetes mellitus (IDDM) [1-4]. Elevated plasma concentrations of GH have been demonstrated in diabetic patients [5,6]. We have shown that renal hyperfiltration, which is probably the earliest symptom in the development of diabetic nephropathy, is correlated with supranormal concentrations of GH [6]. Renal enlargement and hyperfiltration develop soon after the diagnosis of spontaneous or experimental diabetes [7-9]. Prior to this renal enlargement, accumulation of IGF-I in the kidney of diabetic rats has been demonstrated and the amount of kidney IGF-I was related to the severity of hyperglycaemia [10]. These observations suggest that hyperglycaemia is able to stimulate the accumulation of IGF-I in the kidney, and that IGF-I may be the most important factor in explaining kidney growth. The initial increase in kidney IGF-I and kidney growth can be prevented by the administration of either insulin or somatostatin [9,11,12]. The effects of the latter treatment support an important role of IGF-I.

Long-term experimental diabetes in mice and rats eventually leads to renal lesions comparable to human glomerulosclerosis [13]. Both diabetic and non-diabetic transgenic mice with excessive GH-production also develop glomerulosclerosis [13-16]. Remarkably, diabetic mice that express a GH-antagonist which interferes with GH action, do not develop renal lesions [13].

To further investigate the importance of GH and IGF-I in mediating the acute effects of diabetes on the kidney, we used animal models that are novel for these studies. We induced insulin-dependent diabetes in transgenic mice that express large amounts of bovine (b) GH, and in genetically GH-deficient Ames-dwarf mice. These dwarf mice have non-detectable plasma GH and IGF-I levels [17,18]. This is a congenital condition, i.e. it exists throughout their entire life span and develops without any treatment.

## **Material and methods**

### *Animals*

Transgenic mice expressing bGH (2-4½ months of age) and Ames-dwarf mice (4-9 months of age) were used along with their littermate non-mutated controls. Only adult male animals were used in this study. The GH-transgenic animals were derived from a single male founder produced by microinjection of the rat phosphoenolpyruvate carboxykinase (PEPCK)

promoter region (300 bp) fused with the bGH gene, into the male pronucleus of a single-cell embryo, as described previously [19,20]. A 25-copy bGH strain was used in this study. The line is propagated by matings of transgene-bearing males with C57Bl/6J x C3H/J F1 hybrid females. Plasma GH levels are more than 10 times those of normal mice [21]. The Ames-dwarf mice were derived from a closed random-bred colony. Ames-dwarfs are deficient in GH, prolactin and thyrotropin [17,22]. No thyroxine substitution is given, because the animals are in perfectly good health. Moreover, their life span is considerably extended over that of non-dwarf mice (Brown-Borg, Borg & Bartke, unpublished observation), which is worth emphasizing since kidney pathology is a common cause of death in aging normal mice.

The animals were kept according to the principles of laboratory animal care. They were housed in groups of 2-5 mice per cage since weaning (dwarf mice at 40-60 days, non-mutated littermates and GH-transgenic mice at 21 days) and were kept in a temperature-controlled (22 °C) room on a 12-h light/12-h dark cycle. They were fed a standard laboratory rodent diet (Formulab 5008, containing 23% protein and 6.5% fat. PMI Feeds, St.Louis, MO, USA) ad libitum with free access to tap water. The protocol was approved by the animal care committee.

#### *Streptozotocin treatment*

Because acute induction of insulin-dependent diabetes mellitus with a single intraperitoneal (i.p.) injection of Streptozotocin (STZ) is less predictable in mice than in rats [23-25], we performed a pilot study to find the appropriate dose of STZ for each category of mice. On the basis of the results of this study, we have selected the following doses for a single i.p. injection of STZ, dissolved in citrate buffer (pH 4.5): Ames-dwarf and Ames-normal mice: 300 mg/kg; PEPCK-bGH-transgenic mice: 350 mg/kg; PEPCK-normal mice: 250 mg/kg. All injections were given between 8:00 and 10:00 a.m. Mice that did not respond with hyperglycaemia were excluded from the analysis. Glucose levels above 11.1 mmol/l were considered as diabetic. In many Ames-normal mice basal glucose concentrations were already in the diabetic range; therefore we chose a glucose concentration above 16 mmol/l as diabetic in this group. Non-diabetic control mice were studied after administration of a corresponding amount of citrate buffer solution.

The animals were handled daily to record body weight and health. Non-diabetic control mice were killed at day 0 and day 8. At 2, 4 and 8 days after the injection of STZ, 5-12 animals per group were bled by cardiac puncture under ether anesthesia and killed by cervical dislocation. Plasma was

separated by centrifugation and was stored at -20 °C until further assay. Kidneys and liver were removed, weighed and snap frozen in liquid nitrogen and stored at -70 °C until further assay.

In an additional experiment diabetic mice were tested daily for ketonuria to exclude extreme catabolism.

#### *Laboratory methods*

Glucose was measured in plasma by a glucose oxidase/peroxidase reaction with photometric determination of absorption at 450 nm (Sigma kit No. 510, Sigma diagnostics, St.Louis, MO, USA). Plasma IGF-I was measured after extraction of its binding proteins by a combined technique of formic acid/acetone extraction and cryoprecipitation as described before [18,26,27]. Mean recovery was 90%. IGF-I in kidney and liver was extracted with a modified procedure of Lee *et al.* [28]. Tissues were homogenized on ice with 4 volumes of a freshly made solution of 0.5% Tween-20 and formic acid (3.3 mmol/l). After centrifugation at 12000 rpm for 30 min, 150  $\mu$ l of the supernatant were heated at 90 °C for 30 min, after which 350  $\mu$ l acetone were added. After centrifugation for another 30 min the supernatant was neutralized with Tris-base 20% and placed in -20 °C for cryoprecipitation. To the remaining supernatant 3 volumes of IGF-RIA buffer (20 mg protamine sulphate in 100 ml phosphate buffer solution (PBS), pH 7.5, with 50  $\mu$ l Tween-20) were added; the solution was stored at -20 °C until the RIA. Recovery was 82%. The plasma, kidney and liver IGF-I levels were measured by a validated RIA as described in our previous publication [18]. Briefly, human (h) IGF-I was used as a reference preparation and used to prepare the (<sup>125</sup>I)-iodinated trace. Antiserum prepared against hIGF-I (developed by Drs. L.E. Underwood and J.J. van Wyk, University of North Carolina at Chapel Hill, NC and obtained from The Pituitary Hormone Distribution Program, NIH, Baltimore, MD) was utilized in this RIA. The sensitivity of this assay was 32 pg/tube. All samples (plasma or kidney or liver extracts) were included in the same assay to avoid interassay variability. The mean intraassay coefficient of variation was 3.6%.

Ketonuria was measured using Chemstrip 10 with SG Urine Test Strips (Boehringer-Mannheim).

#### *Statistical analysis*

Since the age of the animals varied, which had a significant independent effect on organ weights, data on body and organ weights and IGF-I concentrations were corrected for age by regression analysis. Except for

age, non-diabetic control animals on day 0 and day 8 did not differ significantly, so data of these animals are taken together as 'baseline values'. Baseline values in the Table are expressed as mean  $\pm$  SEM. Differences between transgenic/dwarf mice and their respective non-mutated controls at baseline were analyzed by Wilcoxon's Mann-Whitney-U test.

The data in the figures are displayed as mean (SEM) changes from baseline, and were analyzed by ANOVA. Pearson's correlation coefficients were calculated for individual variables. Linear regression analysis was performed to assess independent effects of blood glucose, age, plasma and liver IGF-I, and kidney weight on the renal IGF-I concentration. All statistical tests were two-tailed. Differences were considered statistically significant with  $p < 0.05$ .

## Results

In Table 1, baseline data for non-diabetic animals are shown. PEPCK-bGH-transgenic animals were 1½ times heavier than their non-mutated littermate controls ( $p < 0.001$ ) while dwarfs were only 1/3 of the weight of their non-mutated littermate controls ( $p < 0.001$ ). IGF-I concentrations were practically non-detectable in dwarf mice, while GH-transgenic animals had IGF-I concentrations more than twice as high as non-mutated animals.

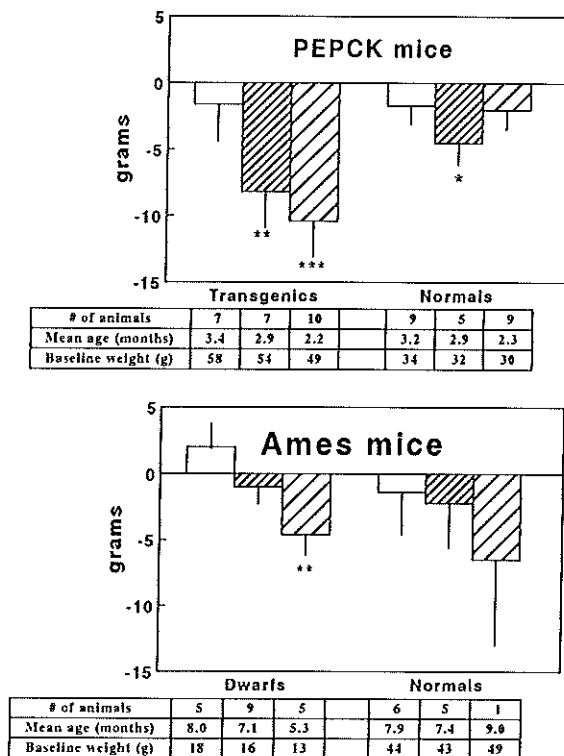
**Table 1. Non-diabetic data of PEPCK and Ames mice**

	PEPCK		Ames	
	GH-transge- nics n = 12	Non-mutated normals n = 11	Dwarfs n = 9	Non-mutated normals n = 10
body weight (g)	52 $\pm$ 2 <sup>a</sup>	33 $\pm$ 2	14 $\pm$ 1 <sup>a</sup>	43 $\pm$ 3
liver weight (g)	4.5 $\pm$ 0.2 <sup>a</sup>	1.5 $\pm$ 0.06	0.6 $\pm$ 0.02 <sup>a</sup>	2.3 $\pm$ 0.2
kidney weight (g)	0.42 $\pm$ 0.03 <sup>a</sup>	0.23 $\pm$ 0.01	0.09 $\pm$ 0.00 <sup>a</sup>	0.30 $\pm$ 0.02
plasma IGF-I (ng/ml)	543 $\pm$ 34 <sup>a</sup>	254 $\pm$ 16	0 <sup>a</sup>	252 $\pm$ 13
liver IGF-I (ng/g liver)	578 $\pm$ 33 <sup>a</sup>	260 $\pm$ 31	0 <sup>a</sup>	133 $\pm$ 23
kidney IGF-I (ng/g kidney)	483 $\pm$ 24 <sup>a</sup>	184 $\pm$ 33	0.09 $\pm$ 0.00 <sup>a</sup>	177 $\pm$ 13

<sup>a</sup>  $p < 0.001$  vs. non-mutated normal littermates

In the diabetic mice, mean glucose concentrations were  $23 \pm 5$  mmol/l (range 12-34 mmol/l). At day 8, only one Ames-normal mouse had reached our criteria for diabetes; all other groups consisted of 5-12 mice. Insulin concentrations were reduced to approximately 10%-20% of normal concentrations (data not shown).

In Figure 1 the age-corrected changes in body weight of PEPCK and Ames mice have been plotted. After 8 days of diabetes, body weight was significantly decreased in GH-transgenic and dwarf mice ( $p < 0.001$ ).



**Fig. 1.** Age-corrected changes in body weight of transgenic PEPCK-bGH mice, Ames-dwarf mice, and the respective normal non-mutated littermate controls.

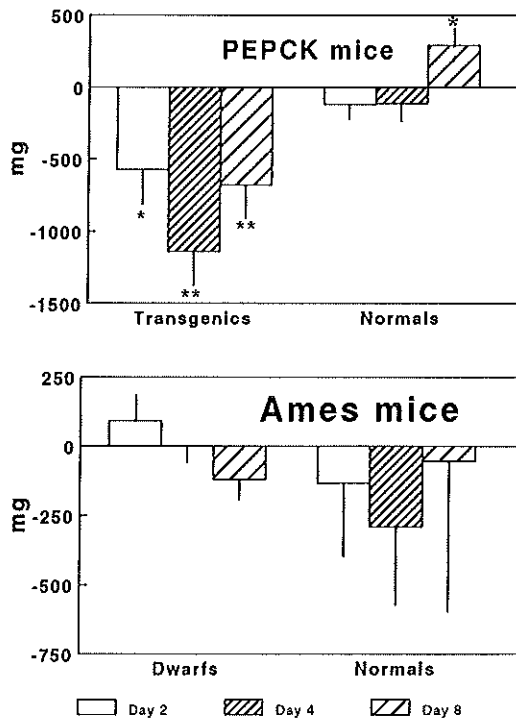
Bars represent mean (SEM) changes from non-diabetic baseline data given in Table 1.

Open bars: 2 days, fine-hatched bars: 4 days, wide-hatched bars: 8 days after the injection of streptozotocin. Statistically significant differences from baseline are indicated by asterisks

(\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ).

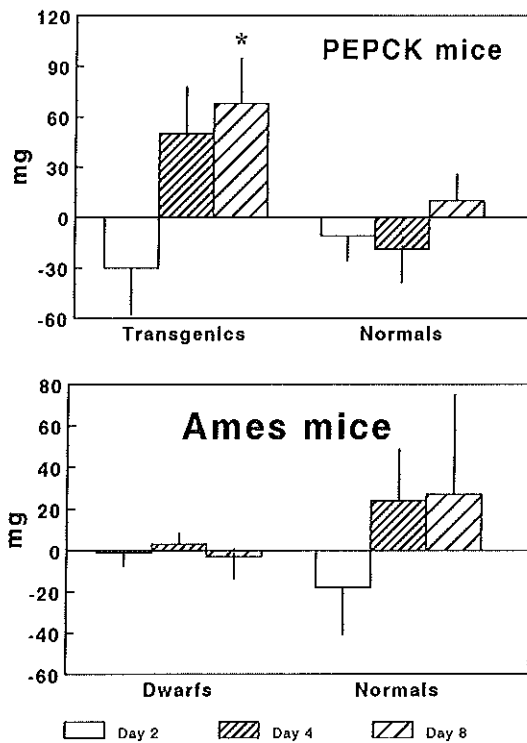
There was a significant correlation between the level of plasma glucose and the decrease in body weight ( $r = -0.65$ ;  $p < 0.001$ ). In dwarf mice the relative decrease in body weight was most striking (35%).

Diabetes also induced a decrease in liver weight, as shown in Figure 2, but the changes were only significant in PEPCK-bGH-transgenic mice ( $p < 0.01$ ). PEPCK-normal mice had increased liver weight at day 8 ( $p < 0.05$ ).



**Fig. 2.** Age-corrected changes in liver weight of transgenic PEPCK-bGH mice, Ames-dwarf mice, and the respective normal non-mutated littermate controls. See Fig.1 for explanation of the symbols.

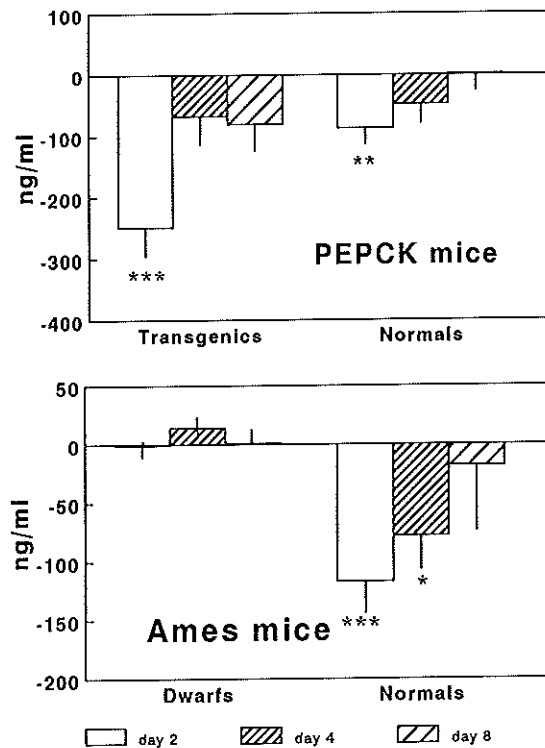
Age-corrected changes in kidney weight are shown in Figure 3. No significant change in kidney weight was seen after STZ injection in normal and dwarf mice. GH-transgenic mice, however, had significantly increased kidney weight at 8 days after STZ injection ( $p < 0.02$ ).



**Fig. 3.** Age-corrected changes in kidney weight of transgenic PEPCK-bGH mice, Ames-dwarf mice, and the respective normal non-mutated littermate controls.

See Fig.1 for explanation of the symbols.

As shown in Figure 4, plasma IGF-I concentration decreased significantly 2 days after the onset of diabetes ( $p < 0.01$ ). By day 8, the plasma IGF-I concentration no longer differed from baseline. Plasma IGF-I level was negatively correlated with blood glucose level, but this relation reached significance only in Ames-normal mice ( $r = -0.59$ ;  $p < 0.01$ ). In Ames-dwarf mice, plasma IGF-I was not detectable except at day 4.

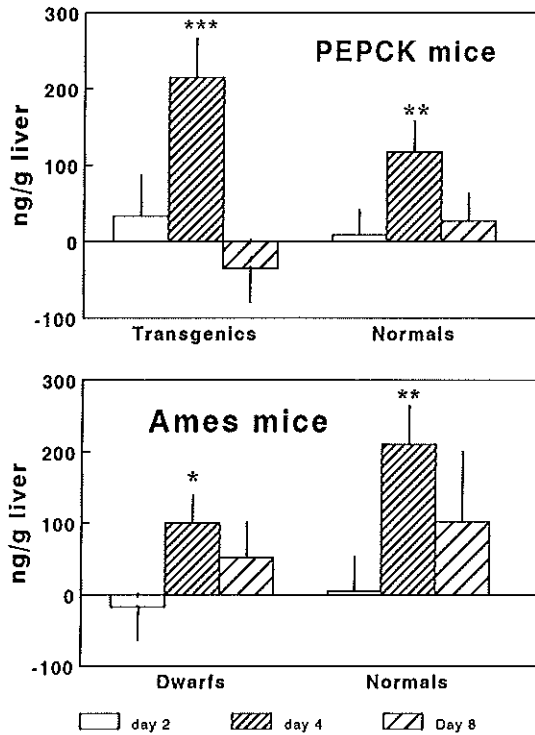


**Fig. 4. Age-corrected changes in plasma IGF-I concentration in transgenic PEPCK-bGH mice, Ames-dwarf mice, and the respective normal non-mutated littermate controls.**

See Fig.1 for explanation of the symbols.

As shown in Figure 5, an increased liver concentration of IGF-I was measured after 4 days of diabetes in all categories of animals compared to non-diabetic controls ( $p < 0.01$  for GH-transgenics and both normal groups, respectively;  $p < 0.05$  for dwarf mice). Total liver IGF-I content increased significantly in Ames-normal and Ames-dwarf mice, remained stable in PEPCK-normal mice, and decreased in PEPCK-bGH-transgenic mice. Liver IGF-I concentration was positively correlated with plasma glucose levels, but only in Ames-normal mice this was statistically significant ( $r = 0.52$ ;  $p < 0.02$ ).

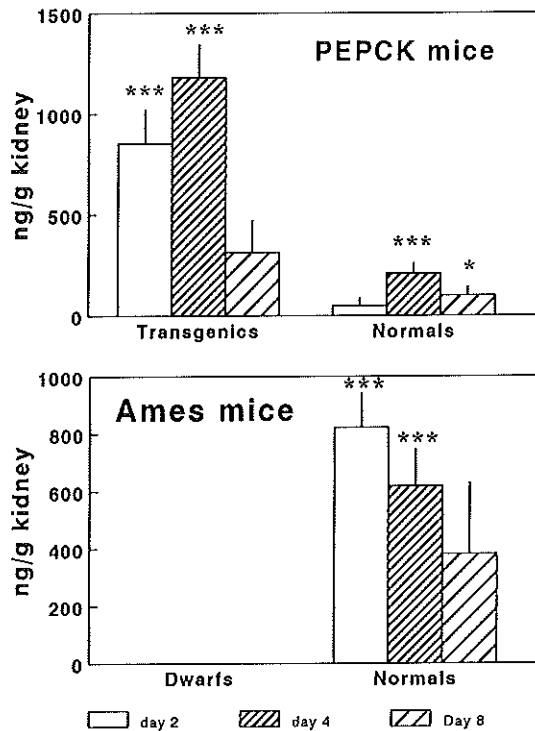




**Fig. 5.** Age-corrected changes in liver IGF-I concentration in transgenic PEPCK-bGH mice, Ames-dwarf mice, and the respective normal non-mutated littermate controls.

See Fig.1 for explanation of the symbols.

Figure 6 demonstrates the changes in kidney IGF-I concentration. Dwarf mice had non-detectable renal IGF-I concentrations, which did not change after the onset of diabetes. The other groups showed a significant increase of kidney IGF-I content after STZ injection, with the highest increase of more than 3-fold measured at day 4 in the PEPCK-bGH-transgenic mice ( $p < 0.001$  vs. day 0). In non-dwarf mice, we found a positive correlation between glucose level and kidney IGF-I content ( $r = 0.74$ ,  $r = 0.53$  and  $r = 0.49$  for Ames-normals, PEPCK-transgenics and PEPCK-normals, respectively;  $p < 0.005$ ).



**Fig. 6. Age-corrected changes in kidney IGF-I concentration in transgenic PEPCK-bGH mice, Ames-dwarf mice, and the respective normal non-mutated littermate controls.**

See Fig.1 for explanation of the symbols.

Multivariate regression analysis revealed that plasma glucose level, kidney weight and liver IGF-I content were all independent factors contributing to the kidney IGF-I concentration ( $p < 0.001$ ). With kidney weight as the dependent variable, IGF-I concentrations in plasma, liver and kidney were all positive significant contributors ( $p < 0.01$ ), while age and glucose level were not.

Ketonuria existed in 7 of 17 PEPCK-bGH-transgenic diabetic mice (41%), 2 of 16 diabetic PEPCK-normals (13%), 2 of 6 diabetic Ames-dwarfs (33%) and none of three diabetic Ames-normals.

## **Discussion**

The results of this study indicate that the increase in kidney IGF-I concentration early after the onset of experimental diabetes is a GH-dependent phenomenon. STZ-treated insulin-deficient diabetic transgenic mice expressing bovine GH showed an excessive increment in kidney IGF-I concentration prior to kidney growth, while Ames-dwarf mice with complete GH deficiency had no detectable IGF-I in the kidney and no kidney growth. The induction of diabetes in normal mice was associated with an increment of IGF-I in the kidney without significant changes in kidney weight.

There is growing evidence for the relation between GH/IGF-I and the development of kidney lesions in diabetes mellitus. It has been demonstrated that GH-transgenic mice develop a type of glomerulosclerosis comparable to diabetic glomerulosclerosis in humans [13-16,29]. Remarkably, transgenic mice expressing high plasma levels of IGF-I do not develop renal lesions [14,15]. IGF-I transgenic mice have very low plasma GH levels [14], which may prevent local IGF-I synthesis. Therefore, elevated local but not systemic IGF-I concentrations appear to be the determinant factors in the induction of diabetic nephropathy.

The cause of the increased kidney IGF-I concentration in diabetes is not known. Local production of IGF-I in the kidney has been suggested by the demonstration of increased IGF-I mRNA levels after the induction of diabetes in rats [30,31]. However, this observation could not be confirmed by others [32]. Entrapment of plasma IGF-I by binding proteins in the kidney is a more likely explanation for the increase in renal IGF-I. IGF-I receptors in the kidney are present [33] and increased expression of IGF binding proteins in the kidneys of diabetic rats has been demonstrated [34-36]. Direct stimulation by GH is unlikely, since STZ-diabetic mice have suppressed GH secretion [37]. Nutritional factors, insulin or other GH-independent factors may play a role in the local IGF-I accumulation [38,39].

The low plasma IGF-I levels invariably found in diabetic states, in humans as well as in animals [this study, 6,7,13], might also be explained by the increased renal uptake. Alternatively, deficient protein synthesis in the liver may be responsible for the decrease in plasma IGF-I. This may be due to cellular malnutrition [40-42], or to the decreased plasma GH-concentrations in

diabetic rodents [37], since the liver is the major producer of IGF-I in a GH-dependent fashion [43]. The finding of decreased IGF-I mRNA concentrations in the diabetic rat liver supports this hypothesis [38,44,45]. However, although liver weights tended to decrease, IGF-I protein concentrations per unit liver weight in our study were higher in diabetic mice compared to non-diabetic control mice. Increased translation efficiency, changes in half-life of either the messenger or the protein, reduced protein secretion, or uptake from the blood may explain the discrepancy between low gene transcription and high protein concentration, which has been described before [43].

The diabetes-induced increase in kidney IGF-I concentration and renal growth in mice are in accordance with the findings in rats [9,10,13,32,46-48]. However, not all our mice had increased kidney weights. Ectopic renal expression of the aberrant GH [14,19] might be an explanation for the selective renal growth in our GH-transgenic mice. However, all morphologic and physiologic effects of other GH-transgenes are the same, and similar to endogenous GH-overproduction [14,19]. This argues against a direct role of renal GH as the cause of kidney growth in diabetic mice. The lack of renal growth in the normal mice is surprising, since normal rats have kidney growth after the induction of diabetes [9,10,13,32]. The difference may be due to the different species we used. Two other studies on diabetic mice did not mention kidney growth after the induction of diabetes, although glomerular volume increased in non-transgenic mice, but not in GH-transgenic mice or in dwarf mice [13,49]. Our normal mice may have had glomerular growth as well. Alternatively, some degree of catabolism as a cause for the lack of renal growth cannot be excluded, since body weights decreased after the induction of hyperglycaemia, and ketonuria was found in some animals. However, the lack of renal growth can not be due to severe catabolism, since ketonuria was most prevalent in GH-transgenic animals that did have renal growth. Also, production of IGF-I in the liver increased in all mice after the induction of diabetes, which would have been unlikely in catabolic mice. Besides, glucose levels in our study were comparable to or even lower than those in other studies [12,46], where increased kidney weight in diabetic rats was reported. Apparently, for renal growth to occur, a certain balance between plasma glucose level, plasma GH or IGF-I, and kidney IGF-I concentration has to be achieved. Due to the small blood amounts available in mice, we could not measure plasma GH concentrations, so acute changes in the GH/IGF-I ratio could not be determined. However, the complete absence of kidney IGF-I might explain the lack of kidney growth in our diabetic dwarf mice, although other factors cannot be excluded. Administration of GH might have stimulated

kidney growth in these dwarf animals. Although we did not perform this experiment, Flyvbjerg *et al.* [50] have demonstrated that administration of hGH increased kidney weight in diabetic dwarf rats compared to their non-treated controls.

Suppression of GH by somatostatin administration reduced kidney IGF-I concentration and kidney growth in diabetic rats [11,12,47], although Muntzel *et al.* [8] did not find a suppressive effect of somatostatin on growth of diabetic rat kidneys. In our study, induction of diabetes in normal mice increased kidney IGF-I concentrations to the high levels found in GH-transgenic mice at baseline. Thus, diabetes might indirectly cause renal lesions via stimulation of the IGF-I content of the kidney. Increased IGF-I could induce glomerulosclerosis by persistent hyperfiltration and especially increased glomerular capillary pressure, which causes endothelial damage and eventually sclerosis [51,52]. The fact that diabetic dwarf mice seem to be protected from renal lesions, as has been demonstrated in long-term experiments [13,49], is an important argument in favour of the causal role of GH and IGF-I in inducing these pathological changes. In our short-term study, we provide evidence that this protection may be caused by complete lack of early local IGF-I accumulation in the kidney after the induction of diabetes.

We conclude from our data that the diabetes-induced increase in renal IGF-I is dependent on the presence of GH. GH deficiency may protect diabetic animals from early changes in the kidney. Furthermore, we speculate that entrapment of IGF-I in the kidney and not deficient IGF-I synthesis in the liver is the cause of low plasma concentrations of IGF-I in diabetes.

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3.3.2 Insulin-like growth factor (IGF)-I and IGF binding protein mRNA expression in the kidneys of normal, growth hormone (GH) transgenic, and GH-deficient diabetic mice.

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*Submitted*

## Summary

Growth hormone (GH) and its mediator insulin-like growth factor (IGF)-I are suggested to play a role in the development of diabetic nephropathy. We investigated whether early diabetic kidney growth and kidney IGF-I accumulation are due to local production of IGF-I or IGF binding proteins (IGFBPs), and whether these events are GH dependent. Therefore, we determined the *in situ* mRNA expression of the IGF and IGFBP probes in the kidneys of normal mice, and of GH-transgenic and GH-deficient mice, before and after the onset of streptozotocin induced diabetes. Two days after the onset of experimental diabetes, an increased expression of IGF-I mRNA was seen in the kidneys of normal, GH-transgenic and GH-deficient mice. Messenger RNA expression of IGFBP-2, IGFBP-3 and IGFBP-5 tended to increase, while IGFBP-4 mRNA decreased. In conclusion, we found increased IGF-I mRNA expression in the kidneys of diabetic mice, suggesting local IGF-I production. This expression is GH independent, while effective translation of IGF-I mRNA into protein seems to be GH dependent.

## Introduction

Renal complications are found in approximately 30% of the patients with diabetes mellitus [1]. Although strict glycaemic control is able to reduce the risk of developing diabetic nephropathy [2], other factors like growth hormone (GH) and its mediator insulin-like growth factor (IGF)-I have also been suggested to play a role in the development of this complication [3]. High serum concentrations of GH and IGF-I are associated with glomerulosclerosis [4] and ultimately renal failure [5]. To prevent this late organ damage, knowledge of the pathological process is mandatory.

Increased concentrations of IGF-I have been demonstrated in rodent kidneys after the onset of streptozotocin (STZ) induced diabetes [6-10]. Although the liver is the major site of IGF-I synthesis [11,12], local production may be the cause of the renal accumulation of IGF-I in experimental diabetes, as suggested by increased expression of IGF-I mRNA [13,14]. Alternatively or in addition, entrapment of plasma IGF-I by increased expression of IGF binding proteins (IGFBPs) may also play a role [15-17]. IGFBPs are considered to modulate IGF-I bioavailability and activity in the serum and at tissue level [12,18,19]. The precise role of GH on the accumulation of IGF-I in kidneys of diabetic mice has not yet been elucidated. We previously demonstrated that GH-transgenic mice had increased kidney IGF-I concentrations and renal growth after the onset of STZ induced diabetes, while GH-deficient dwarf mice had no detectable IGF-I in the kidneys and no renal growth [10].

The aim of the present study is to investigate the possible origin of the increased kidney IGF-I in diabetic mice, and the influence of GH in this process. To address this issue, we determined the effects of STZ induced diabetes on the mRNA expression of IGF-I and its binding proteins in the kidneys of normal mice, and of GH-transgenic and GH-deficient dwarf mice.

## Material and Methods

### *Study design*

Adult male transgenic mice expressing bovine GH (bGH) and Ames-dwarf mice were used along with their littermate controls. The transgenic animals were derived from a single male founder produced by microinjection of the rat phosphoenolpyruvate carboxykinase (PEPCK) promoter region (300 bp) fused with the bGH gene, into the male pronucleus of a single-cell embryo, as described previously [20,21]. A 25-copy bGH strain was used in this study. The line is propagated by matings of transgene-bearing males with

C57Bl/6J x C3H/J F1 hybrid females. Plasma bGH levels are more than 10 times those of normal mice [22]. The Ames-dwarf mice were derived from a closed random-bred colony. They have non-detectable plasma GH and IGF-I levels [23]. The GH-deficient state is a congenital condition, i.e. it exists throughout their entire life span and develops without any treatment.

The animals were kept according to the principles of laboratory animal care. They were housed in groups of 2-5 mice per cage since weaning and were kept in a temperature-controlled (22 °C) room on a 12-h light/12-h dark cycle. They were fed a standard laboratory rodent diet (Formulab 5008, containing 23% protein and 6.5% fat. PMI Feeds, St.Louis, MO, USA) *ad libitum* with free access to tap water. The protocol was approved by the animal care committee.

We induced diabetes mellitus by a single i.p. injection of STZ in citrate buffer (pH 4.5), as previously described [10]. The optimal doses of STZ to achieve blood glucose levels of 15-25 mmol/l were determined in a pilot study, and were as follows: Ames-dwarf and Ames-normal mice 300 mg/kg body wt; PEPCK-bGH-transgenic mice 350 mg/kg; PEPCK-normal mice 250 mg/kg. All injections were given between 8:00 and 10:00 a.m. Mice that did not respond with hyperglycaemia were excluded from the analysis. Glucose levels above 11.1 mmol/l were considered as diabetic. In approximately 70% of the Ames-normal mice basal glucose concentrations were already above 11 mmol/l (mean  $\pm$  SD:  $13.8 \pm 2.7$  mmol/l); therefore we chose a glucose concentration above 16 mmol/l as diabetic in this group. Control mice were given the corresponding amount of citrate buffer solution.

The animals were handled daily to record body weight and health. Non-diabetic control mice were killed at day 0 and day 8. At 2, 4 and 8 days after the injection of STZ, 4-12 animals per group were bled by cardiac puncture under ether anesthesia and killed by cervical dislocation. The left kidney was removed, decapsulated, weighed, fixed in formaldehyde and embedded in paraffine according to standard procedures. Sections of 4  $\mu$ m were cut on a microtome and mounted on 3-aminopropyl trioxysilane-coated slides, and dried at 37 °C for 3 days. For each group of mice, the kidneys of two representative mice on each day of the study were used for *in situ* hybridization with mRNA probes. At least two slides per mouse were studied.

### *Probe preparation*

The IGFBP-2 to IGFBP-6 cRNA probes were transcribed from templates described by Schuller et al. [24]. As template for the IGFBP-1 cRNA probe the mouse cDNA fragment Sph1-Sac1 was cloned into pTZ18R or

pTZ19R (Pharmacia, Uppsala, Sweden) for the antisense and sense probes, respectively. cDNAs encoding mouse IGF-I and IGF-II were kindly provided by Dr. G.I. Bell (Howard Hughes Medical Institute, Chicago, IL, USA). Fragments were subcloned into pTZ18 and pTZ19 (EcoR1 for IGF-I and BamH1/Sac1 for IGF-II). Digoxigenin-11-UTP (DIG) labeled RNA probes were prepared according to the manufacturer's prescription (Boehringer Mannheim GmbH, Biochemica, Mannheim, Germany) using T7 or SP6 RNA polymerase.

#### *In situ hybridization*

Sections were dewaxed, hydrated and incubated in the following solutions: 0.2 N HCL, 0.3% Triton-X 100 in PBS, 5  $\mu$ g/ml proteinase K (37 °C), 4% formalin in PBS and finally acetylated with acetic anhydride diluted in 0.1 M triethanolamine (750  $\mu$ l/200 ml). Until hybridization, sections were stored in a solution of 50% formamide in 2x SSC (1xSSC=150 mM NaCl/15 mM sodium citrate solution, pH 7.0) at 37 °C. For hybridization, probes were diluted in hybridization solution (50% deionized formamide, 10% dextran sulfate, 2x SSC, 1x Denhardt's solution, 1  $\mu$ g/ml tRNA, 250  $\mu$ g/ml hering sperm DNA) to a concentration of 100 ng/ml, incubated at 68 °C for 15 min and layered onto the sections. Sections were hybridized overnight at 55 °C in a humid chamber. Posthybridization washes were performed at 45 °C using the following steps: 50% formamide in 2x SSC, 50% formamide in 1x SSC, and 0.1x SSC. A 15 min incubation with RNase T1 (2 U/ml in 1 mM EDTA in 2x SSC) at 37 °C was followed by washes of 0.1x SSC at 45 °C and 2x SSC at room temperature. The DIG-labeled hybrids were detected by antibody incubation performed according to the manufacturer's description (Boehringer Mannheim GmbH, Biochemica, Germany) with following modifications. A 1:2000 dilution of alkaline phosphatase conjugated anti-digoxigenin (Fab) was used for a 2.5 h incubation at room temperature. Sheep serum (5%) and Triton (0.1%) were added to the diluted antibody solution in order to diminish background staining. Afterwards, the prepared were washed in 0.025% Tween in Tris-buffered saline pH 7.5. For staining, sections were layered with detection buffer (0.1 M Tris-HCl, 0.1 M NaCl, 0.05 M MgCl<sub>2</sub> pH 9.5) containing 0.33 mg/ml NBT (4-nitroblue tetrazolium chloride), 0.16 mg/ml BCIP (5-bromo-4-chloro-3-indolyl-phosphate), 7.5% PVA (polyvinylalcohol, mol wt 31,000 - 50,000, Aldrich Chemical Co., Milwaukee, WIS, USA) and 1 mM levamisole (Sigma Chemical Co., St.Louis, MO, USA). The colour reaction was performed in the dark and was stopped after 3 to 40 h incubation when the desired intensity of the resulting blue precipitate was reached.

Sections were washed in 10 mM Tris-HCl, 1 mM EDTA pH 8.0,

counterstained with PAS and Nuclear Red solution, dehydrated with ethanol gradients and mounted with an ethanol based mounting medium Euparal (Chroma-Gesellschaft, Stuttgart, Germany). Control sections for morphological analysis were stained with hematoxylin and eosin.

## Results

### EFFECT OF DIABETES IN NORMAL MICE

#### *Body and kidney weight, kidney morphology and IGF-I content*

After STZ injection, body weight decreased in relation with the degree of hyperglycaemia ( $r = -0.66$ ;  $p < 0.001$ ). No significant change in kidney weight was seen after the induction of diabetes in PEPCK-normals and Ames-normal mice. However, kidney IGF-I content increased 3-4 fold after STZ injection, both in PEPCK-normals and in Ames-normals [10].

From the fourth day after the onset of diabetes, dilation of renal tubules was seen, with accumulation of PAS-positive staining material.

#### *Kidney IGF-I and IGFBP mRNA (Table 1)*

At baseline, IGF-I mRNA was expressed in the renal tubules of all mice. Two days after the induction of diabetes, the distal tubules stained more intensely compared to non-diabetic mice, but after 4 days the expression of IGF-I mRNA decreased and was more evenly distributed between proximal and distal tubules. Expression of IGF-I mRNA was also found in the glomerular cells of diabetic mice of all groups, but not in non-diabetic controls.

IGFBP-2 mRNA was expressed in the medulla (thin limbs of Henle's loop) of non-diabetic mice, and in some glomeruli of diabetic mice. IGFBP-3 mRNA was abundant in the peritubular capillaries of the cortex in non-diabetic mice. From day 4 after the induction of diabetes, IGFBP-3 expression was also seen in the peritubular capillaries of the outer medulla. IGFBP-4 mRNA staining was diffuse in the proximal tubules of the very outer cortex, and in Bowman's capsule of healthy mice. After the induction of diabetes, we saw a persistent decrease in IGFBP-4 mRNA expression. At baseline, IGFBP-5 mRNA was prominent in the glomerular mesangial cells, in the peritubular capillaries of the medulla, and to a lesser extent in the cortical capillaries. Scattered cells of distal tubules contained IGFBP-5 mRNA as well. After the induction of diabetes, the expression of IGFBP-5 mRNA was transiently increased on day 2 and 4 in the glomeruli of normal mice. IGF-II, IGFBP-1 and IGFBP-6 mRNA was not detected in either control or diabetic mice.

**Table 1. Expression of IGF-I, IGF-II, and IGFBP mRNA in the kidneys of healthy and diabetic normal mice, GH-transgenic mice, and GH-deficient dwarf mice**

probe	location in nephron	normal mice		PEPCK-bGH-transgenic mice		Ames-dwarf mice	
		DM—	DM +	DM—	DM +	DM—	DM +
IGF-I	glomerulus	+ /—	+	+	+	+ /—	+
	tubuli/Henle <sup>a</sup>	+	++	+	++	+	++
	vascul./interst.	—	—	—	+	—	—
IGF-II	glomerulus	—	—	—	—	—	—
	tubuli/Henle	—	—	—	—	—	—
	vascul./interst.	—	—	—	—	—	—
IGFBP-1	glomerulus	—	—	—	—	—	—
	tubuli/Henle	—	—	—	—	—	—
	vascul./interst.	—	—	—	—	—	—
IGFBP-2	glomerulus	—	—	—	+	—	+ /—
	tubuli/Henle	+	+	+	++	na	+ /—
	vascul./interst.	—	—	—	—	—	—
IGFBP-3	glomerulus	—	—	—	— (ill: +)	—	—
	tubuli/Henle	—	—	—	—	—	—
	vascul./interst. <sup>b</sup>	+	+	+	+	+	+
IGFBP-4	glomerulus	+	+	+	+	—	—
	tubuli/Henle	+ /—	—	+	+ /—	+ /—	—
	vascul./interst.	—	—	—	—	—	—
IGFBP-5	glomerulus <sup>c</sup>	+	++	+	++	+	+
	tubuli/Henle	+	+	+	+	+	+
	vascul./interst. <sup>d</sup>	+	+	+	+	+	+
IGFBP-6	glomerulus	—	—	—	—	—	—
	tubuli/Henle	—	—	—	—	—	—
	vascul./interst.	—	—	—	—	—	—

— expression not detectable, + /— weak expression, + expression present, ++ strong expression

<sup>a</sup> peak on day 2; back to normal at day 8; <sup>b</sup> from day 4, medullary expression increased;

<sup>c</sup> increased expression on 2 and 4 days after the onset of diabetes, thereafter weaker expression;

<sup>d</sup> medulla ++, cortex + /— ; na: tissue not available

## EFFECT OF GROWTH HORMONE

### *Body and kidney weight, kidney morphology and IGF-I content*

As described before [10], Ames-dwarf mice had non-detectable renal IGF-I concentrations, which did not change after the onset of diabetes. No increase in kidney weight was seen after the induction of diabetes in Ames-dwarf mice. PEPCK-bGH-transgenic mice had the highest concentrations of kidney IGF-I ( $p < 0.001$  compared to all other mice), which increased more than 3-fold on day 4 after the onset of diabetes ( $p < 0.001$  vs. non-diabetic controls on day 0). Kidney weight increased significantly after the onset of diabetes in PEPCK-bGH-transgenic mice [10].

Ames-dwarf mice had smaller glomeruli and tubules compared to other mice. Diabetes induced similar changes in the tubuli of these dwarf mice compared to normal mice. Glomeruli of PEPCK-bGH-transgenic mice were larger and stained more intensely with PAS, and had thicker basal membranes and Bowman's capsules compared to other mice. Widening of tubuli started on day 2 after the onset of diabetes in GH-transgenic mice, with abundant staining of PAS positive material within the tubuli.

### *Kidney IGF-I and IGFBP mRNA (Table 1)*

Like normal mice, both PEPCK-bGH-transgenics and Ames-dwarf mice had IGF-I mRNA expression in the tubules, which increased after 2 days of hyperglycaemia. Bowman's capsule stained also positive for IGF-I mRNA in PEPCK-bGH-transgenic mice. Diabetes induced IGF-I mRNA expression in peritubular capillaries in PEPCK-bGH-transgenic mice, but not in Ames-dwarf mice.

At baseline, IGFBP-4 mRNA expression was less abundant in Ames-dwarf mice compared to GH-transgenic mice. The other probes had similar expression in all groups of mice.

After the onset of diabetes, IGFBP-2 mRNA expression in the thin limbs of Henle's loops was more abundant in GH-transgenic mice compared to other mice. GH-transgenic mice also had more expression of IGFBP-4 mRNA, since 'nests' of IGFBP-4 mRNA staining tubules were seen after 2 and 4 days of diabetes in these mice. After 8 days, the expression of IGFBP-4 mRNA had returned to a more diffuse staining pattern. Glomeruli of ill GH-transgenic animals expressed IGFBP-3 mRNA on day 4, but no other obvious differences were seen between PEPCK-bGH-transgenic mice, GH-deficient Ames-dwarf mice, and normal mice in the expression of IGFBP-3 and IGFBP-5 mRNA before or after the onset of diabetes mellitus.



## Discussion

We and others have described that IGF-I accumulates in the kidneys of rodents early after the onset of experimental diabetes mellitus [6-8,10,25]. IGF-I is considered to play an important role in the early diabetes-induced kidney growth [7,8]. It is not known whether the renal accumulation of IGF-I is due to local production or to binding of plasma IGF-I by kidney IGFBPs. In this study we demonstrate that the expression of IGF-I mRNA is increased on the second day after the induction of diabetes. Since accumulation of the protein was maximal after 4 days [10], the increase in IGF-I mRNA apparently precedes the rise of IGF-I protein [13]. This suggests that the accumulation of kidney IGF-I in diabetic mice is due to local production.

We also showed that diabetes did not induce uniform increments in the renal mRNA expression of IGFBPs. This further supports the theory that diabetes induces local IGF-I production. However, it has not been demonstrated that mRNA production is linearly associated with protein synthesis, and we did not measure IGFBP concentrations. Therefore, we can not definitely exclude increased IGF-I bioavailability through subtle changes in local concentrations of binding proteins. It is known that IGFBP-2, IGFBP-3 and IGFBP-5 can potentiate IGF-I action, while IGFBP-4 has largely inhibitory effects [18]. The slight increase in mRNA of the former binding proteins and the persistent decrease in IGFBP-4 mRNA that we have found, might contribute to an increased IGF-I protein concentration.

Abnormal serum GH concentrations are considered to affect the diabetes-induced kidney growth and kidney IGF-I concentration [10,25]. GH suppression by somatostatin analogues decreases kidney growth [26,27]. We have shown that GH-deficient diabetic Ames-dwarf mice have absent accumulation of renal IGF-I and reduced kidney growth, while GH overproduction in PEPCK-bGH-transgenic mice resulted in increased kidney IGF-I and kidney growth [10]. In the present study we saw no difference in IGF-I mRNA production between GH-deficient mice and GH-transgenic mice. On the other hand, we found slightly more renal IGFBP-2 and IGFBP-4 mRNA expression in GH-transgenic mice compared to non-transgenic mice, while the expression of these mRNAs was less in GH-deficient Ames-dwarf mice. This suggests at least partial GH dependency of the transcription of these genes in the mouse kidney, although mRNA expression was not absent in dwarfs. The effect of GH was more marked after the induction of diabetes. Kidney expression of IGFBP-3 and IGFBP-5 appears to be less GH dependent. Some ill GH-transgenic animals with extensive tubular and glomerular destruction had

IGFBP-3 mRNA expression in glomeruli. This suggests a regression to less differentiated stages as a compensatory repair mechanism, which has been described before after tissue damage [12,28]. In the fetal mouse kidney, IGFBP-3 mRNA expression was seen in the developing glomerular bud [Lindenbergh-Kortleve, submitted], but no longer in the mature glomerulus. An increased need for IGF-I might be the stimulus for increased IGFBP-3 expression.

Most striking in our study was the presence of IGF-I mRNA in GH-deficient Ames-dwarf mice, that do not have any detectable IGF-I in the kidney [10]. This suggests that IGF-I gene transcription in the mouse kidney is not GH dependent, as has been described by others [11]. The difference between the presence of IGF-I mRNA and the absence of IGF-I protein in dwarf mice must be explained by GH dependency of posttranscriptional events [29]. GH deficiency might induce alternative IGF-I gene transcription in dwarf mice, since multiple IGF-I mRNA transcripts exist [11,19,29,30]. These transcripts are expressed in a tissue specific manner, and are under variable GH regulation [11,14,19,29,31]. Insulin, protein intake and local factors influence IGF-I gene transcription as well [32]. We used a relatively long IGF-I probe, which is supposed to be highly specific, but different promoter regions or other changes in the transcript not covered by the probe, may cause defective translation of the mRNA. Lack of negative feedback by the IGF-I protein in dwarf mice may be another cause of persistent gene transcription and mRNA expression.

In conclusion, we found an increased expression of IGF-I mRNA in the kidneys of diabetic mice, suggesting local IGF-I production. This IGF-I mRNA expression is not dependent on the presence of GH, but effective translation of the mRNA into protein seems to be GH dependent.

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### 3.4 Suppression of growth hormone in patients with diabetes mellitus

Effect of long-acting somatostatin analogue (Somatulin) on renal hyperfiltration in patients with IDDM.

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## Summary

**Objective.** To investigate whether long-acting somatostatin (SMS) can suppress renal hyperfiltration in patients with IDDM.

**Research design and methods.** A double-blind, randomized treatment of nine patients with IDDM was used. Selection criteria were renal hyperfiltration (glomerular filtration rate [GFR]  $\geq 129$  ml/min/1.73m<sup>2</sup>) and absence of hypertension and macroalbuminuria. Treatment was either with a long-acting SMS analogue (Somatulin, 30 mg) or with placebo, given by intramuscular injections every 10 days for 9 months. GFR, effective renal plasma flow (ERPF), IGF-I, and 24-h growth hormone (GH) profiles were used as evaluation parameters.

**Results.** Five patients were randomized to Somatulin, four patients to placebo. One of the patients treated with Somatulin stopped after 3 months because of persistent abdominal discomfort after the injections. Somatulin treatment for 3 months lowered GFR and ERPF compared with placebo ( $p < 0.05$ ). After 9 months, the differences were no longer significant. After 3 months, IGF-I concentrations were decreased in all Somatulin-treated patients. GH secretion tended to increase in the placebo group.

**Conclusions.** The administration of long-acting Somatulin to patients with IDDM and renal hyperfiltration leads to only a temporary reduction of ERPF/GFR.

## Introduction

Of patients with IDDM, ~30-40% develop diabetic nephropathy (DNP) [1]. Renal hyperfiltration is considered to be the first abnormality in the diabetic kidney [2] and might be a risk factor for DNP [3-5]. A role for growth hormone (GH) in hyperfiltration is suggested, based on the observation that the glomerular filtration rate (GFR) increases in patients with IDDM who received GH injections [6] and in healthy volunteers treated with IGF-I intravenously [7]. Acromegalic patients also have increased renal function [8]. Elevated GH concentrations have been found in patients with poorly and moderately controlled IDDM and are associated with renal hyperfiltration [9-11].

Treatment of these diabetes-associated abnormalities in the GH/IGF-I axis might offer an alternative treatment option to prevent DNP. Remission of DNP was seen after the onset of hypopituitarism [12]. Subcutaneous injections of octreotide, a somatostatin (SMS) analogue, led to a reduction in GFR in patients with acromegaly [8]. In a short-term study, GFR was reduced by continuous subcutaneous infusion of SMS in patients with IDDM [13]. We have investigated whether the administration of a long-acting SMS analogue (Somatulin, BIM 23014), which has proven its efficacy to lower GH levels in patients with acromegaly [14], could achieve reduction of renal hyperfiltration.

## Research design and methods

### *Patients and study design*

Nine patients with IDDM and renal hyperfiltration ( $\text{GFR} \geq 129 \text{ ml/min/1.73m}^2$ ) participated in the study. They had no hypertension, macroalbuminuria, medication other than insulin, or oral contraceptives in women.

Five patients were allocated to treatment with the long-acting SMS analogue Somatulin (30 mg) [14], and four patients to placebo therapy (both supplied by Ipsen Biotech, Paris, France), in a double-blind randomized fashion. Trial medication was administered by a study nurse, the patients themselves, or a spouse by intramuscular injections every 10 days. Compliance to the treatment was checked by examination of the empty vials. At baseline, 3, and 9 months, all patients were admitted to the ward for the assessment of serum GH concentrations every hour during 24 h, gallbladder ultrasound, measurement of 24-h albumin excretion and renal function [GFR

and effective renal plasma flow (ERPF)].

All patients were treated with insulin by the basal/prandial regimen with appropriate adjustments of insulin doses. The study was approved by the Medical Ethical Committee, and all patients gave written informed consent.

#### *Laboratory techniques*

Renal function was measured during normoglycaemia after a period of at least 8 h fasting. To maintain good hydration, a glucose 5% (w/v) infusion was given with an appropriate amount of insulin added. Blood glucose levels were measured at 30-min intervals and kept constant between 4 and 8 mmol/l. GFR and ERPF were measured simultaneously by the continuous infusion technique with  $^{125}\text{I}$ -thalamate and  $^{131}\text{I}$ -hippuran sulphate [15] and standardized to  $1.73\text{ m}^2$  body surface area. Normal GFR in our laboratory is 88-130 ml/min/ $1.73\text{ m}^2$  [10].

Plasma GH was measured by a solid phase two-site immunoradiometric assay (CIS bio international, ORIS group, Gif-sur-Yvette, France; detection limit  $0.04\text{ }\mu\text{g/l}$ ; normal values are  $<10\text{ }\mu\text{g/l}$ ; intra-assay and interassay coefficients of variation [CV] 3 and 4%, respectively). IGF-I was removed from its binding proteins by the acid-ethanol extraction method [16] and was measured by radioimmunoassay (RIA) (Medgenix, Fleurus, Belgium; reference range 9-64 nmol/l; intra-assay and interassay CV 7 and 10%, respectively). HbA1c was determined by high-performance liquid chromatography (HPLC) (reference range 5.0-6.3%). Glucagon was measured after extraction by RIA (Euro-diagnostica; sensitivity 3 ng/l; normal values are  $<80\text{ ng/l}$ ; CV 17-30%), serum creatinine by colorimetry (reference range 60-110  $\mu\text{mol/l}$ ), and albumin excretion by immunonephelometry. Microalbuminuria is defined as albumin excretion between 30 and 300 mg/24h.

Dilated funduscopy was performed by an experienced ophtalmologist and was graded as no diabetic retinopathy (DRP), background DRP, or proliferative DRP.

#### *Statistical analysis*

Differences between the medians (ranges) in the Somatulin and placebo group were analyzed by the (exact) Mann-Whitney U test. Spearman rank correlation coefficients were calculated between individual variables. The two-sided level of significance was set at  $p < 0.05$ .



## Results

There were no statistically significant differences in baseline characteristics between the two groups (Table 1).

**Table 1. Baseline characteristics of the patients**

	Somatulin	Placebo
n	5	4
sex (M/F)	3/2	3/1
age (years)	25 (19-45)	28 (21-50)
duration of diabetes (years)	14.0 (5-25)	15.5 (9-44)
BMI (kg/m <sup>2</sup> )	28.2 (21.8-37.4)	22.5 (20.8-28.4)
insulin dose (U/day)	72 (46-78)	65 (44-72)
DRP (n)		
no	4	2
background	1	1
proliferative	-	1
DNP (n)		
no	3	4
microalbuminuria	2	-
HbA1c (%)	8.8 (7.6-10.1)	7.7 (7.0-9.3)
serum creatinine ( $\mu$ mol/l)	55.0 (50-73)	54.5 (45-65)
glucagon (ng/l)	4 (3-56)	14 (3-47)
IGF-I (nmol/l)	24.7 (15.1-28.6)	23.1 (12.4-27.6)
mean 24-h GH concentration ( $\mu$ g/l)	2.8 (1.0-3.5)	2.8 (1.2-8.3)
GH area under the curve ( $\mu$ g/l per 24 h)	1602 (536-2087)	1334 (703-4032)
albuminuria (mg/24 h)	22 (9-65)	10 (6-14)
GFR (ml/min/1.73m <sup>2</sup> )	144 (129-154)	142 (139-159)
ERPF (ml/min/1.73m <sup>2</sup> )	587 (540-598)	658 (553-687)
gallbladder ultrasound (n)		
normal	5	3
polyp	-	1

Data are median (range) or number (n) of patients.

Side effects were reported both by Somatulin- and placebo-treated patients: abdominal discomfort and diarrhea for 1 to 2 days after the injection (temporary), painful induration at the injection site, and decreased blood glucose concentration in the evening or day after injection (recurrent). One patient in the Somatulin group stopped after 3 months because of persistent abdominal discomfort after each injection. No gallstones developed, but after 3 months asymptomatic gallbladder sludge was seen in one patient.

Individual data on GH area under the curve (GH-AUC), plasma IGF-I concentration, GFR, and ERPF are depicted in Fig.1. Compared with baseline, GH-AUC had decreased in four of the five patients after 3 months treatment with Somatulin (Fig.1A), whereas the four placebo-treated patients all had increased GH-AUC (Fig.1B). IGF-I decreased in all patients treated with Somatulin (Fig.1C). The changes from baseline in GH and IGF-I concentrations were not significantly different between the two treatment groups (Table 2).

**Table 2. Median changes from baseline in GH/IGF-I secretion and renal function tests**

	Somatulin (n = 5)	Placebo (n = 4)
<b>After 3 months</b>		
n	5	4
GH area under the curve ( $\mu\text{g/l}$ per 24h)	-515 (-1163 to 1656)	602 (533 to 3979)
mean 24-h GH concentration ( $\mu\text{g/l}$ )	-1.0 (-1.9 to 2.6)	1.7 (1.0 to 6.4)
IGF-I (nmol/l)	-4 (-9.8 to -0.9)	-3.4 (-7.2 to 0.6)
GFR (ml/min/1.73m <sup>2</sup> )	-3 (-23 to 5)*	12 (-1 to 17)
ERPF (ml/min/1.73m <sup>2</sup> )	-77 (-130 to -27)*	14 (-34 to 85)
<b>After 9 months</b>		
n	4	4
GH area under the curve ( $\mu\text{g/l}$ per 24h)	68 (-931 to 340)	424 (-384 to 1267)
mean 24-h GH concentration ( $\mu\text{g/l}$ )	0.2 (-1.6 to 0.6)	0.1 (-1.0 to 1.7)
IGF-I (nmol/l)	-8.3 (-10.2 to -0.4)	-6.0 (-8.2 to -0.7)
GFR (ml/min/1.73m <sup>2</sup> )	-6 (-16 to 9)	5 (-8 to 32)
ERPF (ml/min/1.73m <sup>2</sup> )	-61 (-82 to -41)	-23 (-99 to 52)

Data are median (range) change from baseline.

Differences were analyzed by the exact Mann-Whitney U test; \*  $p < 0.05$  versus placebo

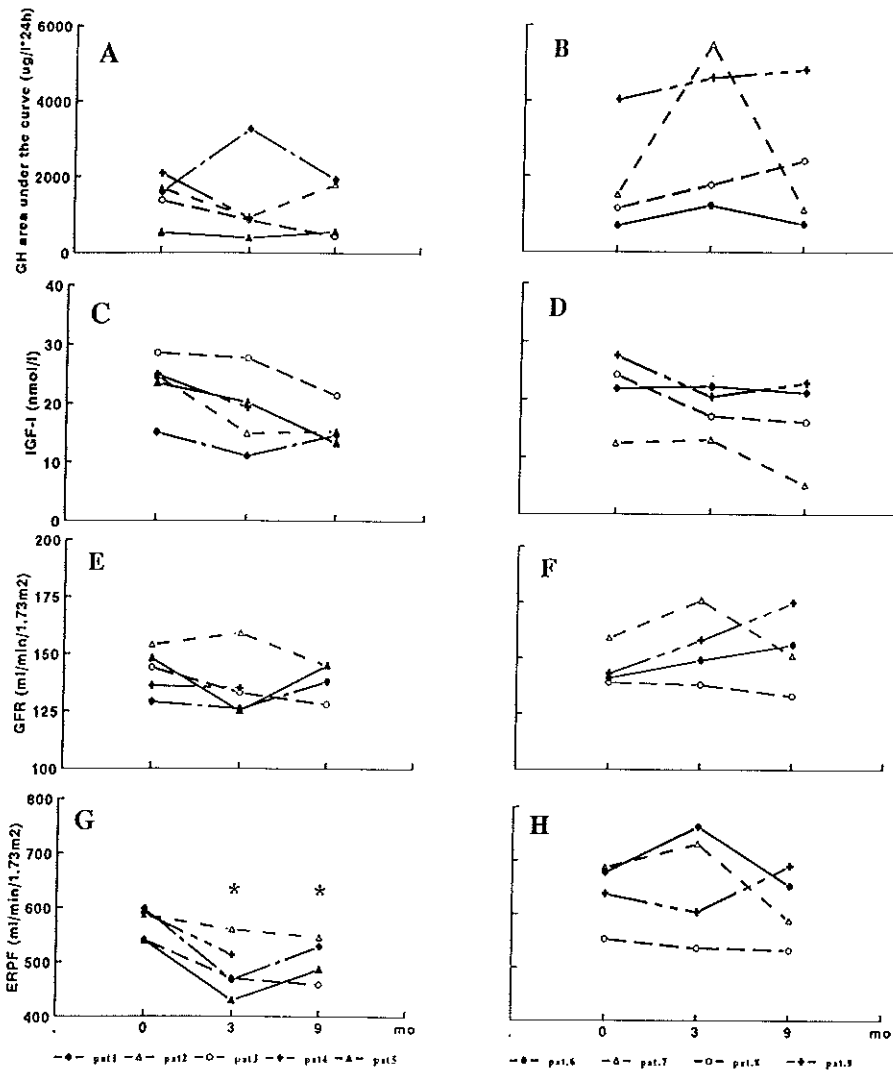


Fig. 1. Area under the 24-h GH curve, IGF-I concentrations, GFR, and ERPF of the individual patients before and during treatment with Somatulin (A, C, E and G) or placebo (B, D, F and H).

\*  $p < 0.05$  vs. placebo

GFR was decreased in four of the five Somatulin-treated patients at 3 months, but the decrease was sustained until 9 months only in patient 3 (Fig.1E). The responding patient was one of the patients with microalbuminuria; reevaluation at 3 months after the last injection showed a return of his GFR to the level at baseline. There was a tendency to a further increase of hyperfiltration in the placebo group (Fig.1F). Median change from baseline was significantly different in Somatulin compared to placebo-treated patients at 3 months (-3 vs. 12 ml/min/1.73m<sup>2</sup>;  $p < 0.05$ ), but not at 9 months (Table 2). As shown in Figs.1G and 1H, ERPF was significantly different between the study groups at 3 and 9 months ( $p < 0.05$ ). All patients treated with Somatulin had a decrease in ERPF from baseline to 3 months. At 9 months, values tended to return to pre-treatment levels (Fig.1G). Median ERPF in placebo-treated patients did not change (Fig.1H). The change in ERPF from baseline was significantly different between Somatulin- and placebo-treated patients at 3 months only (Table 2).

Median insulin dose decreased during the study period, but not significantly differently in the Somatulin- versus placebo-treated patients (-12 vs. -16 U/day). At 3 months, the median change in HbA1c was significantly different in Somatulin- versus placebo-treated patients (-0.8% vs. 0.5%;  $p < 0.05$ ). The change in HbA1c was significantly correlated with the change in ERPF ( $r = 0.69$ ;  $p < 0.05$ ). At 9 months there was no significant difference in HbA1c between the two treatment groups. Blood pressure, body weight, albumin excretion, serum creatinine, and plasma glucagon concentration did not change in either group.

## Conclusions

A role for GH in the development of diabetic renal hyperfiltration and eventually DNP has been suggested previously [6-8,10]. In this study we demonstrate that patients with IDDM and renal hyperfiltration show decreased GFR and ERPF after 3 months of treatment with Somatulin compared with placebo. The effect was lost after 9 months of Somatulin therapy in four of the five patients. Although our study population is small, our findings at 3 months are in accordance with other acute and short-term studies [17,13].

The mechanisms by which SMS reduces ERPF and/or GFR are not fully understood. Indirect effects of SMS on renal function can be assumed by suppression of GH/IGF-I and glucagon. GH suppression leads to a decrease in total body water [18], which might have caused a seeming decrease in ERPF. A reduced systemic and local IGF-I concentration leads to diminished renal

vasodilation [7] and thereby to a reduction of ERPF, possibly by suppression of nitric oxide [19] or kinin [20] activity. The small and temporary suppression of GH in our study might have been sufficient to suppress IGF-I in the kidney and thereby hyperfiltration, although the changes in plasma IGF-I were only marginal. Reduction of plasma glucagon and insulin concentrations, other possible effects of SMS therapy with renal consequences, could not be demonstrated in our study. Somatulin led to a small improvement of glycaemic control, which might have contributed to the reduction in GFR [9].

A direct effect of SMS on the kidney has been demonstrated *in vitro*, where SMS could relax cultured human mesangium cells by antagonizing the constricting effect of angiotensin II [21]. This would induce an increase rather than a decrease in renal function, which might explain the return to high GFR after 3 months of Somatulin treatment.

Because we could not reduce GFR in all patients, resistance to the GH suppressive effect of SMS is suspected [22,23]. Increased doses of Somatulin might have overcome this problem, although acromegalic patients had persistent and effective GH reduction by less frequent administration of the same dose [14]. Development of resistance to SMS by receptor adaptation (desensitization) [24,25] is supported by the lack of long-term persistent beneficial effect in our study and in a study on diabetic retinopathy [26].

The increase of GH secretion and GFR at 3 months in the placebo-treated patients suggests that the maximum level of renal hyperfiltration has not yet been reached, even after a diabetes duration of at least 9 years. This increase was prevented by Somatulin treatment, but the effect was only temporary. However, due to the small number and clinical heterogeneity of patients, lack of statistical significance does not exclude a small real effect.

We conclude that administration of Somatulin does not seem suitable for long-term treatment of renal hyperfiltration in patients with IDDM. Persistent side effects and resistance to the GH suppressive effects are seen after treatment for more than 3 months. A larger long-term study, maybe with higher dosages of this or another analogue, is needed to confirm our data.

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### 3.5 Conclusions

Growth hormone is inappropriately secreted in patients with IDDM, while plasma concentrations of its mediator, IGF-I, are relatively low. In diabetic mice, plasma IGF-I concentrations are low as well, but local IGF-I concentrations in the kidneys increase dramatically, shortly after the induction of diabetes. While transgenic mice expressing bovine GH develop extensive kidney lesions and kidney growth in the first days after the induction of hyperglycaemia, GH-deficient dwarf mice seem to be protected from these changes. They also do not have IGF-I in the kidney, although IGF-I mRNA was expressed. Apparently local IGF-I is of main importance for the induction of renal lesions, and this is dependent on the availability of GH in the body.

Humans with diabetes and renal hyperfiltration might benefit from suppression of the inappropriate GH levels. We tried to achieve this with the administration of a long-acting Somatostatin analogue. Although treatment for 3 months appeared to be successful, the effect was lost after long-term treatment for 9 months. This might be due to gradual adaptation to the physiological effects of SMS.



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## **Chapter 4**

### **Concluding remarks**

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## Concluding remarks

Treatment of acute symptoms and prevention of long-term complications are the goals in diabetes care. Aiming at (near-)normoglycaemia should be the primary action to achieve these goals, since hyperglycaemia is the pathogenetic basis for the development of all microvascular complications [1,2]. This thesis described two important elements in the development of diabetic complications that are apt to treatment, namely the level of glycaemic control, and the role of growth hormone (GH). Because diabetic nephropathy (DNP) can be life-threatening, the focus has mainly been on this complication. Early kidney growth and hyperfiltration are risk factors for the development of DNP [3,4], and are considered as the earliest abnormalities in the diabetic kidney.

As described in Chapter 2, glycaemic control is poor in routine diabetes care, both in general practice and in outpatient clinics, both with oral hypoglycaemic agents and with insulin, both for IDDM and for NIDDM patients. Only consistent application of treatment guidelines and strict adherence to the treatment goals can lead to a significant improvement of glycaemic control (§2.3.2). Insulin pens, self-control equipment and a specialized diabetes nurse - all available in routine diabetes care - may be helpful tools to improve diabetes management, but the personal attitude of the doctor seems to be of major importance. Sincere interest in the patient's well-being, permanent education, frequent doctor-patient contacts and psychological support are necessary to achieve the desired level of glycaemic control [1]. Whether this kind of intensive treatment will be able to prevent or postpone all complications in IDDM as well as in NIDDM patients, remains to be proven.

At present the above-mentioned conditions are only fulfilled in strictly-controlled studies with selected and highly-motivated patient groups. However, the majority of the diabetic patients is treated in routine settings, where less time, money and motivated personnel is available. This implicates that large numbers of patients with diabetes are in suboptimal glycaemic control for the greater part of their lifetime. In association with this, an increased risk for the development of long-term complications exists in these patients.

Although hyperglycaemia is the basic pathophysiological mechanism leading to diabetic complications, it is not the only abnormality found in patients with diabetes. As described in Chapter 3, disturbance of the GH/IGF-

I axis is also an important and potentially treatable finding, even in patients with moderately controlled diabetes mellitus (§3.2). If glycaemic control cannot be achieved, GH reduction might possibly be an alternative treatment option for poorly-controlled patients with diabetes.

Experiments with diabetic rats have demonstrated a beneficial effect of GH suppression by somatostatin (SMS) on the early changes in the diabetic kidney [5,6]. We found that complete lack of GH activity in genetic dwarf mice was also protective against these early kidney changes, i.e. IGF-I accumulation and kidney growth (§3.3.1). Although IGF-I gene transcription in the mouse kidney was not GH dependent (§3.3.2), the effective translation of the mRNA transcript into IGF-I protein was defective in GH-deficient dwarf mice. This suggests that GH is a necessary factor for the diabetes-induced kidney changes.

In humans we suspect a similar relation between diabetes, GH and renal IGF-I accumulation. The resulting renal hyperfiltration is considered as a risk factor for the development of DNP [3,4]. However, when we tried to suppress the abnormal GH secretion in patients with IDDM and renal hyperfiltration, only a temporary effect was seen. After three months treatment with a long-acting SMS analogue we observed a significant decrease in GFR and ERPF, paralleling a tendency to decreased GH/IGF-I secretion. After nine months, however, the effect was lost. The results of this study suggest that successful suppression of GH is possible, with concomitant effects on the kidney, even in patients with long-standing diabetes. For the successful prevention of DNP, we need a drug suitable for long-term treatment, with the potential to suppress GH action permanently, e.g. GH receptor blockers. The main goal should be local suppression of GH action, since systemic levels of GH do not influence kidney function directly. The harmful effect of GH in patients with diabetes mellitus is exerted via induction of tissue IGF-I (§3.3), so this must be the main target of treatment.

In summary, unless more intensive diabetes management will be realized in routine diabetes care, achievement of strict glycaemic control seems out of reach for the majority of patients with diabetes mellitus. Therefore, adjuvant therapy by suppression of GH action might be a tool to prevent the development of DNP and maybe other long-term complications.

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**Summary**

**Samenvatting**

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## Summary

Many patients with diabetes mellitus develop long-term complications, in variable severity. The risk of these complications increases linearly with the level of glycaemic control. Diabetic nephropathy (DNP) occurs in 30-40% of the patients, and has enormous consequences for their survival and quality of life. Once manifest DNP has developed, with proteinuria, hypertension and declining renal function, no treatment is available to halt or reverse the progression to end-stage renal failure. Therefore, in order to reduce the number of diabetic patients developing this complication, intervention during early stages of the disease is the only option. In **Chapter 1** the role of hyperglycaemia is discussed, and the concept of renal hyperfiltration is introduced, one of the earliest abnormalities found in diabetic patients. In the presence of hyperglycaemia, renal hyperfiltration is a risk factor for the development of DNP. Apart from poor metabolic control, enhanced growth hormone (GH) secretion with local accumulation of insulin-like growth factor (IGF)-I seem the main factors responsible for hyperfiltration. IGF-I in the kidney induces selective afferent arteriolar vasodilation resulting in glomerular hypertension, the basis of glomerulosclerosis and DNP. To prevent DNP, either hyperglycaemia or the abnormal GH/IGF-I secretion accompanying diabetes mellitus should be treated effectively.

In **Chapter 2** the current state of glycaemic control in patients with diabetes mellitus is examined. As described in §2.2.1, not all the patients with NIDDM are known with the disease. Approximately 50% of all patients with the disease are unrecognized and remain untreated. Screening by random glucose measurement in an unselected population without specific symptoms was not an efficient method to improve this number. Under the age of 40, no new patients were diagnosed. All the newly diagnosed patients had at least one additional risk factor for the development of cardiovascular complications, so casefinding in a subgroup of the population would be more effective. Hypertension, hypercholesterolaemia, obesity and cardiovascular symptoms were present in the majority of the newly-diagnosed patients. In this study we also found that patients known with the disease were not sufficiently treated: glycaemia, body mass index (BMI), smoking habits and blood cholesterol levels were poorly controlled in this group. Although the results of this study might not be representative for all patients with NIDDM, routine diabetes care by the general practitioner could be improved.

In a study with both NIDDM and IDDM insulin-using patients from

14 hospitals in the Rotterdam region, glycaemic control was investigated in relation to body weight and the daily number of insulin injections and insulin dose (§2.2.2). Obesity was associated with poor glycaemic control in patients with NIDDM. Higher insulin doses were associated with higher HbA1c values, both in IDDM and in NIDDM patients. Treatment of the patients with 'intensive therapy' (4 or more insulin injections per day) did not result in better glycaemic control than treatment with conventional therapy (1 to 3 injections per day). We conclude the failure of routine diabetes care in achieving good glycaemic control, even when multiple injections are given.

At present, most patients with NIDDM will be referred to an internist when installation of insulin therapy has become necessary. These internists usually are specialized in the treatment of diabetes, and they have various tools available for the initiation and control of insulin therapy, like a specialized diabetes nurse, insulin pen systems, and self-control equipment. Regular appointments at 3-month intervals are usual in this routine outpatient setting. However, installation of insulin therapy does not appear to be a panacea. As described in §2.3.1, patients with NIDDM were studied for one year in a crossover study with oral hypoglycaemic agents and insulin therapy. The patients were recruited from 14 hospitals within the Rotterdam region, and were included in normal routine diabetes care at the outpatient clinic of each hospital. Poor glycaemic control, obesity and hypertension were present in three quarters of the patients. Six months treatment with insulin did not result in better glycaemic control. Underdosage of insulin, but also lack of strict supervision are thought to be responsible for the negative result of this study.

The possible ways to achieve good metabolic control in general practice are described in §2.3.2. We studied the effectiveness of a computerized treatment schedule, based on strict application of the Dutch General Practitioners guidelines for the treatment of NIDDM (NHG-standard). The general physician working with the strict, computerized guidelines was capable of achieving a significantly lower HbA1c in patients with NIDDM compared with general physicians not strictly applying these guidelines. Since installation of strict metabolic control is not a goal in itself, the prevalence of micro- and macrovascular complications was investigated in these patients. No significant difference between the well- and poorly-controlled patient groups was found, except for an even higher prevalence of neuropathy in the general practice with good glycaemic control. Although this is a disappointing finding, it can be explained by the relatively short time since the program was implemented.

Since routine diabetes care results in suboptimal glycaemic control,

other treatment options must be examined to prevent patients from developing complications. Whether GH plays a role in moderately controlled diabetes, and which are the possible effects of GH suppression, was investigated in **Chapter 3**.

IDDM patients with moderate hyperglycaemia have elevated GH concentrations when tested by GH stimulation tests (§3.2). When correction is done for confounding factors like BMI, age and sex, no difference is found between patients and healthy controls. Still, in view of the hyperglycaemia present in these patients, 'normal' GH concentrations must be considered too high, since increased glucose levels in non-diabetic persons would suppress GH secretion by feedback mechanisms. The results of this study prove that humans with diabetes mellitus have abnormal GH secretion.

Experimental diabetes was induced in transgenic mice expressing large quantities of bovine GH, and in dwarf mice with genetic GH deficiency. The effect of high and low plasma GH concentrations on IGF-I accumulation in the diabetic kidney was studied (§3.3.1). Kidney IGF-I concentrations increased within 2 days after the induction of diabetes in mice with normal or bovine GH production. No IGF-I was detected in the kidneys of dwarf mice. Kidney growth was absent in GH-deficient dwarf mice as well, and this supports the concept that GH deficiency is protective against the development of diabetic renal complications.

In the study described in §3.3.2 we investigated whether IGF-I accumulation in diabetic kidneys is the result of local production, or of entrapment of plasma IGF-I by local IGF-I binding proteins (IGFBP). Therefore, *in situ* hybridization was performed with mRNAs of the six IGFBPs and of IGF-I in the kidneys of GH-transgenic mice and dwarf mice. We found an increase in IGF-I mRNA after the induction of diabetes, in normal mice, GH-transgenic mice and GH-deficient dwarf mice. This implies that IGF-I gene transcription is not GH dependent. However, in view of the lack of IGF-I protein in dwarf mice, apparently effective translation of the IGF-I mRNA is dependent on the presence of GH.

In §3.4 the results of a pilot study in humans with IDDM and renal hyperfiltration are described. Treatment of the abnormal GH secretion with a long-acting somatostatin analogue resulted in effective suppression of the renal hyperfiltration after 3 months, but not after 9 months. Although this is disappointing in view of the possible prevention of DNP, we learn from this study that successful GH suppression is effective in normalizing renal function.

As concluded in **Chapter 4**, the studies described in this thesis

indicate that good glycaemic control is not yet being achieved in routine diabetes care. Participation of engaged doctors with special interest in the diabetic patient is necessary. Adjuvant therapy by antagonizing GH action might offer an alternative treatment option to prevent the development of DNP and maybe other long-term complications in our patients with diabetes mellitus.

## Samenvatting

Veel patiënten met diabetes mellitus ontwikkelen lange-termijn complicaties, in wisselende ernst. De kans op deze complicaties neemt toe naarmate de bloedsuikerregulatie slechter is. Diabetische nefropathie (DNP) treedt in 30-40% van de patiënten op, en heeft enorme gevolgen voor de overlevingskans en kwaliteit van leven. Wanneer manifeste DNP zich eenmaal heeft ontwikkeld, met eiwitverlies in de urine, hoge bloeddruk en achteruitgang van de nierfunctie, is geen behandeling meer mogelijk om de voortgang tot het eindstadium van nierfalen te voorkomen. Om het aantal diabetespatiënten dat deze complicatie ontwikkelt te verkleinen, is ingrijpen in vroege stadia van de ziekte de enige mogelijkheid. In **Hoofdstuk 1** wordt de invloed van hyperglycaemie besproken, en wordt het begrip "renale hyperfiltratie" geïntroduceerd, een van de eerste afwijkingen bij patiënten met diabetes. In aanwezigheid van hyperglycaemie is hyperfiltratie een risicofactor voor de ontwikkeling van DNP. Naast een slechte bloedsuikerregulatie zijn een toegenomen groeihormoon (GH) secretie met locale ophoping van "insulin-like growth factor" (IGF)-I de belangrijkste oorzaak van de renale hyperfiltratie. IGF-I in de nier veroorzaakt selectieve vasodilatatie van de afferente arteriolen, met glomerulaire hypertensie als resultaat, de basis van glomerulosclerose en DNP. Om DNP te voorkomen moeten ofwel de hyperglycaemie ofwel de abnormale GH/IGF-I secretie effectief worden behandeld.

In **Hoofdstuk 2** wordt de huidige staat van de diabetesregulatie onderzocht. Zoals beschreven staat in §2.2.1, blijken niet alle patiënten met NIDDM als zodanig gediagnostiseerd te zijn. Ongeveer 50% van het totale aantal patiënten is nog niet herkend en wordt dus niet behandeld. Screenen van een ongeselecteerde bevolking zonder specifieke klachten door middel van een willekeurige glucosemeting was geen efficiënte manier om het aantal gevallen van NIDDM te vergroten. Onder de leeftijd van 40 jaar werden geen nieuwe ziektegevallen gevonden. Alle nieuw-gevonden patiënten hadden tenminste één aanvullende risicofactor voor de ontwikkeling van hart- en vaatziekten, dus opsporen van patiënten in een subgroep van de bevolking lijkt effectiever. Hoge bloeddruk, verhoogd cholesterol, overgewicht, en tekenen van hart- en vaatziekten waren bij de meeste nieuw-ontdekte diabetespatiënten aanwezig. Uit deze studie bleek ook dat de reeds bekende diabetespatiënten onvoldoende behandeld werden: bloedsuikerspiegel, Queteletindex, rookgewoonten en cholesterolspiegels waren slecht

gereguleerd in deze groep. Hoewel de resultaten van deze studie misschien niet representatief zijn voor alle patiënten met NIDDM, lijkt de routine diabeteszorg bij de huisarts voor verbetering vatbaar.

In een studie van insulinegebruikende patiënten met NIDDM of IDDM uit 14 ziekenhuizen in de regio Rotterdam werd de diabetesregulatie onderzocht, in relatie tot het lichaamsgewicht en het aantal insuline-injecties en de insulinedosis per dag (§2.2.2). Overgewicht bleek gerelateerd aan slechte diabetesregulatie bij patiënten met NIDDM. Hogere insulinedoseringen waren gerelateerd aan hogere HbA1c-waarden bij zowel IDDM- als NIDDM-patiënten. “Intensieve behandeling” (4 of meer insuline-injecties per dag) leverde geen betere diabetesregulatie op dan conventionele behandeling (1 tot 3 injecties per dag). Opnieuw constateren we falende routine diabeteszorg met betrekking tot de glucoseregulatie, zelfs met multiële insuline-injecties.

Heden ten dage worden de meeste patiënten met NIDDM naar de internist verwezen wanneer insulinebehandeling noodzakelijk is geworden. Deze internisten zijn meestal gespecialiseerd in diabetes en ze hebben diverse hulpmiddelen voor het instellen op en controleren van insulinetherapie, zoals een gespecialiseerde diabetesverpleegkundige, insulinepennen en zelfcontrole-middelen. Regelmatige afspraken om de 3 maanden zijn normaal in de routine diabeteszorg op de polikliniek. Toch blijkt insulinetherapie geen panacee. Zoals beschreven staat in §2.3.1 werden NIDDM-patiënten gedurende een jaar bestudeerd in een crossover studie met bloedsuikerverlagende tabletten en insuline. De patiënten waren afkomstig van 14 ziekenhuizen in de regio Rotterdam, en werden met de gewone diabeteszorg behandeld op de poliklinieken van die ziekenhuizen. Slechte glucoseregulatie, overgewicht en hoge bloeddruk waren bij driekwart van de patiënten aanwezig. Zes maanden behandeling met insuline resulteerde echter niet in een betere diabetesregulatie. Te lage insulinedosering, maar ook tekortschietende begeleiding worden verantwoordelijk geacht voor de negatieve bevinding van deze studie.

Mogelijke manieren om een goede diabetesregulatie in de huisartspraktijk te bewerkstelligen, worden beschreven in §2.3.2. We onderzochten de effectiviteit van een geautomatiseerd behandelplan, dat gebaseerd is op stricte toepassing van de NHG-standaard voor de behandeling van NIDDM. De huisarts die de geautomatiseerde richtlijnen streng toepaste, bereikte in zijn patiënten met NIDDM een significant lager HbA1c dan de huisartsen die deze richtlijnen niet streng toepasten. Aangezien het bereiken van een goede diabetesregulatie geen doel op zichzelf is, werd de aanwezigheid van micro- en macrovasculaire complicaties in deze patiënten eveneens onderzocht. Er werd geen significant verschil gevonden tussen de



goed en niet goed gereguleerde praktijken. Neuropathie bleek zelfs vaker voor te komen in de huisartspraktijk met goede glucoseregulatie. Hoewel deze uitkomst teleurstellend is, kan ze verklaard worden uit de relatief korte tijd dat het programma nog maar in gebruik was.

Omdat routine diabeteszorg blijkbaar geen adequate glucoseregulatie kan bewerkstelligen, moeten andere behandelmogelijkheden worden onderzocht om patiënten te beschermen tegen de ontwikkeling van complicaties. Of GH een rol speelt ook in matig-gereguleerde diabetes, en wat de mogelijke effecten van GH-onderdrukking zijn, werd onderzocht in **Hoofdstuk 3**.

Patiënten met matig gereguleerde IDDM hebben verhoogde GH-spiegels als dit met GH-stimulatietesten wordt onderzocht (§3.2). Als wordt gecorrigeerd for "confounding factors" zoals Queteletindex, leeftijd en geslacht, wordt echter geen verschil gevonden tussen patiënten en gezonde proefpersonen. Toch moeten de "normale" GH-spiegels als te hoog worden beschouwd, gezien de hyperglycaemie van de diabetespatiënten, want dergelijke hoge bloedsuikers in niet-diabeten zouden de GH-uitscheiding onderdrukken door middel van terugkoppelingsmechanismen. De resultaten van deze studie tonen aan dat mensen met diabetes mellitus een abnormale GH-uitscheiding hebben.

In een proefdiermodel werd diabetes opgewekt in transgene muizen met sterk verhoogde expressie van runder-GH, en in dwergmuizen met erfelijk GH-gebrek. Het effect van deze hoge en lage plasma GH-spiegels op de IGF-I ophoping in de diabetische nier werd bestudeerd (§3.3.1). De nierconcentraties van IGF-I stegen binnen 2 dagen na het opwekken van de diabetes in muizen met normale of verhoogde GH-productie. In de nieren van dwergmuisjes werd geen IGF-I gevonden. Ook was er geen niergroei bij de dwergmuizen, wat een ondersteuning is voor de veronderstelling dat GH-gebrek bescherming biedt tegen de ontwikkeling van diabetische niercomplicaties.

In de studie die beschreven wordt in §3.3.2, onderzochten we of de IGF-I ophoping in diabetische nieren het gevolg is van locale productie, of van het wegvangen van plasma IGF-I door locale IGF-I bindende eiwitten (IGFBPs). Daarom werd *in situ* hybridizatie verricht met mRNAs van de zes IGFBPs en IGF-I, in de niertjes van GH-transgene muizen en dwergmuizen. We zagen een toename van de IGF-I mRNA expressie na de inductie van diabetes mellitus, zowel in normale muizen, muizen met GH-overproductie, als in GH-deficiënte dwergmuizen. Dit houdt in dat transcriptie van het IGF-I gen niet afhankelijk is van de aanwezigheid van GH. Gezien de afwezigheid van het IGF-I eiwit in de

niertjes van GH-deficiënte dwergmuizen, is effectieve translatie van het IGF-I mRNA blijikbaar wel GH-afhankelijk.

In §3.4 worden de resultaten beschreven van een pilootstudie met mensen met IDDM en renale hyperfiltratie. Onderdrukking van de abnormale GH-uitscheiding door middel van een langwerkend somatostatine-analoog leidde tot succesvolle vermindering van de hyperfiltratie na 3 maanden behandeling, maar niet meer na 9 maanden. Hoewel dit teleurstellend is met het oog op het voorkómen van DNP, leert deze studie ons dat geslaagde GH-onderdrukking in staat is om de verhoogde nierfunctie te normaliseren.

Zoals in **Hoofdstuk 4** wordt geconcludeerd, blijkt uit de studies van dit proefschrift dat goede diabetesregulatie in de routine diabetespraktijk niet wordt bereikt. Het is noodzakelijk dat enthousiaste artsen met veel interesse voor de persoon met diabetes aan het werk gaan. Als alternatieve behandeling kan onderdrukking van GH een methode zijn om de ontwikkeling van DNP en misschien ook van andere lange-termijn complicaties te voorkomen bij onze patiënten met diabetes mellitus.

## List of abbreviations

ACE	angiotensin converting enzyme
BMI	body mass index
CSII	continuous subcutaneous insulin infusion
DCCT	diabetes control and complications trial
DNP	diabetic nephropathy
DNeuP	diabetic neuropathy
DRP	diabetic retinopathy
ECV	extracellular volume
ERPF	effective renal plasma flow
ESRF	end-stage renal failure
GFR	glomerular filtration rate
GH	growth hormone
GHRH	growth hormone releasing hormone
HbA1c	glycated haemoglobin
HPLC	high-performance liquid chromatography
IDDM	insulin-dependent diabetes mellitus, type I diabetes
IGF-I	insulin-like growth factor-I
IGT	impaired glucose tolerance
IGFBP	insulin-like growth factor binding protein
K <sub>f</sub>	glomerular ultrafiltration coefficient
mRNA	messenger ribonucleic acid
NHG	Nederlands Huisartsen Genootschap
NIDDM	non-insulin-dependent diabetes mellitus, type II diabetes
OGTT	oral glucose tolerance test
OHA	oral hypoglycaemic agents
PAH	paraimmunohippuran
PEPCK	phosphoenolpyruvate carboxykinase
RPF	renal plasma flow
SMBG	self-monitoring of blood glucose
SMS	somatostatin
SNGFR	single nephron glomerular filtration rate
STZ	streptozotocin
UAE	urine albumin excretion
WHR	waist-to-hip girth ratio
$\Delta P$	transcapillary hydraulic pressure
$\Delta \pi$	transcapillary oncotic pressure

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10. **Jacobs ML**, Lindenbergh DJ, Van Neck JW, Drop S, Bartke A, Weber RFA. Insulin-like growth factor (IGF)-I and IGF binding protein mRNA in the kidneys of normal, growth hormone (GH) transgenic and GH-deficient diabetic mice. Submitted
11. **Jacobs ML**, Boet AJF, Wigard MEH, Holovecz JM, Van Doorn BA, Kneepkens-Wouda MF, Stolk RP, Prins A, Weber RFA. Screening for NIDDM in a general practice. Submitted
12. **Jacobs ML**, van Ouwerkerk BM, Hoogma RPLM, Stijnen Th, Weber RFA and the Studygroup Diabetes Rijnmond. Failure to achieve optimal glycaemic control in patients with NIDDM with routine diabetes care. Submitted

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Marloes

## Curriculum vitae

De schrijfster van dit proefschrift werd geboren op 17 juni 1965 te Middelharnis. Na het behalen van het Atheneum-B diploma in 1983, aan de Rijksscholengemeenschap Goeree-Overflakkee, ging zij in datzelfde jaar geneeskunde studeren aan de Erasmus Universiteit te Rotterdam. Tevens studeerde zij enkele jaren Filosofie van de Geneeskunde.

Het artsexamen werd behaald in 1991 (Cum Laude). Na ruim een jaar werkzaamheid als AGNIO op de afdeling Interne van het St. Franciscus Gasthuis te Rotterdam, begon zij in augustus 1992 met het in dit proefschrift beschreven onderzoek, op de afdeling Inwendige Geneeskunde III van het Dijkzigt ziekenhuis. Tevens hield zij wekelijks spreekuur voor diabetespatiënten. Van april tot juli 1995 werd het dierexperimentele werk in Carbon-dale, aan de Southern Illinois University, bij Prof. A. Bartke verricht.

In maart 1997 is zij begonnen met de opleiding tot huisarts, aan het Instituut Huisartsgeneeskunde te Rotterdam.