

**MARKERS OF  
MICROVASCULAR  
COMPLICATIONS IN  
ADOLESCENTS WITH  
TYPE I DIABETES**

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OULU 2002





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Academic Dissertation to be presented with the assent of the Faculty of Medicine, University of Oulu, for public discussion in the Auditorium L12 of the Department of Paediatrics, University Hospital of Oulu, on January 10th, 2003, at 12 noon.

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## **Tossavainen née Riihimaa, Päivi, Markers of microvascular complications in adolescents with type 1 diabetes**

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### ***Abstract***

The markers of microvascular complications of type 1 diabetes were evaluated in adolescents in a cross sectional survey of 100 out of 138 eligible patients aged 9-19 years with a duration of diabetes over two years who visited the Paediatric Outpatient Clinic at Oulu University Hospital in 1997-1999, and one hundred healthy controls.

Two patients in early or mid-puberty had non-proliferative diabetic retinopathy, but no other signs of microvascular complications.

The five patients with persistent microalbuminuria were all girls; one prepubertal, one late pubertal and three postpubertal. Their mean glycated haemoglobin A1c (HbA1c) was higher, but they had a similar duration of diabetes and age distribution to those without microalbuminuria.

The adolescent patients were predisposed to higher fasting serum total and low-density lipoprotein cholesterol and triglyceride levels and higher diastolic blood pressure than the control subjects. The proportional total body fat was highest in the girls with diabetes by the end of puberty, while serum leptin levels did not differ between the patients and healthy controls. The patients had low fasting serum insulin levels and high insulin-like growth factor-binding protein 1 levels, related to hypoinsulinaemia.

Distal motor nerve function in the lower extremities were already affected before puberty, and distal and proximal nerve function deteriorated as puberty advanced. Ten patients had neurophysiologically confirmed distal diabetic polyneuropathy, and they were older and they had longer duration of diabetes and higher HbA1c than patients without polyneuropathy.

Although cardiovascular function was in the main well preserved in the adolescents with type 1 diabetes, the power spectrum analysis of heart rate variability showed attenuated autonomic nervous system reactivity.

Taken together these data show that a relatively small proportion of adolescents with type 1 diabetes have signs of microvascular complications. The prevalences of diabetic retinopathy, persistent microalbuminuria and distal diabetic polyneuropathy were 2%, 6% and 10%, respectively. Pubertal maturation seems to promote the progression of early signs of microvascular complications in patients affected by type 1 diabetes.

*Keywords:* adolescence, heart rate variability, insulin dependent diabetes mellitus, micro-angiopathy, puberty



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Oulu, November 2002

Päivi Tossavainen



## Abbreviations

ACE	angiotensin converting enzyme
AER	albumin excretion rate
AGEs	advanced glycation end products
ANS	autonomic nervous system
B	breast staging
CI	confidence interval
CMAP	compound muscle action potential
CV	coefficient of variation
DAG	diacylglycerol
G	genital staging
GFR	glomerular filtration rate
HbA1c	haemoglobin A1c
HDL	high-density lipoprotein
HF	high frequency
HRV	heart rate variability
IA	insulin antibodies
IGF-I	insulin-like growth factor-I
IGFBP-1	insulin-like growth factor-binding protein 1
LDL	low-density lipoprotein
LF	low frequency
PKC	protein kinase C
RU	relative units
SNAP	sensory nerve action potential
T I – T V	Tanner pubertal stages I – V
TGF	transforming growth factor
VEGF	vascular endothelial growth factor
VLF	very low frequency
VPT	vibration perception threshold



## **List of original articles**

This thesis is based on the following publications, which are referred to in the text by their Roman numerals:

- I Riihimaa P H, Knip M, Hirvelä H & Tapanainen P (2000) Metabolic characteristics and urine albumin excretion rate in relation to pubertal maturation in Type 1 diabetes. *Diabetes/Metabolism Research and Reviews* 16: 269-275
- II Riihimaa PH, Knip M, Ruokonen A & Tapanainen P (2002) Lack of physiological suppression of IGFBP-1 in puberty in patients with insulin dependent diabetes mellitus. *European Journal of Endocrinology* 147: 235-241
- III Riihimaa P H, Suominen K, Tolonen U, Jäntti V, Knip M & Tapanainen P (2001) Peripheral nerve function is increasingly impaired during puberty in adolescents with Type 1 diabetes. *Diabetes Care* 24: 1087-1092
- IV Riihimaa PH, Suominen K, Knip M, Tapanainen P & Tolonen U (2002) Cardiovascular autonomic reactivity is decreased in adolescent patients with type 1 diabetes. *Diabetic Medicine* 19: 932-938

In addition, some unpublished data are presented



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# 1 Introduction

Type 1 diabetes is characterised by insulin deficiency despite regular subcutaneous insulin administration. Prolonged exposure to high levels of blood glucose affects the microvascular vessels in various tissues such as the retina, kidney and neurons resulting in functional and structural changes. Manifestation of type 1 diabetes precedes the development of retinopathy, nephropathy and neuropathy by several years, the total glycaemic exposure over time being a major factor in the development and progression of the microvascular complications (1,2).

Although some patients are affected by microvascular complications of type 1 diabetes only after several decades, the advanced tissue changes may have their roots in early presentation with clinical diabetes, and patients with type 1 diabetes manifested in childhood have a greater lifetime risk of late complications (3).

The incidence of type 1 diabetes in children and adolescents has increased worldwide over the past decades (4), and a greater number of patients present with type 1 diabetes at a very young age. This is likely to lead to higher lifetime cumulative glycaemic exposure and a greater risk of microvascular complications than in patients with adult onset of type 1 diabetes. Although microvascular complications are rare in young children, several authors have pointed to the appearance of these problems during pubertal maturation (5,6), and it has been suggested that puberty may enhance the degenerative processes characteristic of type 1 diabetes (7).

## 2 Review of the literature

### 2.1 Hormonal and metabolic factors in puberty in type 1 diabetes

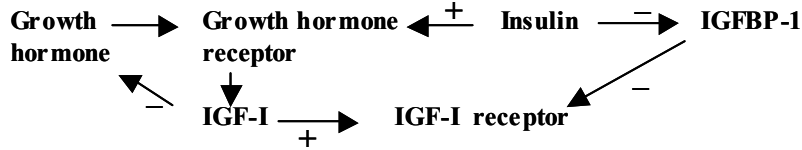
Several factors are implicated in decreased insulin bioavailability during puberty. Most importantly, the pulse amplitudes of growth hormone secretion are increased in response to early changes in sex steroids (8), while the relative insulin resistance in diabetes may lead to an adverse serum lipid profile with respect to macrovascular changes. Finally, serum insulin levels are linked with serum leptin concentrations, which reflect the function of adipose tissue (9).

*Growth hormone / IGF-I axis.* The increased peripheral effects of growth hormone during puberty in healthy adolescents lead to increased insulin secretion (10) to meet the heightened demand caused by peripheral insulin resistance. This has been demonstrated experimentally by means of the euglycaemic insulin clamp or hyperglycaemic clamp technique (11), or with intravenous glucose tolerance tests (10). Insulin requirements increase early in puberty and are highest in mid and late puberty. Healthy adolescent boys have a higher response in terms of insulin secretion than girls (10).

Insulin-like growth factor I (IGF-I) mediates the effects of growth hormone in peripheral tissues, and its function is also enhanced by insulin. Increased IGF-I levels have a negative feedback on growth hormone secretion. In case of low IGF-I levels in type 1 diabetes the negative feedback to growth hormone is attenuated (12,13). The increasing growth hormone levels initiate a cascade, as represented in Figure 1.

Specific binding proteins regulate the bioavailability of circulating IGF-I. IGF-binding protein 1 (IGFBP-1), which is synthesized in liver, is directly related to insulin levels in the portal circulation. Fasting IGFBP-1 levels become lower in the course of puberty, owing to increased insulin secretion during maturation (14). In contrast, the relative hypoinsulinaemia in the liver leads to inefficient suppression of IGFBP-1 levels (15). Although IGFBP-1 binds only about 10% of the circulating IGF-I, the increased levels may further decrease the bioactivity of IGF-I (8).





**Fig. 1. Schematic representation of the interaction between insulin and growth hormone, insulin-like growth factor I (IGF-I) and IGF binding protein -1 (IGFBP-1).**

*Serum lipid profile.* Serum total cholesterol and triglyceride levels increase slowly during early childhood and remain relatively stable up to the beginning of puberty. During puberty the total cholesterol and low-density lipoprotein (LDL) cholesterol levels fall with advancing maturation and high-density lipoprotein (HDL) also decrease especially in the boys (16). In type 1 diabetes, the overall lipid levels in children and adolescents are in the normal range (17), but when glycosylated haemoglobin levels are high, total and LDL cholesterol and triglyceride levels are elevated and HDL cholesterol levels depressed (17). Total cholesterol levels are lower in intensively treated adolescents with type 1 diabetes than in conventionally treated patients (18).

*Serum leptin.* Leptin, which is a 167-amino-acid peptide, is a product of the ob gene. Leptin inhibits food intake and increases thermogenesis in brown adipose tissue (19). Leptin is expressed in adipocytes, and its production is stimulated by insulin (9). It does not play a very central role as far as planning the care of adolescents with type 1 diabetes is concerned, but it may reflect adiposity and insulin bioavailability. Leptin levels remain low in children with type 1 diabetes before insulin treatment (20), but increase rapidly after its initiation (21). Later, leptin levels may be similar to (20) or higher than (22) those in healthy children and adolescents. Girls with type 1 diabetes have higher leptin levels than boys, a difference that is related to increased body fat in pubertal girls (22) and possibly the suppression of leptin levels by androgens in boys (23).

## 2.2 Pathology and evaluation of microvascular complications

The functional and structural changes entailed in late complications of type 1 diabetes are characterised by abnormalities in the microvasculature of various organs. The main feature is thickening of the basement membranes. The primary reason and initiator of these changes is hyperglycaemia. Although experimental and animal studies, together with clinical and genetic assessments have provided new information on the pathology of the late complications of type 1 diabetes and their pathogenetic mechanisms, there are several areas where the exact pathogenesis is not yet fully understood.

### ***2.2.1 Diabetic retinopathy***

Diabetic retinopathy relates to alterations in the function and structure of the microvessels of the retina and retinal nerve fibre layer. The progression of the structural changes occurs in succession, and this can be utilized for follow-up and treatment purposes. Retinopathy may be divided into two main stages, non-proliferative and proliferative stage (24).

The earliest changes are vascular microaneurysms, resulting from endothelial proliferation, and blot haemorrhages. In mild non-proliferative retinopathy the capillaries leak due to increased permeability. The pericytes, which support the structure of the vessel walls die, and the capillary basement membrane becomes thickened. Soft cotton-wool spots are also seen, reflecting ischaemic stasis in the nerve fibres (24,25).

In the middle stages of non-proliferative diabetic retinopathy the venous calibers are variable and show beading. There are intraretinal microvascular abnormalities and ischaemic areas, which relate to the closure and loss of capillaries. Furthermore, haemorrhages and microaneurysms become more extensive. These haemorrhages appear in the inner layer of the retina (dot-shaped haemorrhages), or into the nerve fibre layer (flame-shaped haemorrhages) (24,25). Increased vascular permeability may result in fluid accumulation in the retina and cause macular oedema at any stage (24,26).

As retinopathy advances into the proliferative stage, large vessels are also occluded, leading to proliferation of new vessels growing into the optic disc or other parts of the retina and vitreous. Haemorrhages occur in the preretinal area and vitreous body, and the iris may also neovascularise, resulting in glaucoma. Retinal traction and tears may occur, as well as retinal detachment. This stage entails a high risk of blindness (24).

#### ***2.2.1.1 Evaluation of diabetic retinopathy***

Direct evaluation and follow-up of the microvascular tissue changes in the retina is possible, but cataracts and glaucoma may occur in addition to the retinal changes. Thus the retinal examination should also include measurements of visual acuity and intraocular pressure (27). Dilatation of the pupils is recommended before retinal examination (24,27).

Direct or indirect ophthalmoscopy may be used, but these methods have restrictions that may cause loss of information on the retinal status (27). Direct ophthalmoscopy is not recommended as a screening method for retinopathy in children (28). Intravenous fluorescein angiography with a retinal fundus photograph is a sensitive method for detecting not only structural but also early functional abnormalities, but it may have adverse effects (27), and the earliest functional changes seen with this technique are not necessarily progressive (29). Fluoroangiography is not recommended for screening retinopathy in otherwise healthy patients (24). Fundus photographs are used most frequently in clinical work, and they are helpful for documenting and evaluating retinal changes at follow-up (24). A variable number of visual fields can be used (27).

Screening for diabetic retinopathy with fundus photography through dilated pupils is recommended for children and adolescents with type 1 diabetes. This should be started five years after the presentation of diabetes, at the age of 11 years or at puberty – whichever is the earlier – with annual follow-ups thereafter. In the case of pubertal onset diabetes, the first evaluation is recommended two years after onset (28).

The follow-up of retinal changes is important, since retinopathy may progress rapidly. Bonney *et al.* (30) observed that 11% of their adolescent patients with type 1 diabetes having early diabetic retinopathy showed progression during the median follow-up time of 1.3 years. It is recommended that cases of mild and moderate non-proliferative retinopathy without macular oedema in the adult patients should be re-examined within 6-12 months because of the increased risk of progression to the proliferative stage (31).

## 2.2.2 Diabetic nephropathy

Diabetic renal disease is characterised first by increased kidney size and glomerular filtration rate (GFR), followed by a progressive increase in urinary albumin excretion. Later on, glomerular filtration decreases and blood pressure levels increase. Persistently elevated urinary albumin excretion rate (AER) in type 1 diabetes is predictive of progression to nephropathy (32). Albuminuria reflects tissue changes in other organ systems as well (33), which makes the evaluation of kidney function in type 1 diabetes extremely important. Diabetic kidney disease can be indirectly assessed by clinical variables such as AER and functional tests such as GFR, while structural changes are seen directly in biopsy. Factors like increased vascular permeability in the retina bear similarities to features of diabetic renal disease.

### 2.2.2.1 Urinary albumin excretion rate

AER is enhanced by an increased GFR, or by a saturated tubular reabsorption capacity. Such conditions prevail in connection with exercise, upright posture, hyperglycaemia, infection and pregnancy (34). AER is approximately 30% higher in a 24-h urine sample than in an overnight urine collection (35), and there is considerable variation between consecutive samples. AER increases with puberty in healthy adolescents (37), and is elevated in many teenage patients with poor metabolic control (38).

AER in the range 20–200 µg/min in an overnight timed urine sample can be considered to represent microalbuminuria. There are several methods for screening like the albumin-to-creatinine ratio in a random spot sample, a 24-h sample with measurement of creatinine clearance, and a timed overnight urine collection (38). Because of the day-to-day variation, two out of three urine samples taken within three to six months should be abnormal to meet the definition of persistent microalbuminuria (28,38). The

recommendations regarding screening of children and adolescents with type 1 diabetes for microalbuminuria are identical to those for retinopathy (28).

### *2.2.2.2 Functional and structural aspects*

Increase in GFR is one of the earliest functional kidney changes in diabetes (39). In an Italian study GFR in adolescent patients with type 1 diabetes elevated during the first six years of diabetes followed by a slow decline (40).

The capillary endothelial cells, glomerular basement membrane and podocytes form a filtration barrier between the blood and the urinary space. The glomerular mesangial cells provide structural support for the capillary loops, and the surrounding matrix is composed of sulphated glycosaminoglycans, fibronectin and laminin (34). The structural kidney changes occurring in diabetes can be evaluated quantitatively from the thickness of the glomerular basement membrane and the mesangial and matrix volume as a proportion of the glomerular volume (41).

The renal mesangium, although not the mesangial cellularity, normally increases in relation to glomerular size in healthy children and adolescents (42). The basement membrane width and relative mesangial and matrix volume increase in normoalbuminuric children and adolescents with type 1 diabetes. These changes are detectable soon after presentation of diabetes (43), and they increase with disease duration (43) and with any deterioration in the glycaemic control (44). The glomeruli are usually not enlarged in early type 1 diabetes (43).

### *2.2.2.3 Natural history*

Like retinopathy, diabetic renal disease also develops in stages, and the progression can be divided into five phases (39). The initial silent kidney changes are functional ones, including glomerular hyperperfusion and hyperfiltration. Kidney size and glomerular volume also increase. These changes are most probably related to the ambient glucose level and thus reversible after initiation of insulin treatment (39).

Secondly, subtle morphological changes take place, such as thickening of the glomerular basement membrane, glomerular hypertrophy and mesangial and mild tubulointerstitial expansion (39). The AER may be within the normoalbuminuric range at this stage (43).

In the third phase, which may also be called incipient nephropathy, microalbuminuria is persistent, the glomerular basement membrane becomes thicker, and the mesangial matrix is further expanded. During the fourth phase AER continues to increase to reach macroalbuminuria, i.e. AER over 200 µg/min, and the GFR decreases. This phase represents overt nephropathy. If no therapy is provided to protect the kidneys, this will lead to the fifth phase, i.e. end-stage renal failure (39). The course of diabetic renal

disease is variable between patients, however, so that some may experience only mild disease even in the fourth decade of diabetes (45).

### ***2.2.3 Diabetic neuropathy***

The term diabetic neuropathy includes either a clinical or subclinical disorder without any additional causes of peripheral neuropathy other than diabetes (46). Since diabetes affects the somatic sensory and motor nerves and also the autonomic nerves, diabetic neuropathy is actually a group of neuropathies (47). It can be divided into reversible “hyperglycaemic neuropathy” and more persistent progressive neuropathy, where the latter includes symmetric focal, multifocal and distal diabetic polyneuropathy and mixed forms. Distal diabetic polyneuropathy is the most common of the diabetic neuropathies and implies diffuse symmetric damage to the peripheral small sensory and large motor nerves with or without changes in the function of the autonomic nervous system (ANS) (47,48).

#### ***2.2.3.1 Pathology***

The number of nerve fibres decreases and the myelin sheath degenerates, and changes also appear in the endoneurial connective tissue, endoneurial vessels and perineurium (49). An abnormal vasa nervorum may cause local nerve ischaemia (50), since the endoneurial capillary basement membrane is thickened, and the tight junctions between the cells decrease in number (51). The progression of nerve demyelination leads first to a decrease in conduction velocity and then to a nerve conduction block. The process of nerve demyelination may progress to Wallerian degeneration, in which the nerve axon is also injured and the distal part of the axon dies (49).

#### ***2.2.3.2 Evaluation***

*Peripheral nervous system.* Dysfunction of peripheral small nerve fibres involves diminished pain and temperature perception and it may include paraesthesias, dysaesthesias and neuropathic pain. If the large nerve fibres are affected, vibration, light touch and joint position senses are impaired, and the tendon reflexes are attenuated. Peripheral nerve function can be assessed objectively in terms of the vibration perception threshold (VPT), temperature thresholds and nerve conduction studies (52).

Subclinical diabetic neuropathy can only be detected by sensitive nerve function tests or nerve biopsy (46,53). Quantitative sensory testing, nerve conduction studies and testing of ANS function may be needed in addition to clinical examinations in order to

stage diabetic neuropathy (46). In the presence of unfavourable metabolic control children and adolescents should be examined in relation to symptoms of neuropathy, skin sensation, vibration sense, light touch and ankle reflexes (28).

*Autonomic nervous system.* Symptoms of ANS dysfunction, e.g. bladder complaints, intermittent diarrhoea, gastroparesis (gastric fullness), orthostatic light-headedness and gustatory sweating are rare in children and adolescents, but the symptoms are variable in patients with autonomic neuropathy (54,55).

Cardiovascular autonomic parasympathetic and sympathetic responses in heart rate and blood pressure during various stimuli can be evaluated with standard tests (56): heart rate with the Valsalva -ratio, orthostatic test and deep breathing test and reactions in blood pressure with the orthostatic test and sustained handgrip. There are several other tests available that are suitable for evaluating ANS function in children and adolescents, e.g. pupil reflex adaptation after dark and sympathetic skin response (57-60). Some of the cardiovascular tests are too demanding for children and adolescents (61).

Time domain and power spectrum analyses of heart rate variability (HRV) based on long-term or short-term are used frequently (62-65) and are considered more extensive than conventional cardiovascular reflex tests (66,67). The power (variance) spectrum of HRV contains three main frequency peaks. The high frequency (HF) power (0.15–0.4 Hz) of the HRV is related to respiration and efferent vagal activity, and increases during controlled respiration and cold stimulation of the face, while the low frequency (LF) power (0.04–0.15 Hz) is related to both sympathetic and vagal activity, and increases upon standing up. The very low frequency (VLF) power (<0.04 Hz) is related to cyclic fluctuations in peripheral vasomotor tone, but its physiological correlates are largely unknown (66,68).

### 2.3 Hyperglycaemia in the pathogenesis of microvascular complications

Hyperglycaemia is a major risk factor for the development of diabetic microvascular complications (69), and it promotes many functional changes in the microvasculature that lead to structural tissue changes. The mechanisms that mediate the adverse effects of hyperglycaemia include extracellular nonenzymatic glycation processes, sorbitol accumulation through aberrant aldose-reductase enzyme activation and alterations of various signal pathways like diacylglycerol (DAG) – protein kinase C (PKC) pathway (Figure 2).

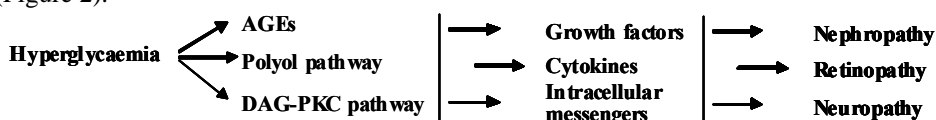
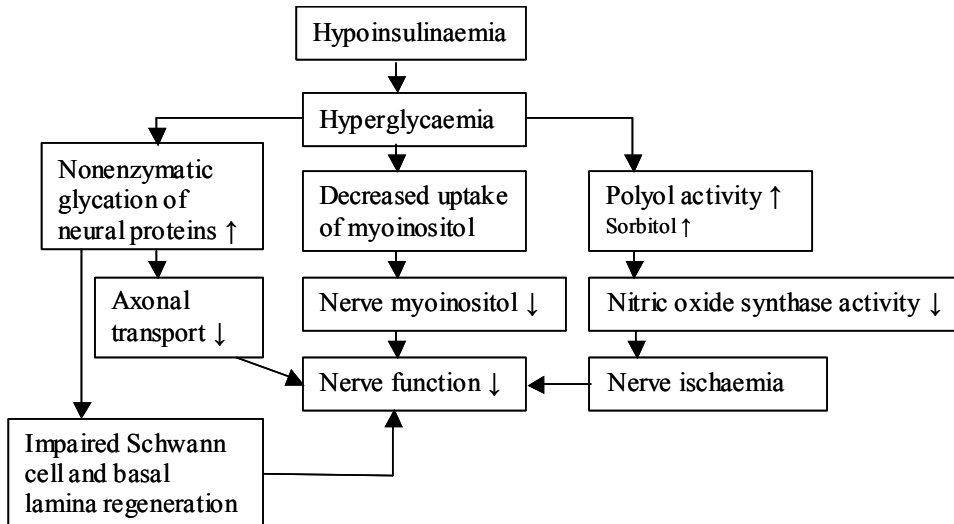


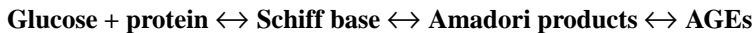
Fig. 2. Pathways of microvascular complications initiated by hyperglycaemia. AGEs, advanced glycation end products; DAG, diacylglycerol; PKC, protein kinase C.

In the kidneys, for example the earliest change is the increase of GFR, which is parallel with the increasing size of the kidneys (39,70). In the nervous system, experimental studies have shown that nerve conduction velocity and nerve amplitude decrease soon after manifestation of clinical diabetes. These changes correlate with nodal swelling, which relates to decreased paranodal sodium permeability, and retarded increase in the initial Na<sup>+</sup> current of the evoked potential (71). These early changes in different organs are reversible after initiation of insulin treatment and correction of hyperglycaemia. Some of the mechanisms involved in peripheral nerves are shown in Figure 3.



**Fig. 3. Some of the pathogenetic mechanisms responsible for the development of microvascular complications in diabetes (diffuse neuropathy).**

*Advanced glycation end products.* Extracellular glucose, fructose and early glycolytic intermediates may react with primary amines such as arginine and lysine in a non-enzymatic process forming advanced glycation end products (AGEs).



AGEs are irreversibly chemically damaged proteins, and in animal models the levels increase in kidneys, vascular tissue and skin within a few weeks of the induction of diabetes (72,73). They are useful biomarkers of tissue damage in diabetes, and they may themselves exacerbate the pathogenetic processes of late complications, by mediating increased extracellular mesangial matrix deposition, for example, which leads to mesangial expansion (74). They cause trapping and cross-linking of proteins, or they may bind to specific receptors on the cell surface and modulate cell functions (74-77). The activities and functions of the receptors *in vivo* remains unclear, although some of them are well characterised (78).

*Protein kinase C.* Hyperglycaemia activates the DAG - PKC signal pathway. DAG is a second messenger for activating protein kinases such as PKC (79,80). This pathway regulates many actions in vascular cells, such as permeability, contractility, cell growth and angiogenesis (79). PKC affects the activation of a number of growth factors and change the expression or function of vasoactive factors like endothelin-1 and nitric oxide (70,81).

*Aldose reductase activation.* Only a small proportion of glucose is metabolised to sorbitol during normoglycaemia, while in hyperglycaemia the enzyme aldose reductase is activated, leading to an accumulation of intracellular sorbitol that increases the flux through the polyol pathway. Accumulation of sorbitol is associated with nerve dysfunction and cataract and has been ascribed a potential role in the pathogenesis of the above complications. However, several trials have been performed that involve the inhibition of aldose reductase in patients with neuropathy without any conclusive results (59).

*Growth factors.* The abnormal glycaemic exposure that takes place in type 1 diabetes changes the expression and local effects of several growth factors, such as IGF-I, transforming growth factor (TGF) and vascular endothelial growth factor (VEGF).

The two main growth factors, which may affect the retinal microvasculature are IGF-I and VEGF. IGF-I stimulates the migration and proliferation of retinal vascular endothelial cells (82). The role of IGF-I in proliferative diabetic retinopathy is not clear, since IGF-I concentrations in the retina or vitreous body may increase secondarily as a result of retinal ischaemia (82,83). VEGF is normally expressed in the retinal vessel walls and retinal pigment epithelium, but its expression changes in incipient retinopathy. This leads to hyperpermeability and endothelial cell proliferation (84), while at the proliferative stage it induces compensatory formation of new vessel in the retina, disc or iris (84,85).

TGF is the main growth factor responsible for the pathological changes in the kidneys in diabetes. TGF normally mediates many functions including the synthesis of extracellular matrix and inhibition of the endothelial cell growth, while in diabetes its expression leads to accumulation of extracellular matrix components, i.e. fibronectin, collagen IV and VI (86). Besides hyperglycaemia, hypertension, AGEs and vasoactive hormones such as angiotensin II in human and rodent mesangial cells enhance the expression of TGF (74,86).

Neurotrophins participate in nerve growth, maturation and function, with retrograde neurotrophin flow along nerve fibres serving as the factor that allows nerve growth and regeneration (86,87). Nerve regeneration is impaired in type 1 diabetes at least partly due to altered retrograde transport of growth factors and decreased growth factor synthesis (86).



## **2.4 Epidemiology of diabetic microvascular complications**

### ***2.4.1 Retinopathy***

Considerable variations have been reported in the prevalence of retinopathy in young patients (Table 1). Mild non-proliferative changes are quite frequent, while proliferative retinopathy is rare (88). Bonney *et al.* (30) demonstrated that 11% of their adolescents with diabetic retinopathy progressed during a relatively short follow-up (range 0.5 – 3.0 years), while 5% showed regression. Seventy to 90% of the patients will develop retinopathy during 20 years duration of diabetes (89), and the cumulative incidence rate of proliferative diabetic retinopathy in patients over 15 years of age is 17.3 per 1000 patient years (90). Loss of vision occurs in about 2.5% of adult patients with type 1 diabetes, and it is related to long diabetes duration, poor glycaemic control and the grade of retinopathy (91).

### ***2.4.2 Microalbuminuria and nephropathy***

As with retinopathy, the reported prevalence of persistent microalbuminuria in adolescent patients with type 1 diabetes is also variable (89,92-94) (Table 2). In the Oxford Regional Prospective Study the cumulative probability for persistent microalbuminuria in children and adolescents was 18% after 11 years of diabetes (95). The cumulative probability of microalbuminuria during the prepubertal years has been reported to be 9% in patients under 5 years of age at presentation with diabetes, and the incidence density increases more during adolescence in these patients than in patients diagnosed between 5 and 11 years (95). Approximately two thirds of patients with persistent microalbuminuria during the first ten years of diabetes show further progress in AER during the next decade (96). The annual incidence of development of persistent microalbuminuria has been observed to be 2% in adult patients (97).

Nephropathy is seldom observed in children and adolescents until the second decade of type 1 diabetes (98), a situation which is substantially different from that in adult patients, 4% of whom develop nephropathy during ten years of diabetes (45). The incidence of nephropathy peaks during the second decade of type 1 diabetes and declines thereafter. The cumulative incidence of nephropathy after 40 years of diabetes is around 40% (45).

The incidence of nephropathy in type 1 patients is declining (99) as a consequence of improved glycaemic control over recent decades. Intensive insulin therapy reduces the risk of microalbuminuria and delays the onset of diabetic nephropathy and retards its progression (18,100). The prevalence of proteinuria is lower in European than in American patients, irrespective of the duration of diabetes (101).

Table 1. Studies on the prevalence of retinopathy in children and adolescents with type 1 diabetes.

Study (reference number)	Number of patients / boys	Age (years)	Duration of diabetes (years)	Retinopathy (%)	
				Non-proliferative	Proliferative
Olsen <i>et al.</i> 1999 (114)	339 / 180	21.1 <sup>1</sup> (12.0 – 26.9)	13.2 <sup>1</sup> (8.9 – 24.5)	58	1 patient
Donaghue <i>et al.</i> 1997 (115)	188 / 97	(15 – 22)	11.5 (9.2 – 13.5) <sup>2</sup>	52	-
Joner <i>et al.</i> 1992 (129)	371	18.3 (4.9)	10.1 (2.9)	33	-
Bognetti <i>et al.</i> 1997 (133)	228	15.9 (4.4)	8.8 (3.7)	20	3
Bonney <i>et al.</i> 1995 (30)	203	14.5 <sup>1</sup> (10.4 – 20.6)	6.6 (1.1 – 16.3)	41	-
Kernell K <i>et al.</i> 1997 (88)	557 / 278	14.6 (12.4 – 17.0)	5.4 (3.6 – 7.8)	15	2.3
D'Antonio <i>et al.</i> 1989 (17)	65 / 25	14.3 (5.8 – 20.9)	5.0	13	-
Falck <i>et al.</i> 1993 (158)	194 / 107	11.8 (4.6 – 16.6)	4.5 (0 – 14.2)	11	-

Data are means (range or SD), unless otherwise stated. <sup>1</sup> Median (range) <sup>2</sup> Patients with retinopathy; patients without retinopathy: 8.5 (6.0 – 11.7) years.

Table 2. Studies on the prevalence of persistent microalbuminuria in adolescent patients with type 1 diabetes.

Study (reference number)	Number of		Age (years)	Duration of diabetes (years)	Reference limit for		Prevalence (%)
	patients / boys	boys			urinary AER ( $\mu\text{g}/\text{min}$ )	urinary AER ( $\mu\text{g}/\text{min}$ )	
Rudberg <i>et al.</i> 1996 ** (140)	155 / 67		17.0 (SE 0.3)	9.8 (SE 0.3)	20 $\mu\text{g}/\text{min}$	T (2)	15
Rowe <i>et al.</i> 1984 * (92)	86 / 47		13.2 (7 - 19)	5.0 (3.4)	12.2 $\mu\text{g}/\text{min}$	T (1)	15
D'Antonio <i>et al.</i> 1989 * (17)	62 / 29		14.3 (3.7)	5.0	20 $\mu\text{g}/\text{min}$	D (1)	21
Salardi <i>et al.</i> 1990 * (29)	125 / 62		11.9 (5.0)	4.9 (4.7)	17 $\mu\text{g}/\text{min}$	D (2)	10
Dahlquist <i>et al.</i> 1987 * (159)	179 / 89		17 (4)	(5 - 20)	18.5 $\mu\text{g}/\text{min}$	D (1)	20
Mathiesen <i>et al.</i> 1986 * (93)	102 / 50		15 <sup>1</sup> (7 - 18)	> 2 years	14 $\mu\text{g}/\text{min}$	T (2)	20
Janner <i>et al.</i> 1994 ** (6)	164 / 81		10.2 (5.0 - 13.5)	(1 - 12)	20 $\mu\text{g}/\text{min}$ / 1.73 $\text{m}^2$	T (3)	20
Mortensen <i>et al.</i> 1990 * (94)	1020 / 546		(1.4 - 18.9)	(0.0 - 17.6)	20 $\mu\text{g}/\text{min}$	T (2)	4

Data are means (SD or range), unless otherwise stated. SE; standard error of the mean, <sup>1</sup>Median (range). \* Cross-sectional study, \*\* Follow-up study.

AER: albumin excretion rate, T; timed overnight urine sample D; 24-hour urine sample, number of abnormal samples required for diagnosis in parenthesis.

### ***2.4.3 Peripheral and autonomic neuropathy***

*Peripheral somatic neuropathy.* The prevalence of subclinical distal diabetic neuropathy varies between 0 and 70% in studies on children and adolescents. This wide variation may be due to the variety and number of measurements included in the definition of neuronal dysfunction (102-105) (Table 3). Peripheral nerve dysfunction in children and adolescent patients with type 1 diabetes progresses slowly (106). In an Australian study, re-testing of peripheral nerve function in response to hot, cold and vibration sensations in adolescent patients did not reveal any increase in the number of abnormalities over a 3-year period, nerve dysfunction being unrelated to age, duration of diabetes and glycaemic control (106). On the other hand, an epidemiological study in Pittsburgh showed the prevalence of symmetric clinical diabetic polyneuropathy to be 18% in patients with type 1 diabetes between 18 and 29 years of age (107).

*Autonomic neuropathy.* The definitions of ANS dysfunction in children with type 1 diabetes have been variable, partly due to the number and quality of the individual tests used, and partly to the considerable variability in the normal ranges for ANS function in young patients (Table 4). The prevalence of autonomic dysfunction, based on one or more abnormal cardiovascular reflex tests varies between 0–40% (106,108-110), while figures based on two or more test abnormalities range up to 24 % (111). Some series suggest a very early onset of ANS dysfunction (112), while other studies have observed only mild deterioration in the cardiovascular reflex tests, or none at all, during several years of follow-up (61,106,113). There are only few reports of permanent changes in ANS in children and adolescents with type 1 diabetes (61).

*Table 3. Studies on the prevalence of peripheral nervous system dysfunction according to the definition used in adolescent patients with type 1 diabetes compared with the control subjects.*

Study (reference number)	Number of subjects	Age (years)	Duration of diabetes (years)	Definition of dysfunction	Abnormal (%)
Bognetti <i>et al.</i> 1997 (133)	292	15.9 (4.4)	8.8 (3.7)	Nerve conduction tests > ± 2 SD for age	19
Hyllienmark <i>et al.</i> 1995 (182)	75	15.4 (3.6)	8.2 (3.5)	Peroneal nerve conduction velocity < - 2 SD	41
Bao <i>et al.</i> 1999 (105)	38	12.7 (4 – 21)	7.2 (1 – 15)	Nerve conduction tests > ± 2 SD	69
Duck <i>et al.</i> 1991 (103)	66	12.7 (6.2 – 18.2)	6.1 (1.2)	1 or more nerve conduction tests > ± 3 SD <sup>1</sup>	0
Käär <i>et al.</i> 1983 (131)	161	12.5 (3.6)	5.3 (3.9)	Peroneal nerve conduction velocity < -2 SD	30
Donaghue <i>et al.</i> 1993 (57)	181	15.0 (1.9)	7.8 (3.2)	Temperature or VPT tests > 95 % reference limit	9
Olsen <i>et al.</i> 1994 (180)	61	15.5 (10 – 21)	6.9 (1 – 19)	VPT > 95th percentile	20
Barkai <i>et al.</i> 1998 (160)	92	14.2 (2.1)	5.8 (3.0)	1 or more current perception threshold above normal	23
Davis <i>et al.</i> 1997 (104)	307	13.3 (4.6)	5.1 (4.9)	VPT > 97th percentile	9

Data are mean (SD or range). VPT; vibration perception threshold. <sup>1</sup> in 2 or more nerves + ankle reflexes or VPT reduced

Table 4. Studies on the prevalence of autonomic nervous dysfunction in cardiovascular reflex tests in children and adolescents with type 1 diabetes compared with the control subjects.

Study (reference number)	Number	Age (years)	Duration of diabetes (years)	Cardiovascular reflex tests	Abnormal (%) (number of tests)
Holder <i>et al.</i> 1997 (109)	204	16.5 (4.3)	7.9 (5.3)	Valsalva ratio, DB, 30:15 ratio	34 (1 or over)
Verrotti <i>et al.</i> 1995 (108)	110	14.9 (7.1)	6.1 (4.0)	Valsalva ratio, DB, standing, sustained handgrip < 2.3 th percentile	43 (1 or over)
Barkai <i>et al.</i> 1995 (111)	110	13.0 (2.4)	6.0 (3.8)	Heart rate at rest, DB, standing, sustained handgrip <sup>1</sup>	15 (1); 24 (2 or over)
Ringel <i>et al.</i> 1993 (61)	248	11.6 (2.6)	4.0 (3.2)	DB, HRV during standing <sup>2</sup>	3 (2)
Karavanaki <i>et al.</i> 1997 (112)	129	11.9 (3.4)	3.9 (3.2)	HRV during rest and standing, DB, Valsalva ratio <sup>1</sup>	16 (1); 8 (2 or more)

Data are means (SD). <sup>1</sup>> 95% or < 5% confidence limit <sup>2</sup> < 5th percentile. DB; deep breathing test, HRV; heart rate variability.

## **2.5 Risk factors for microvascular complications in diabetes**

### ***2.5.1 Glycaemic control***

The rate of non-proliferative diabetic retinopathy in the Berlin Retinopathy Study was found to increase with deteriorating glycaemic control, the greatest progression being observed in patients with long-term HbA1c within the highest quartile (116). Bonney *et al.* (30) reported that progression of diabetic retinopathy was twice as likely in adolescent patients with high HbA1c and long duration of diabetes as in patients without these risk factors. The model presented by Javitt *et al.* (117) predicted that 72% of patients with type 1 diabetes and inadequate metabolic control would develop proliferative retinopathy at some stage in their life-time. There are also reports of transiently progressive retinopathy in the presence of strictly improved metabolic control (100,118).

The overall increase in AER and the development of persistent microalbuminuria is primarily determined by poor long-term metabolic control (119), and poor control during the first years of diabetes in particular may later predispose to microalbuminuria (120). In a large prospective study from Denmark the relative risk for progression from normoalbuminuria to micro- or macroalbuminuria was 1.13 (95% confidence interval, CI 1.04–1.23) for patients with poor metabolic control (121). A direct association between microalbuminuria and high HbA1c has been established especially in adolescent girls with type 1 diabetes (94), while there is no such gender difference in adults with microalbuminuria (119). Incipient structural changes in the kidneys of children and adolescents do not necessarily relate to long-term glycaemic control (43), but high HbA1c is an independent risk factor for increasing basal membrane thickness (122).

Nerve conduction, which is slightly reduced at the time of diagnosis of type 1 diabetes, improves with the improvement of metabolic control (123,124), and long-term normoglycaemia retards the development of peripheral nerve dysfunction (2,125). The Oslo study, for example, showed decreases in motor and sensory nerve conduction velocities along with deterioration of glycaemic control, independently of other parameters (125). In some studies reduction in nerve conduction has been related to poor metabolic control more strongly in male than in female patients (126).

### ***2.5.2 Duration of diabetes***

Although structural changes in the retina may be found shortly after the diagnosis of diabetes, they are usually not reproducible (127). Retinopathic lesions may improve and fluctuate (30,127). There are a few young patients who experience retinopathy within a couple of years of diabetes. Prevalence of retinopathy increases with increasing duration of the disease, since one third of patients with a duration between 10 and 12 years have retinopathy (88), and nearly all of those with a duration of 20 years or over have it to

some degree (89,128). Conversely, it has also been demonstrated that patients with more advanced retinopathy have a long duration of diabetes. Patients with type 1 diabetes diagnosed between 10 and 30 years of age may experience significant retinal changes within ten years thereafter (129), and proliferative retinopathy appears to 4% of those who have had diabetes for 10 years, 25% of those with 15 years of diabetes (130), and 30% of those who have had it for over 20 years (128).

The duration of diabetes may have less influence on diabetic nephropathy and microalbuminuria than on retinopathy. The incidence peak of diabetic nephropathy occurs during the second decade of type 1 diabetes and declines thereafter (45), while only a slight increase in the prevalence of microalbuminuria has been observed during the second decade of diabetes in young adult patients (129).

Many previous series and some recent ones have showed that duration of diabetes in addition to poor glycaemic control have a negative impact on peripheral nerve function in children and adolescents with type 1 diabetes (69,103,131-133).

### ***2.5.3 Blood pressure***

An increase in blood pressure is most closely related to diabetic renal disease. Whether blood pressure is a primary or secondary factor in the development of diabetic nephropathy was for a long time questionable. Originally it was thought that blood pressure increases as a consequence of the renal lesions, since an elevated AER seemed to precede any increase in blood pressure in type 1 diabetes and the diastolic blood pressure was shown to increase before the systolic blood pressure level (97). This view differed, however, from the findings of two previous studies, which showed that parental familial hypertension increased the risk of persistent microalbuminuria (134,135) suggesting that genetic susceptibility to high blood pressure is associated with the development of diabetic nephropathy. The latter view was supported by a more recent 24-hour blood pressure monitoring study of Finnish and Danish patients with type 1 diabetes (136).

The view today is that subtle changes in blood pressure precede the development of nephropathy. An increased blood pressure level is a consistent finding related to diabetic renal disease, and blood pressure lowering has a dramatic effect on the progression of nephropathy (137). The use of angiotensin converting enzyme (ACE) inhibitors further retards disease progression relative to blood pressure control alone (138).

Hypertension has also observed to influence on the progression to clinical neuropathy in adult patients with type 1 diabetes, in addition to other risk factors such as poor metabolic control, smoking, duration of diabetes and height (139).



### ***2.5.4 Microalbuminuria***

Microalbuminuria at the level of 30 µg/min seems to predict an increase in AER (140). If microalbuminuria is intermittent, it is reversible in a considerable proportion of patients, but it can still be regarded as a risk factor for AER progression, since one fourth of adolescent patients still progress to persistent microalbuminuria during the subsequent years (141). Although the types of samples and cut-off levels for microalbuminuria vary between reports (Table 2), it has been accepted that persistent microalbuminuria in type 1 diabetes increases the risk of the later development of overt nephropathy (32,142,143).

AER *per se* may enhance the progression of albuminuria, as has been demonstrated in normoalbuminuric adult patients who later developed persistent microalbuminuria, in that they had previously had significantly increased AER, although within normal range, compared with non-progressors (97). Furthermore, AER has been reported to increase about 20% per year in patients who will later develop persistent proteinuria (144).

Persistent microalbuminuria is a risk factor for diabetic retinopathy, in addition to poor glycaemic control, long diabetes duration and elevated blood pressure (17,116,130).

### ***2.5.5 Genetic factors***

Consideration of the influence of genetic factors on microvascular complications has largely been focused on diabetic nephropathy. Nephropathy affects only one third of patients with type 1 diabetes, and in contrast to retinopathy and neuropathy, has an incidence peak during the second decade of diabetes, declining thereafter (45). The risk of nephropathy is increased in patients who have siblings with type 1 diabetes and nephropathy (145,146). This familial clustering suggests that genetic factors are important in the development of renal disease. Furthermore, parental hypertension (134,136), parental type 2 diabetes (147) and increased parental cardiovascular mortality (148) all favour some measure of genetic susceptibility. Many candidate genes conferring susceptibility to diabetic nephropathy have been implicated, but without any conclusive results so far (149).

### ***2.5.6 Other risk factors***

*Smoking.* There are reports that patients with persistent microalbuminuria or nephropathy smoke more than those without renal problems (45). In an Australian study, smoking was more prevalent among adolescent patients with microalbuminuria than among those without, and smoking was directly related to the progression of microalbuminuria (150). Interestingly, maternal smoking during pregnancy has also been showed to be associated

with persistent microalbuminuria in young adults with type 1 diabetes. This may be associated with low birth weight and elevated blood pressure (151).

*Gender.* Men have a higher risk of nephropathy than women (45), whereas no such clear relationship with sex has been demonstrated for elevated urinary AER or microalbuminuria (90,111). The EURODIAB Study reported moderate non-proliferative diabetic retinopathy to be significantly less frequent in women than in men, but mild non-proliferative and proliferative diabetic retinopathy did not differ between the sexes (91).

## 2.6 Impact of puberty on microvascular complications

The impact of pubertal maturation on diabetic microvascular complications has attracted much interest in recent years. Studies on hormonal profiles in adolescents with type 1 diabetes have provided new approaches for estimating the relation between puberty and tissue changes related to the development of microvascular complications. There are several variables, however, which may be directly associated with pubertal maturation, e.g. age, increase in HbA1c, height, duration of diabetes, daily insulin dose, and not least, psychosocial problems and adherence to diabetes care, and these interrelated factors may further complicate the possible conclusions to be reached on the impact of the pubertal years on diabetes.

It is possible to examine pubertal maturation closely by means of pubertal staging (152). Few studies in this area have, however, been designed to evaluate the Tanner stages of pubertal maturation (7,88,106,111), but instead the tendency has been to choose an arbitrary age limit for the onset of puberty (63,94,110,153,154).

*Retinopathy.* Diabetic retinopathy has been reported in prepubertal patients with type 1 diabetes, but the prevalence is much lower than in the late pubertal period (155,156), and it is extremely rare in children younger than 10 years, regardless of the duration of diabetes (157). In a Swedish multicenter study, diabetic retinopathy regardless of stage, was detected in 6% of the prepubertal patients compared with 18% of the postpubertal patients with a similar duration of diabetes (88).

Although patients with type 1 diabetes diagnosed before 10 years of age have a longer retinopathy-free latency period than those diagnosed close to or in puberty (127), the long-term risk for diabetic retinopathy is greater in adolescent and young adult patients diagnosed before puberty than in patients with onset during or after puberty (90,128,157,158), and patients with very early onset i.e. under eight years of age, also have an increased risk of proliferative retinopathy as young adults (128).

*Persistent microalbuminuria.* Prepubertal metabolic control in type 1 diabetes has been reported to contribute to the risk of microalbuminuria during and after puberty (95) and pubertal development seems to initiate (159) or enhance the progression of microalbuminuria (7,95,150). Some studies have emphasized that microalbuminuria is more frequent in early than late puberty (6). The progression of AER and the risk of persistent microalbuminuria are higher in pubertal patients than in prepubertal or

postpubertal ones (7), while if microalbuminuria is diagnosed at the end of puberty, it is more likely to progress than regress (140).

*Peripheral and autonomic nervous system.* It is obvious that puberty may have an enhancing effect on nerve dysfunction, since at least peripheral sensory nerve dysfunction has shown to deteriorate with advancing puberty, in addition to poor glycaemic control (111,160). Advanced puberty (160) and height gain (161,162) have negative influence on peripheral nervous system function in type 1 diabetes. Pubertal staging has seldom been performed on series that have undergone tests for peripheral nervous system or ANS function (106,111), age having been used as a marker of puberty in earlier power spectrum analyses of HRV and blood pressure (63,64,110,154).

### **3 Purpose of the research**

The purpose of the present work was to evaluate the impact of puberty on microvascular complications of type 1 diabetes. The more specific aims of the individual papers were:

1. to assess the prevalence of retinopathy and persistent microalbuminuria and to evaluate the role of metabolic and hormonal factors and lipid profile in microvascular complications during puberty in type 1 diabetes (I)
2. to assess insulin bioavailability and evaluate the effect of insulin treatment on adolescent patients with type 1 diabetes (II)
3. to evaluate the relationship between pubertal maturation and peripheral neuronal function in type 1 diabetes (III)
4. to evaluate cardiovascular ANS function in adolescent patients with type 1 diabetes (IV)

## **4 Subjects and methods**

### **4.1 Subjects**

The initial population consisted of all the patients with type 1 diabetes who attended the Paediatric Diabetes Outpatient Clinic at Oulu University Hospital between March 1997 and February 1999, together with healthy age and sex-matched control subjects. The clinic takes care of all patients with type 1 diabetes diagnosed before the age of 16 years in the region, and follows the patients up to the age of 16–18 years. The inclusion criteria for the present series were duration of diabetes over two years and age over nine years in girls and over ten years in boys. The characteristics of the subjects are detailed in Table 5.

One hundred and one of the 138 eligible patients agreed to participate, one of whom was excluded from the analyses because of Down syndrome and hypothyroidism. Age and sex-matched healthy children were recruited from nearby schools, their records being checked at the school health service clinic to exclude any with chronic illness. Around one third of the invited control subjects actually agreed to participate, but none withdrew their participation once the research had started.

All of the children and adolescents concerned and at least one parent of each gave informed and written consent. The protocol was approved by the Ethics Committee of the Medical Faculty at the University of Oulu (patients), and by the Ethics Committee of the Oulu Municipal Health Care Service (control subjects). The research was conducted according to the provisions of the Declaration of Helsinki.

The patients who refused to participate did not differ from the participants with respect to age, duration of type 1 diabetes or glycaemic control (Table 5 and Table 9).

*Table 5. Characteristics of the patients and the control subjects and the non-participants. T I – T V are Tanner pubertal stages.*

	Patients	Control subjects	Non-participants
N (boys / girls)	101 (49 / 52)	100 (49 / 51)	37 (21 / 16)
N (boys / girls) at Tanner stages			
T I <sup>1</sup>	26 (15 / 11)	15 (8 / 7)	-
T II	15 (11 / 4)	17 (10 / 7)	-
T III	13 (4 / 9)	13 (8 / 5)	-
T IV	27 (11 / 16)	26 (11 / 15)	-
T V	19 (8 / 11)	29 (12 / 17)	-
Age (years)	13.8 (9.3 – 19.0)	13.8 (9.2 – 18.8)	14.9 (10.7 – 16.5)
Age at pubertal stages			
T I	11.2 (9.3 – 12.8)	11.3 (9.2 – 12.0)	-
T II	12.9 (10.9 – 14.9)	11.8 (9.8 – 13.5)	-
T III	13.8 (11.8 – 16.6)	12.9 (11.6 – 14.0)	-
T IV	14.5 (12.4 – 16.0)	14.4 (12.5 – 15.7)	-
T V	16.1 (14.6 – 19.0)	15.7 (14.1 – 18.8)	-
Duration of diabetes (years)			
All	6.2 (2.1 – 15.6)	.	6.5 (2.6 – 13.4)
T I	5.7 (2.1 – 10.7)	.	-
T II	5.7 (2.7 – 10.1)	.	-
T III	6.6 (2.7 – 10.7)	.	-
T IV	8.3 (2.2 – 14.5)	.	-
T V	7.6 (2.4 – 15.6)	.	-

Data are medians (range). <sup>1</sup> Five of the patients at Tanner stage I had advanced to stages T II or T III by the time of the analyses in papers III and IV.

## 4.2 Methods

All patients and control subjects were hospitalised for a 22-24 h period at the beginning of the investigation for interviews and clinical examinations conducted by the author.

### 4.2.1 Clinical examination (I-IV)

The pubertal staging (stages T I – T V) was based on genital maturation (G 1-5) in the boys and breast staging (B 1-5) in the girls (152). Testes volume (ml) was calculated as  $0.52 \times \text{length (cm)} \times \text{width squared (cm)}$  (163). Volume of the larger testis of more than 2.0

ml or testicular length of more than 20 mm was regarded as indicating the start of pubertal development in the boys.

The anthropometric measurements were performed three times consecutively and the mean value used as the actual measurement. Weight (kg) was measured once to the nearest 0.1 kg with an electronic scale, and height (m) to the nearest 1.0 mm with a wall-mounted Harpende stadiometer. The Finnish growth charts were used to assess the relative height for age (SD) and relative weight for height (%) (164). Body mass index was calculated as weight (kg) divided by height (m) squared ( $\text{kg}/\text{m}^2$ ). Waist and hip circumferences were assessed to the nearest 1.0 mm (165), and the waist to hip –ratio was calculated. The biceps, triceps and subscapular skin folds were measured to the nearest 0.1 mm with a Harpende skinfold caliper (John Bull, UK). Body density was estimated from regression equations based on the triceps and subscapular skin folds (166), and total body fat (%) was calculated (167).

The insulin injection sites were evaluated for possible lipohypertrophy or lipoatrophy, and the distal and proximal interphalangeal joints and the metacarpal joints of the fingers were examined to assess possible joint changes associated with type 1 diabetes.

The tendon reflexes in the quadriceps, gastrocnemius, biceps and triceps muscles were examined bilaterally. The sense of vibration was assessed using a 260 Hz vibrating fork. The sense of light touch, pain and joint position in the index finger and big toe were evaluated bilaterally with a round cotton-wool stick, pinprick and change in joint position, respectively. The tendon reflexes and all the sense modalities were classified as present or absent.

Blood pressure (mmHg) was measured with a random zero sphygmomanometer (Hawksley, UK) three times consecutively after 5 minutes rest in supine position and with two minutes rest between the measurements. Korotkoff's first and fifth phases were taken to represent the systolic and diastolic blood pressures, respectively.

#### ***4.2.2 Background information (I-IV)***

All patients were receiving intensive insulin treatment, with three to five daily injections, except for one girl at Tanner stage I who was treated with two daily injections. The history for possible other diseases as well as for other drugs than insulin was taken. One patient had epilepsy and was being treated with oxcarbazepine and lamotrigine. One boy and one girl had coeliac disease and two girls had bronchial asthma regularly treated with fluticasone and nedocromil. One boy at Tanner stage II had had a Wilms' tumour, the treatment of which had been completed at the age of 5 years. Smoking status and alcohol habits were recorded. Similarly the daily insulin dose, injection sites and injection times were recorded.

A questionnaire regarding type 1 diabetes, type 2 diabetes and hypertension in first and second-degree relatives was completed at the beginning of the research by the patients and their parents and checked by the author. A standard direct questionnaire regarding possible symptoms and signs of peripheral somatic or autonomic neuropathy, modified from previous studies (168,169), was completed at registration by the author.

### ***4.2.3 Eye fundus examination (I)***

Bilateral fundus photographs were taken by a photographer at the Department of Ophthalmology if the latest examination had been performed more than three months before the research began. After dilating the pupils with one drop of cyclopentolate (Oftan-Syklo®) 10 mg/ml in each eye, a Canon CF-60Z fundus camera with a Kodak DCS 100 digital camera pack was used to take a 60° digital colour photograph centred on the fovea centralis. The fundus photographs were evaluated by a senior ophthalmologist who was blinded to the clinical data.

### ***4.2.4 Urine examination (I)***

Timed overnight urine was collected in the ward. The subjects voided in the evening just before going to bed in order to empty the bladder, and the urine collected during the next eight to ten hours was analysed immediately in the morning.

Persistent microalbuminuria was defined as urinary AER between 20–200 µg/min in at least two successive urine samples. Normoalbuminuria was defined as an AER < 20 µg/min. A separate urine sample was taken to exclude urinary tract infection and haematuria. The subjects were asked to avoid vigorous exercise during the day before the examination in order to avoid false positive albuminuria. If a girl had menses at the time, urine was collected later at home.

Altogether 76 patients (40 boys, 36 girls) who were initially normoalbuminuric subsequently collected one or two timed overnight urine samples at home; 29 of them within 6 months before or after the investigation proper, 44 patients between 6 months and 1.5 years after the initial urine sample, and three patients 1.5–2.5 years afterwards. All these patients remained normoalbuminuric. One urine sample was collected from each healthy control subject. The AER reading obtained in the examination itself was used in the statistical analyses.

### ***4.2.5 Electroneurography and vibration perception threshold (III)***

Surface skin electrodes were used to measure the compound muscle action potential (CMAP) and sensory nerve action potential (SNAP). The skin temperature was measured, and the extremities were warmed with warm water, a heating gel-bag or a hot-air fan if necessary, in order to keep the skin temperature at 32 °C. The previously described criterion of two or more nerve conduction tests below the 1st or above the 99th percentile of the control values (46,170) was used for peripheral polyneuropathy in diabetes. The CMAP and SNAP variables reflect the functioning of the distal parts of the peripheral



nerves, whereas the H-reflex latency difference (see below) reflects the functioning of the peripheral nerve proximally (tibial nerve).

*Motor nerve conduction.* The CMAP of the right median nerve was obtained on the abductor pollicis brevis muscle. The nerve was stimulated supramaximally at the wrist 65 mm above the recording electrode and at the elbow. The peroneal nerve CMAP was recorded on both sides of the extensor digitorum brevis muscle after supramaximal stimulation at the ankle 75 mm above the recording electrode and at the popliteal fossa. The distal latency was measured from the onset of the stimulus to the initial negative deflection.

*Sensory nerve conduction.* SNAP of the median nerve was measured by the antidromic technique with ring electrodes placed 2.5 cm apart on the index finger. The sural SNAP was recorded behind the lateral malleolus, with stimulation applied 14 cm proximal to the recording electrode.

*H-reflex.* The H-reflex is an oligosynaptic reflex and it represents the afferent nerve impulses in the sensory fibres extending from the muscle spindles and the efferent nerve impulses in the alpha-motor axons. H-reflex includes the reflex arch of the sensory dorsal root, spinal cord, and motor ventral root, and H-reflex latency difference (total H-reflex latency minus latency from the popliteal fossa to the soleus muscle) reflects the function of the proximal part of the tibial nerve. The H-reflex was measured on the soleus muscle by stimulating the tibial nerve at the popliteal fossa for 0.5 ms at slow stimulus frequency.

*F-wave.* The F-wave of the peroneal nerve was obtained by supramaximal stimulation at the popliteal fossa. The shortest F-wave latency out of 16 responses was selected.

*Vibration perception threshold.* Two consecutive recordings of VPT at 63, 125, 250, and 500 Hz were performed on the second metacarpal and first metatarsal bones bilaterally with a Vibrometry system 9589 (171). VPT was recorded by pressing a button and holding it down until the sense of vibration disappeared. The perception graph was recorded as a vibrogram (dB,  $10^{-6}$  m/s<sup>2</sup>). The mean of two consecutive recordings was taken as the VPT. The room temperature was stable at 22 °C. Skin temperature was recorded, and the extremities were warmed up when necessary.

#### ***4.2.6 Cardiovascular tests and heart rate variability (IV)***

Cardiovascular reflex tests were performed under standardized conditions in a warm (22°C), silent room at the same time of the day for all subjects (6.30 p.m.) after an identical diurnal schedule, including dinner (and short-acting insulin for the patients) 2 to 3 hours before the test. The author performed all the tests according to the same protocol. The subjects were asked not to smoke or consume drinks containing caffeine before the tests.

The cardiovascular reflex tests were performed after 30 min of supine rest. The electrocardiogram and nasal thermistor signals were recorded using a 14-channel electroencephalograph (Neurofax, Japan), with a 12-bit A/D converter having a sampling frequency of 320 Hz. The data was stored in a computer, for analysis after visual on-line and off-line checking of the electrocardiogram and breathing signal (172).

#### *4.2.6.1 Time domain analysis and blood pressure change*

The following time domain heart rate and blood pressure parameters were analysed:

- 1) Standard deviation of all R-R intervals.
- 2) The square root of the mean squared differences between successive R-R intervals during normal breathing for 10 min at rest.
- 3) Paced respiration of six deep but not maximal breaths (5 sec in, 5 sec out) for 1 min during deep breathing tests performed twice at a 3 min interval. The longest and shortest R-R intervals in the six breathing cycles were selected for calculation of the max/min ratio, and the highest median of the max/min ratio was taken as the individual test result.
- 4) After another 15 min of supine rest, the subjects rose quickly. The longest R-R interval around beat 30 (20-40) divided by the shortest R-R interval around beat 15 (10-20) after changing from supine to standing position was taken as the 30:15 ratio.
- 5) The blood pressure response to standing up from a lying position was quantified in terms of the changes in systolic and diastolic blood pressure (Korotkoff first and fifth phase, respectively), as measured with a standard sphygmomanometer at 1, 3, 5, and 7 min of standing. The blood pressure responses to standing were calculated as the differences between the measurements at 1, 3, 5 and 7 min during standing and 3 min before standing.

The heart rate and blood pressure recordings for the subjects who had to sit down during the 7 min of standing were included in the analysis only for the period before sitting.

#### *4.2.6.2 Power spectral analysis and fractal dimension*

Power spectral analysis of the R-R series of HRV was performed using direct Fourier transformation (68,173). R-R intervals at rest and while standing were converted to a smoothed instantaneous R-R time series at 4 Hz after manual editing of ectopic beats, and exact Hanning windowing ( $\cos^2$ ) was applied before direct Fourier transformation. Total power and the VLF (< 0.04 Hz), LF (around 0.04 – 0.15 Hz) and HF (around 0.15 – 0.4 Hz) components were identified for each subject visually from the Fourier series. The HF band was selected manually according to the calculated actual respiration frequency spectrum.

Both absolute and normalized power spectra were calculated, together with the LF: HF -ratio. The normalized units represent the relative value of each power component in proportion to the total power minus the VLF component, e.g.  $normalized\ LF = LF / (total\ power - VLF)$  (66).

The fractal dimension, which describes the random behaviour of the R-R interval dynamics (174), was computed by the Mandelbrot- $\epsilon$ -blanket method.

The data files were analysed by a neurophysiologist who was not aware of the disease status of the subjects.

#### 4.2.7 Laboratory methods

*Urinary albumin.* The urine samples were analysed immediately, without freezing. Urinary albumin concentrations were measured with an immunoturbidimetric analyser (Orion Diagnostica, Espoo, Finland) having a sensitivity of 5 mg/l and an interassay coefficient of variation (CV) of 6% at a concentration of 35 mg/l and of 5% at 100 mg/l.

*Serum lipids.* Serum total and HDL cholesterol and triglycerides (mmol/l) were analysed by standard laboratory methods, and LDL cholesterol levels were calculated with Friedewalds formula.

*Blood glucose.* Fasting blood glucose concentration (mmol/l) was measured for the patients with a bedside glucometer.

*Glycated haemoglobin.* Glycated haemoglobin (HbA1c, %) was measured by high-pressure liquid chromatography, the non-diabetic range being 4.0-6.0 %. Current HbA1c was determined, and the mean of all HbA1c values analysed at regular clinical visits over the preceding 2 years (2-3 measurements) was calculated and taken as the long-term HbA1c.

*Serum free insulin.* Serum free insulin concentrations (mU/l) were quantified with an enzyme-linked immunosorbent assay (DAKO Diagnostics Ltd, Ely, Cambridgeshire, UK) after pretreatment of the sample with polyethylene glycol to precipitate insulin-insulin antibody complexes (175). The sensitivity of the assay was 0.5 mU/l and the intra-assay and inter-assay CV was less than 7.5% and 9.3%, respectively.

*Insulin antibodies.* Insulin antibodies (IA) were analysed with a modified radio-binding micro-assay (176). The cut-off limit for antibody positivity was set at the 99th percentile in 371 non-diabetic Finnish subjects (1.56 relative units, RU). The intra-assay CV for a positive control sample with a medium antibody level was 7% and the inter-assay CV less than 9%.

*Serum IGFBP-1.* Serum IGFBP-1 concentrations ( $\mu$ g/l) were measured with an immunoenzymometric assay (PP12 IEMA test, Medix Biochemica Oy, Kauniainen,

Finland) having a sensitivity of 0.4 µg/l (177). The intra-assay CV was 2.5 % at a serum concentration of 5.5 µg/l and the inter-assay CV 6.4 % at a serum concentration of 4.8 µg/l.

*Serum leptin.* Serum leptin concentrations (µg/l) were quantified with a specific radioimmunoassay for human leptin (Linco Research Inc., St Charles, MO, USA). Intra-assay and inter-assay CV was less than 5 % and 6 %, respectively, at serum concentrations of 7–30 µg/l (178).

#### 4.2.8 Statistical analysis

Continuous variables were expressed as means (SD) or medians (range), and proportions as percentages (I-IV). Cross-tabulation and chi-square statistics or the Fisher test were used when analysing distributions (I, IV). Variables with skewed distribution, e.g. AER, triglycerides, spectral powers of HRV and serial blood pressure measurements were log-transformed before statistical testing (I, IV). Differences between the groups were first assessed by analysis of variance and then tested with the unpaired Student's t-test with Bonferroni correction when normally distributed. Some variables with a skewed distribution were tested with the Mann-Whitney U test.

The variables of nerve conduction studies were adjusted for the mean height and for the skin temperature (32°C) (III), whereas the cardiovascular test results were adjusted for the mean R-R interval (III) or for R-R interval and body mass index (IV), and the differences between the means were assessed with analysis of covariance. 95% CI was calculated for the differences between two means (I-IV). Linear trends in variables with with pubertal maturation were evaluated with one-way analysis of variance (IV), while two-way analysis of variance was performed to reveal any interaction of puberty and diabetes with the variables (III, IV). The serial blood pressure measurements were log-transformed, adjusted for body mass index (IV) and analysed with an analysis of variance for repeated measurements.

Pearson correlation coefficients were calculated in order to analyse simple correlations between variables (II, III). Linear regression analysis was used for assessing correlations, and a stepwise multiple regression analysis was employed to assess the independent associations of the variables with the test parameters (I, III, IV). A *p* value < 0.05 was considered statistically significant. The analyses were performed with SPSS 8.0 (I), 9.0 (III) or 10.1 (II, IV) (SPSS Inc., Chicago, IL, USA).

## **5 Results**

### **5.1 Retinopathy (I)**

Diabetic retinopathy was detected in 2 patients (2%) in the present series. One boy (Tanner pubertal stage II, duration of diabetes 3.7 years, current HbA1c 6.5 %) had three microaneurysms in the left eye, while one girl (pubertal stage III, disease duration 6.0 years, current HbA1c of 7.0 %) had two hard exudates in the left eye. Neither of these patients had any signs of other microvascular complications.

### **5.2 Urinary albumin excretion rate during puberty (I)**

The mean AER increased towards postpuberty in the patients and in the control boys, while the mean AER for the patients was significantly higher than that for the controls (Table 6). None of the patients or controls had urinary tract infection at the time of examination, but one boy and one girl, both postpubertal at the time of the study, had experienced persistent microalbuminuria within the previous year, having reverted to normoalbuminuria before recruitment.

Stepwise multiple regression analysis including duration of diabetes, long-term HbA1c, systolic and diastolic blood pressure and pubertal stage as independent variables showed long-term HbA1c in the girls with diabetes ( $p = 0.002$ ) and pubertal stage in the boys ( $p = 0.013$ ) to be independently associated with urine AER.

### 5.2.1 Persistent microalbuminuria

None of the one hundred patients had overt nephropathy. The prevalence of persistent microalbuminuria was 6 % (5 / 81). Five patients had persistent microalbuminuria, and all these were girls, one prepubertal (T I), one at late puberty (T IV), and three postpubertal (T V). The mean AER of these patients was 62.5  $\mu\text{g}/\text{min}$  (range 24.0–159.0), and they had significantly higher mean long term and current HbA1c than the normoalbuminuric girls with type 1 diabetes, but they did not differ significantly from the others in their mean duration of diabetes, mean age at presentation of type 1 diabetes, chronological age, daily insulin dose or blood pressure (Table 7). A positive family history of hypertension in parents or grandparents was found in one of the five patients with microalbuminuria (20%), 17% of those with normoalbuminuria (16/95) and in 13% of the controls (13/100) ( $p = 0.57$ ).

Table 6. Urinary albumin excretion rate (AER,  $\mu\text{g}/\text{min}$ ) during puberty in adolescents with type 1 diabetes and healthy control subjects.

Subjects	Tanner pubertal stages					All
	T I	T II	T III	T IV	T V	
Boys						
Patients	3.5 (2.3)	4.6 (1.6)	3.5 (1.6)	5.9 (1.8)	6.6 (1.7)	4.8 (2.0)
Controls	3.2 (2.3)	3.2 (1.9)	5.2 (1.7)	4.1 (1.9)	5.5 (1.6)	4.3 (1.9)
Girls						
Patients	8.3 (2.5)	7.3 (1.8)	5.1 (1.5)	6.5 (2.4)	23.2 (3.6)	10.3 <sup>1</sup> (2.5)
Controls	3.4 (1.8)	3.5 (1.8)	8.2 (1.6)	3.6 (1.5)	3.8 (1.8)	4.1 (1.8)
All						
Patients	5.5 (2.4)	5.3 (1.7)	4.7 (1.6)	6.3 (2.2)	16.2 (2.6)	7.6 <sup>2</sup> (2.3)
Controls	3.3 (2.0)	3.3 (1.8)	6.3 (1.7)	3.7 (1.6)	4.5 (1.8)	4.2 (1.8)

Data are means (SD). <sup>1</sup>95% CI 1.0 , 2.0 of the difference between the means,  $p = 0.009$  compared with the control girls <sup>2</sup>95% CI 1.1 , 1.6,  $p = 0.008$  compared with the controls (data from paper I).

### 5.3 Blood pressure (I)

Diastolic blood pressure was higher in the patients than in the controls: 63 (SD 8) mmHg vs. 58 (SD 7) mmHg (95% CI 2.6 , 6.9,  $p < 0.001$ ). The girls with type 1 diabetes had significantly higher diastolic blood pressure than the control girls 64 (SD 9) mmHg vs. 59 (SD 7) mmHg (95% CI 2.3 , 8.5,  $p = 0.001$ ), and a similar difference was also observed in the boys 62 (SD 9) mmHg vs. 57 (SD 7) mmHg (95% CI 1.0 , 7.2,  $p = 0.01$ ). Systolic blood pressure did not differ between the patients and controls either on the total series or within the girls and boys separately (data not shown).

*Table 7. Characteristics of the girls with type 1 diabetes with or without microalbuminuria, and the control girls.*

Variable	Girls with microalbuminuria	Girls with normoalbuminuria	Control girls
N	5	46	51
Age (years)	14.6 (2.9)	13.6 (2.1)	13.6 (2.2)
Age at presentation with diabetes (years)	8.1 (2.0–10.9)	7.0 (0.2–12.6)	.
Relative weight for height (%)	112 (13)	109 (10)	105 (12)
Relative height for age (SDS)	-0.02 (0.6)	+0.16 (1.0)	-0.08 (0.8)
Body mass index (kg/m <sup>2</sup> )	21.5 (3.6)	20.4 (2.5)	19.4 (2.6)
Current HbA1c (%)	10.8 <sup>1</sup> (1.6)	8.5 (1.6)	4.4 (0.3)
Long-term HbA1c (%)	10.7 <sup>2</sup> (1.7)	8.5 (1.3)	.
Duration of diabetes (years)	7.1 (3.9–15.6)	6.4 (2.1–14.5)	.
Daily insulin dose (IU/kg/d)	1.06 (0.6–1.2)	0.90 (0.5–1.4)	.
Systolic blood pressure (mmHg)	114 (7)	114 (10)	112 (9)
Diastolic blood pressure (mmHg)	64 <sup>3</sup> (9)	65 <sup>3</sup> (10)	59 (7)

Data are means (SD) or median (range). <sup>1</sup>95% CI 0.8, 3.8 of the difference between the means,  $p = 0.007$  <sup>2</sup>95% CI 0.9, 3.4,  $p = 0.003$  vs. normoalbuminuric girls with type 1 diabetes <sup>3</sup>95% CI 2.3, 8.5,  $p = 0.001$  all the girls with diabetes vs. the control girls (data from paper I).

## 5.4 Serum lipid profile and glycaemic control (I)

Serum total cholesterol, LDL cholesterol and triglyceride concentrations were higher in the girls with diabetes than in the control girls, but no difference in HDL cholesterol levels was observed (Table 8). The HbA1c levels at various pubertal stages are shown in Table 9.

*Table 8. Serum total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol (mmol/l) and triglyceride concentrations (mmol/l) in the patients with type 1 diabetes and the control subjects.*

Subjects	Serum cholesterol			Triglycerides
	Total	LDL	HDL	
Boys with diabetes	4.1 (0.7)	2.3 (0.5)	1.5 (0.4)	0.87 (0.36)
Control boys	4.1 (0.8)	2.2 (0.6)	1.4 (0.4)	0.91 (0.54)
Girls with diabetes	4.8 <sup>1</sup> (1.0)	2.7 <sup>2</sup> (0.8)	1.5 (0.3)	1.24 <sup>3</sup> (0.80)
Control girls	4.2 (0.7)	2.3 (0.7)	1.4 (0.3)	0.84 (0.29)

Data are means (SD). <sup>1</sup>95% CI 0.3, 1.0,  $p < 0.001$  <sup>2</sup>95% CI 0.1, 0.7,  $p = 0.004$  <sup>3</sup>95% CI 0.16, 0.63,  $p = 0.002$  compared with the control girls (data from paper I and unpublished data).

Table 9. Median (range) glycated haemoglobin A1c (HbA1c, %) in the patients and the control subjects.

HbA1c (%)	Patients	Control subjects	Non-participants
HbA1c measured in the ward	8.5 (5.4 – 13.0)	4.4 (3.8 – 5.4)	
Long -term HbA1c	8.3 (5.6 – 12.3)	.	8.1 (5.6 – 12.1)
Long -term HbA1c at pubertal stages			
T I	8.1 (5.4 – 9.8)	.	-
T II	8.2 (5.9 – 10.8)	.	-
T III	8.8 (7.1 – 10.5)	.	-
T IV	8.6 (6.0 – 12.3)	.	-
T V	8.8 (6.9 – 12.3)	.	-

## 5.5 Metabolic characteristics (II)

### 5.5.1 Serum free insulin and IGFBP-1 concentrations

*Serum free insulin.* The serum free insulin concentrations were significantly lower in the patients than in the controls: 4.7 (SD 2.3) mU/l vs. 6.5 (SD 3.1) mU/l (95% CI -1.8, -1.3,  $p < 0.001$ ), and this was also true separately for the boys, 3.9 (SD 2.2) mU/l vs. 6.3 (SD 3.0) mU/l (95% CI -2.1, -1.2,  $p < 0.001$ ) and girls, 4.6 (SD 2.3) mU/l vs. 6.7 (SD 3.2) mU/l (95% CI -1.8, -1.2,  $p < 0.001$ ), both before and after puberty (Figure 4).

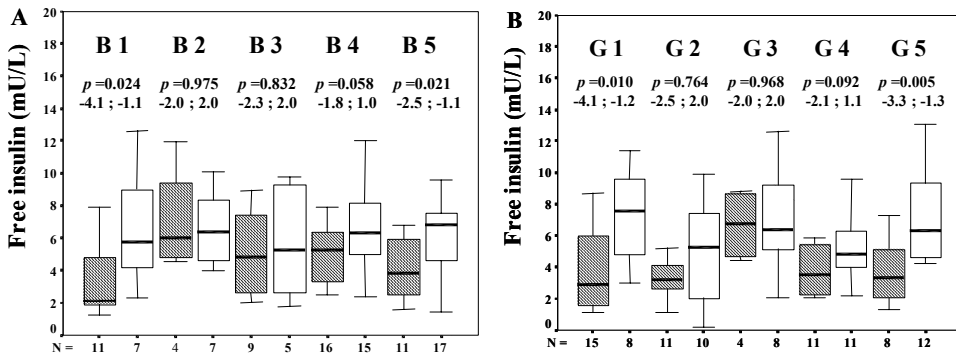
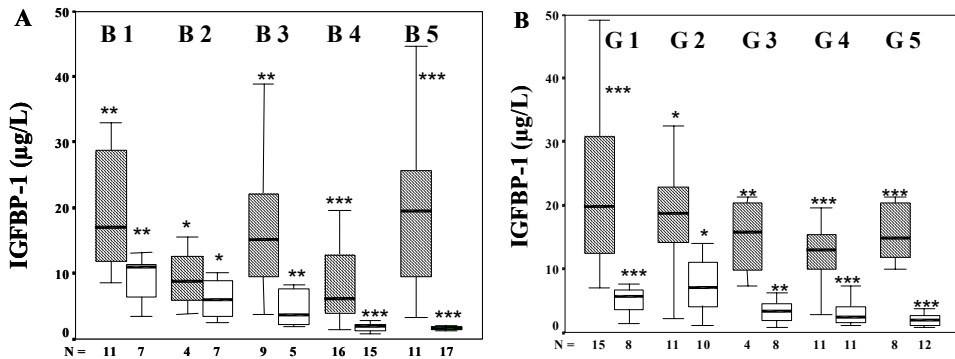


Fig. 4. Serum free insulin concentrations (mU/L) in relation to pubertal maturation in girls (A) and boys (B). B (breast stage in girls) and G (genital maturation in boys) represent pubertal maturation according to Tanner pubertal staging. Striped boxes, patients; open boxes, control subjects. Each box plot represents the median and the 25th and 50th centiles, and the whiskers represent the lowest and the highest values. The 95% CI of the difference between the means is shown under the p-value. Student's *t*-test.



*Serum IGFBP-1.* Serum IGFBP-1 concentrations were significantly higher in the patients than in the controls; 16.5 (10.6)  $\mu\text{g/l}$  vs. 4.0 (SD 3.3)  $\mu\text{g/l}$  (95% CI 3.6 , 5.6,  $p < 0.001$ ), and remained high in the patients throughout pubertal maturation (Figure 5).



**Fig. 5. Serum IGFBP-1 ( $\mu\text{g/L}$ ) concentrations in relation to pubertal maturation in girls (A) and boys (B). Striped boxes, patients; open boxes, control subjects. (A); \* 95% CI -1.5 ; 3.4,  $p = 0.269$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , (B); \* 95% CI 1.0 ; 5.3,  $p = 0.044$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ . Student's *t*-test. B1-5; breast stages, G1-5; genital stages.**

*Serum insulin antibodies.* The median IA level was 96 RU in the boys (mean 187 RU; range 1.0 – 1031 RU) and 108 RU in the girls (mean 200 RU; range 1.0 – 1941 RU). Twelve boys (26%) and 13 girls (25%) had a high IA level ( $> 200$  RU) and the proportion of those with high IA levels was comparable among the prepubertal (27%), pubertal (24%) and postpubertal (26%) patients.

### 5.5.2 Body composition and serum leptin levels (I, II)

*Body mass index and total body fat.* Total body fat was significantly higher in the girls with diabetes than in the control girls by the end of puberty (Table 10), as also was the body mass index, 20.5 (SD 2.6)  $\text{kg/m}^2$  versus 19.4 (SD 2.6)  $\text{kg/m}^2$ , (95% CI 0.1 , 2.1,  $p = 0.04$ ).

*Waist to hip -ratio.* The girls with diabetes had a higher waist to hip -ratio than the female controls; 0.80 (0.05) and 0.78 (0.04) (95% CI 0.004 , 0.04,  $p = 0.015$ ), but no differences were observed among the boys (unpublished data).

*Serum leptin.* Serum leptin levels did not differ significantly between the patients and controls in total, although they were higher in the girls than in the boys both among the patients, girls 14.5 (SD 6.0)  $\mu\text{g/l}$  and boys 7.0 (SD 4.3)  $\mu\text{g/l}$  (95% CI 1.8 , 2.8,  $p < 0.001$ ) and among the control subjects, girls 12.8 (SD 5.7)  $\mu\text{g/l}$  and boys 6.3 (SD 5.3)  $\mu\text{g/l}$  (95% CI 1.9 , 3.1,  $p < 0.001$ ). Serum leptin concentrations increased with pubertal maturation

in the girls with diabetes and in the control girls, but decreased in the boys with diabetes and the control boys.

*Table 10. Body mass index (kg/m<sup>2</sup>) (unpublished data) and total body fat (%) in the patients with type I diabetes and the control subjects at Tanner pubertal stages.*

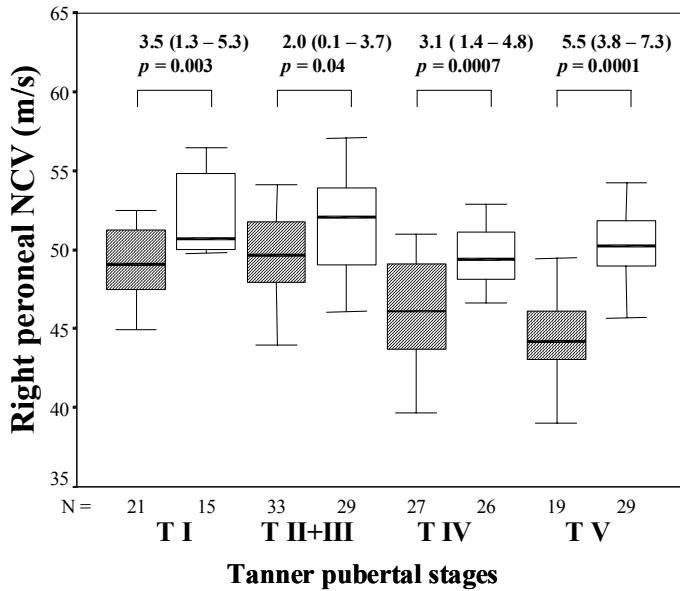
	T I	T II	T III	T IV	T V
Body mass index					
Boys					
Patients	18.7 (1.9)	19.3 (1.7)	18.4 (0.8)	20.2 (1.6)	20.5 (1.7)
Control subjects	18.0 (2.2)	17.2 (1.2)	20.2 (2.5)	19.4 (1.8)	19.9 (2.0)
Girls					
Patients	17.7 (2.1)	18.3 (1.3)	21.0 (1.1)	21.3 (2.1)	22.5 (2.3) <sup>1</sup>
Control subjects	18.1 (3.1)	17.5 (2.6)	17.0 (1.2)	21.3 (1.7)	19.9 (2.0)
Total body fat					
Boys					
Patients	17.7 (3.3)	17.1 (5.1)	16.0 (2.9)	12.1 (3.5)	11.6 (4.3)
Control subjects	18.1 (5.3)	15.7 (2.1)	18.5 (6.0)	13.1 (3.3)	11.1 (3.6)
Girls					
Patients	21.0 (3.1)	20.5 (2.6)	22.3 (3.8)	23.4 (3.8)	24.5 (3.9) <sup>2</sup>
Control subjects	21.0 (4.2)	20.5 (4.7)	19.6 (2.1)	22.1 (1.9)	21.1 (2.4)

Data are means (SD). <sup>1</sup> 95% CI 0.9 , 4.3 of the difference between the means,  $p = 0.004$  <sup>2</sup> 95% CI 1.0 , 5.8,  $p = 0.008$  compared with the control girls at pubertal stage T V (data from paper II; total body fat).

## 5.6 Peripheral nervous system function (III)

*Distal diabetic polyneuropathy.* Diabetic distal polyneuropathy was detected neurophysiologically in ten patients (5 boys / 5 girls): one prepubertal, three pubertal and six postpubertal. Seven of these (3 boys / 4 girls) had symmetrically reduced peroneal nerve conduction velocity, including three patients with persistent numbness of the legs or symmetric loss of deep tendon reflexes in the lower extremities. The patients with neuropathy had significantly higher HbA1c than those without 9.9 (SD 1.8) % vs. 8.3 (SD 1.2) %, (95% CI 0.8 , 2.5,  $p < 0.001$ ) and a longer duration of diabetes 10.8 (SD 3.2) years vs. 6.6 (SD 3.3) years (95% CI 2.0 , 6.4,  $p < 0.001$ ).

*Compound motor nerve action potential.* The peroneal and median motor nerve conduction velocities were significantly lower in the patients than in the controls in total (Table 11), the former decreasing symmetrically in the patients compared with the control subjects as pubertal maturation progressed (Figure 6), but not the latter (data not shown). A significant interaction of diabetes and stage of puberty with peroneal nerve conduction velocity was observed ( $p < 0.05$ ).



**Fig. 6.** Box plots of the right peroneal nerve conduction velocity (NCV, m/s) in the patients (striped boxes) and control subjects. T I, T II + III, T IV and T V are the Tanner pubertal stages. The mean difference of the NCV between the patients and controls (95 % CI) and the  $p$  values are shown above the box plots. N; number of the patients at various pubertal stages.

*Correlations.* A multiple regression model with peroneal nerve conduction velocity as the dependent variable and pubertal stage, duration of diabetes, long-term HbA1c, sex and age at presentation of diabetes as independent variables showed long term HbA1c ( $p = 0.03$ ) and duration of diabetes ( $p = 0.013$ ) to be independently associated with peroneal nerve conduction velocity (adjusted  $R^2 = 0.13$ ).

*Sensory nerve action potential.* The sensory nerve conduction velocity and SNAP amplitude were impaired in the patients as compared with the controls (Table 11). The sural amplitude was 26.0 (SD 1.1)  $\mu$ V in the postpubertal patients, which was significantly lower than in the postpubertal controls, 32.2 (SD 1.4)  $\mu$ V (95% CI 2.6 , 10.2,  $p < 0.001$ , right sural nerve). The median SNAP amplitude did not differ between the patients and controls in postpuberty ( $p = 0.096$ ).

*H-reflex.* The H-reflex latency difference in the patients, 28.3 (SD 0.2) ms, was significantly longer than in the controls; 27.0 (SD 0.1) ms (95% CI 0.5 , 2.1,  $p = 0.002$ , right tibial nerve), and this difference increased as puberty advanced (Table 12). A significant interaction of diabetes and puberty with the H-reflex latency difference was observed ( $p < 0.05$ ).

*F-wave.* The F-wave was symmetrically prolonged in the patients as compared with the controls: 38.1 (SD 0.4) ms versus 36.3 (SD 0.4) ms (95% CI 0.7 , 3.0,  $p = 0.002$ , right peroneal nerve).

Table 11. Nerve conduction velocity (m/s) and sensory nerve action potential (SNAP) amplitude ( $\mu\text{V}$ ) in the patients and the control subjects.

	Peroneal nerve	Sural nerve	Median nerve		Sural SNAP amplitude
			Motor	Sensory	
Patients	47.5 (3.1) 100	47.2 (3.7) 100	55.4 (3.8) 90	50.1 (4.9) 89	28.8 (1.1) 100
Controls	51.0 (3.0) 98	49.2 (3.6) 99	59.0 (3.5) 96	52.1 (3.5) 99	35.8 (1.5) 99
Difference (95% CI)	3.5 (2.7, 4.4)	2.0 (0.9, 3.0)	3.6 (2.6, 4.7)	2.0 (0.8, 3.3)	7.0 (3.5, 10.6)
<i>p</i> value	< 0.0001	0.0004	< 0.0001	0.002	0.0001

Data are means (SD). The results on the right side are shown. Numbers of the subjects at various pubertal stages are shown after nerve conduction velocity results.

Table 12. H-reflex latency difference (ms) at Tanner pubertal stages.

	T I	T II + T III	T IV	T V
Right tibial nerve				
Patients	21.3 (0.2)	23.0 (0.3)	25.6 (0.4)	26.4 (0.5)
Controls	21.4 (0.4)	22.3 (0.2)	24.1 (0.3)	24.7 (0.3)
Difference (95% CI)	-0.1 (-0.9, 0.7)	0.7 (0.05, 1.5)	1.5 (0.4, 2.5)	1.7 (0.7, 2.7)
<i>p</i> value	0.76	0.037	0.006	0.0012
Left tibial nerve				
Patients	21.5 (0.2)	23.0 (0.4)	25.9 (0.5)	26.6 (0.5)
Controls	21.6 (0.4)	22.0 (0.3)	24.0 (0.3)	24.7 (0.3)
Difference (95% CI)	-0.1 (-0.9, 0.8)	1.0 (0.1, 1.9)	1.9 (0.6, 3.1)	1.9 (0.8, 3.0)
<i>p</i> value	0.88	0.032	0.0041	0.0009

Data are means (SD). The H-reflex latency difference (total H-reflex latency minus latency from the popliteal fossa to the soleus muscle) reflects the sensory function of the proximal part of the tibial nerve (data from paper III).

*Vibration perception threshold.* There was a slight tendency for an increase in VPT with puberty in the patients, and a significant difference was observed relative to the controls after puberty at 250 Hz in the left leg ( $p < 0.05$ ) (data not shown).

*Symptoms or signs of peripheral neuropathy.* None of the patients had any loss of the sense of light touch, pain or tactile sense. The questionnaire on symptoms of peripheral neuropathy was completed by 76 patients and 71 controls. In addition to the three patients with symptoms or signs of neuropathy, nine patients without diabetic distal polyneuropathy had peripheral neurological symptoms (tingling or numbness). All the 24 patients who did not complete the questionnaire had a normal sense of vibration, while two (with diabetic polyneuropathy) had an absence of joint reflexes in the lower extremities. Five of these 24 patients had diabetic polyneuropathy neurophysiologically, but they reported no symptoms when asked afterwards. One control girl had occasional numbness of the soles of the feet, but she had no signs of neuropathy and her nerve conduction tests were normal.

## 5.7 Autonomic nervous system function (IV)

### 5.7.1 Cardiovascular reflex tests

No differences in the time domain variables were observed between the patients and controls in the total series or at any of the pubertal stages. Seven patients, i.e. six without diabetic polyneuropathy and one with, and four control subjects had one cardiovascular reflex test under the 5th percentile for the control values, but none of the patients had more than one abnormal test. Altogether 77 patients and 71 controls answered the questionnaire on recurrent diarrhoea, bladder dysfunction or dizziness upon standing up at the beginning of this investigation, whereas no data were available on the subjects' regular physical activity or hypoglycaemic episodes. Twenty patients and 28 of the controls reported dizziness upon standing up ( $p = 0.08$ , chi-square test), but none of the patients or controls had any other symptoms or signs of ANS dysfunction.

*Blood pressure change during standing.* The systolic blood pressure change during 7 min of standing did not differ between the patients and controls, or between the patients with and without distal polyneuropathy. The mean diastolic blood pressure change did not differ between the patients and controls 17.5 (SD 7) mmHg vs. 17.5 (SD 6) mmHg in the total series or at any of the Tanner pubertal stages considered separately (data not shown).

One control subject fainted after two minutes of standing, whereas ten subjects had to sit down during the 7 min period of standing (six controls and four patients). Systolic blood pressure decreased by 20 mmHg or more in another three controls and in one patient without any symptoms (unpublished data).

### 5.7.2 Time and frequency domain analysis of heart rate variability

No differences in the time domain variables were observed between the patients and controls in the total series or at any of the pubertal stages. The mean R-R interval increased along pubertal maturation both in the patients and in the controls: a significant linear trend in the mean R-R interval lengthening was observed in the patients ( $F = 4.5$ ,  $p = 0.037$ ) and in the controls ( $F = 10.1$ ,  $p = 0.002$ ). No linear trend along puberty or differences between patients and controls were observed within the pubertal stages in other time domain variables of HRV (data not shown).

*Supine.* There were no differences between the patients and controls in the total series in any of the frequencies in the power spectrum analysis of HRV in the supine position (Table 13). All the frequency bands of the power spectra, adjusted for body mass index and R-R, decreased with pubertal maturation in both the patients and the controls. A

linear decrease in LF power with puberty was observed both in the patients ( $F = 9.8$ ,  $p = 0.002$ ) and in the controls ( $F = 5.6$ ,  $p = 0.02$ ).

The LF: HF ratio did not differ significantly between the patients and controls (Table 13), neither did the fractal dimension of resting HRV differ between the groups, being 1.92 (SD 0.02) and 1.89 (SD 0.01) in the patients and controls, respectively (95% CI - 0.004 , 0.07,  $p = 0.08$ ).

*Standing.* The VLF power of HRV was significantly lower in the patients, while the other frequencies were comparable between the patients and control subjects in the total series (Table 13). No significant difference in any of the spectral powers was observed between the patients and control subjects within the pubertal stages (data not shown), while there was a linear decrease in HF power with advancing puberty in the control subjects ( $F = 5.8$ ,  $p = 0.018$ ) but not in the patients ( $p = 0.73$ ).

*Supine to standing.* The changes in absolute VLF and LF spectral powers of HRV were significantly lower in the patients (Table 13). However, the changes in the LF or HF bands within the pubertal stages did not differ between the patients and the control subjects (Figure 7).

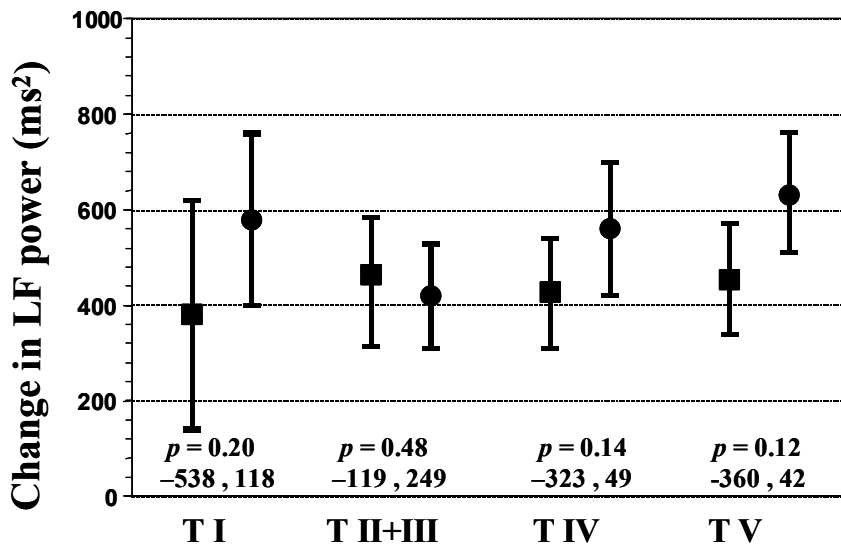


Fig. 7. Power spectra (direct Fourier transformation) of the heart rate variability according to pubertal maturation adjusted for the mean R-R and body mass index ( $\text{kg}/\text{m}^2$ ): change in low frequency (LF) power of HRV between the supine and standing position in patients with type 1 diabetes (square) and control subjects. T I, T II, T III, T IV and T V are the Tanner pubertal stages.

The change in the LF: HF -ratio or changes in the normalised data did not differ between the patients and control subjects (data not shown).

The power spectrum analysis of HRV in the patients with distal polyneuropathy did not differ from that of the other patients when standing or upon changing from a supine to a standing position.

*Table 13. Frequency domain analysis of heart rate variability in a 10 min resting state and 7 min of standing, and the supine to standing difference analysed by direct Fourier transformation in the patients with type 1 diabetes and their control subjects.*

Variable	Patients	Controls	Difference (95 % CI)	<i>p</i>
<b>Supine (ms<sup>2</sup>)</b>				
Total power	1556 (63)	1447 (50)	109 (-47 , 251)	ns
Very low frequency power	186 (7)	173 (7)	13 (-7 , 31)	ns
Low frequency power	512 (23)	455 (19)	57 (-0 , 108)	ns
High frequency power	654 (33)	617 (26)	37 (-46 , 111)	ns
Low frequency : High frequency -ratio	0.78 (0.03)	0.74 (0.03)	0.04 (-0.04 , 0.12)	ns
<b>Standing (ms<sup>2</sup>)</b>				
Total power	2204 (68)	2321 (52)	-117 (-293 , 47)	ns
Very low frequency power	417 (14)	461 (13)	-44 (-85 , -6)	0.023
Low frequency power	939 (35)	990 (30)	-51 (-147 , 36)	ns
High frequency power	559 (28)	588 (20)	-29 (-101 , 36)	ns
Low frequency : High frequency -ratio	1.68 (0.08)	1.68 (0.06)	0 (-0.21 , 0.18)	ns
<b>Change from supine to standing (ms<sup>2</sup>)</b>				
Very low frequency power	240 (17)	295 (15)	-55 (-98 , -12)	0.013
Low frequency power	430 (38)	540 (34)	-110 (-211 , -10)	0.031
High frequency power	-141 (36)	-53 (32)	-88 (-182 , 7)	0.068
Low frequency : High frequency -ratio	1.04 (0.09)	0.99 (0.06)	0.05 (-0.16 , 0.26)	ns

Data are means (SD), adjusted for the mean R-R interval and body mass index (kg/m<sup>2</sup>).

## **6 Discussion**

### **6.1 Methodological aspects**

The cohort comprised 72% of the patients with type 1 diabetes visiting the Outpatient Paediatric Diabetes Clinic at Oulu University Hospital at the beginning of the period concerned who fulfilled the inclusion criteria, and represents about 6% of all the children and adolescents of this age with type 1 diabetes in Finland. The patients who refused participation did not differ from those who took part in terms of age, glycaemic control or duration of diabetes. Since all the children and adolescents diagnosed with type 1 diabetes within the district served by the hospital are treated at this clinic, the series is representative of Finnish patients with type 1 diabetes within the defined age range. A multicentre study would certainly have had the advantage of increasing the number of subjects. In that case one problem might have been the recruitment and retention of healthy control subjects, since only about every third of the potential subjects who were asked to participate agreed to take part in the present cross-sectional study. A follow-up study would have facilitated assessment of the tempo of development of microvascular complications in adolescence.

The protocol included overnight hospitalisation in the ward and three time-consuming assessments, i.e. an electroneurographic examination, a battery of four cardiovascular reflex tests and determination of the VPT. This together with the young age of the patients could have potentially resulted in withdrawals. However, special emphasis was laid on informing the subjects thoroughly beforehand on the course of the examinations, and their compliance was excellent, since none of the diabetic subjects dropped out and only one control subject withdrew from the nerve conduction measurements. This may reflect some selection of subjects in favour of those with high motivation, although there were also a number of other reasons for refusing to participate as well as poor motivation.

There were some variations in the numbers of subjects with results available on conduction studies in the peripheral nerves and on ANS function, but the greatest variation was seen in the examinations for persistent microalbuminuria, the calculation of the prevalence of which had to be based on 81 of the one hundred patients because the



need for several consecutive overnight urine collections to be able to correctly detect true microalbuminuria. Unfortunately, the importance of consecutive urine samples was not emphasized to the normoalbuminuric patients after the study period. Therefore, the study had some methodological limitations (Series I). Nevertheless, the regular follow-up visits to the Outpatient Clinic could be used to confirm normoalbuminuria.

Pubertal staging was performed on all patients and controls before the study period. Because the subjects were matched for age but not for the degree of pubertal maturation, the ages of the patients and controls varied slightly within each pubertal stage. This probably had less impact on the overall results, than the fact that the numbers of subjects within the pubertal stages were rather small, particularly when the boys and girls were analysed separately. The present study was based on pubertal staging, which was however, one of the strengths of the study. The stage of pubertal maturation (152) has seldom been taken into account in series that have studied retinopathy (156), microalbuminuria (6,7,179), or peripheral nervous system and ANS functions (103,111,180), even though observations based on systematic pubertal staging are known to be more accurate than those based on age when assessing the relationship between physiological maturation and the development of microvascular complications in adolescence.

## **6.2 Microvascular complications and pubertal maturation**

### ***6.2.1 Retinopathy (I)***

The two patients in the present series who had diabetic retinopathy were at mid-puberty and had no other signs of microvascular complications, so that it was not possible to see any association between retinopathy and pubertal maturation. This low prevalence of retinopathy is inconsistent with many other reports on children and adolescents (Table 1) and with the relatively high prevalence of retinopathy observed in young adults with type 1 diabetes (88,89). The finding is also surprising in the light of an earlier cross-sectional study performed at the same clinic between the years 1989-1990, where retinopathy was detected in 11% of the children and adolescents, mainly in the late pubertal and postpubertal girls (158). Difference between the studies cannot solely be explained by methodological factors. One explanation might be the change towards more intensified insulin treatment, although two thirds of the children and adolescents with type 1 diabetes in the previous series already had a treatment schedule of three or four daily insulin injections (158).

### ***6.2.2 Persistent microalbuminuria and nephropathy (I)***

About 6% of the patients in this study had persistent microalbuminuria. This finding is largely in line with earlier observations. Interestingly, it was the adolescent girls that experienced persistent microalbuminuria and they were mostly pubertal or postpubertal, as also shown in some earlier studies (95). Thus it seems that the girls with type 1 diabetes have a higher risk of an early elevation of their urinary AER during puberty when compared with boys. This may be at least in part related to their earlier pubertal onset, hormonal changes or maturation. This study further showed a direct association between poor metabolic control and an elevated AER or persistent microalbuminuria, as also has been observed in several other previous series (17,94,95). As a consequence of the rather low prevalence of persistent microalbuminuria and the cross sectional nature of the present study, and a rather limited number of patients in the various pubertal stages, this study does not allow to assess the impact of puberty on persistent microalbuminuria any further. However, other series have shown that pubertal maturation is associated with an increase in microalbuminuria (7,95).

Although adult men with type 1 diabetes have a greater risk of diabetic nephropathy than women (45), it is clear that the relationship between gender and increased urinary AER or microalbuminuria is not straightforward (90,119). In this respect our finding of a predominance of adolescent girls with microalbuminuria is interesting. It may be that the microalbuminuria observed in the present series reflect a combination of structural and functional changes that might be reversible after improved metabolic control. Furthermore, it is known that young adult female patients with type 1 diabetes are known to revert to normoalbuminuria more frequently than male patients as metabolic control improves. On the other hand, male patients with poor metabolic control more often experience progressive microalbuminuria (54).

### ***6.2.3 Peripheral nervous system dysfunction (III)***

Ten percent of the patients in the present series had neurophysiologically proven distal diabetic polyneuropathy. Decreased distal motor nerve conduction velocity could already be detected in the prepubertal subjects with type 1 diabetes, whereas proximal nerve function was increasingly impaired after the onset of puberty, shown in increasing tibial H-reflex latency difference. Moreover, the SNAP amplitude was significantly lower in postpuberty than in prepuberty. These findings imply that mixed sensorimotor neuropathy, which is the commonest form of diabetic polyneuropathy mainly occurs at late puberty or after puberty in adolescents with type 1 diabetes.

Poor metabolic control is one of the most important prognostic factors for neuronal dysfunction both in adolescence (181,182) and in adulthood (107,183), while duration of diabetes may become increasingly important for the progression of nerve dysfunction later in the course of diabetes. The duration of diabetes is related to peripheral nerve dysfunction at least in young adult patients (107). In keeping with the previous findings

the patients with distal diabetic polyneuropathy in the present series not only had higher HbA1c than those without neuropathy but also a longer duration of diabetes. Furthermore, progression with puberty was demonstrated. It is noteworthy that a large number of variables, such as height, weight, age, pubertal stage and glycaemic control, may be interrelated during adolescence, which further complicates the picture.

Although functional changes in the peripheral large myelinated nerve fibres were already detectable before puberty, a slight decrease in VPT was observed only after puberty both among patients and controls. We did not have any data on cold or warm thresholds, which would have been useful for assessing the functioning of small nerve fibres, as this has also been shown to deteriorate in adolescent patients with type 1 diabetes (106).

#### ***6.2.4 Autonomic nervous system dysfunction (IV)***

There were no differences in the cardiovascular reflex test parameters or the time domain variables of the R-R interval between the patients and the controls, whereas the dynamic changes in the VLF and LF spectral powers of HRV were significantly lower in the patients when standing. Signs of cardiovascular ANS dysfunction were also demonstrated in the patients with distal polyneuropathy.

Glycaemic control was not associated with depressed HRV in the present series, while poor metabolic control was inversely associated with peripheral nerve conduction. This discrepancy may be related to the relatively slow deterioration of ANS function compared with the peripheral nerves in adolescents and children with diabetes (106). The ANS may also be more resistant to the physiological changes induced by diabetes. The cardiovascular reflexes are functional measures of complex reactions at multiple sites within the ANS, while the individual nerves in the peripheral nervous system can be evaluated more easily. Thus it may be easier to standardize the examinations on the function of peripheral nervous system than ANS. Moreover, the analyses are impeded by the wide physiological range of HRV and by the possible interrelation between variables such as metabolic control, duration of diabetes, age and puberty. The discrepancies between the time domain and frequency domain parameters in the patients with distal polyneuropathy may argue against any structural changes in the ANS. Although deteriorations were observed in both the peripheral nervous system and ANS, the question of whether the changes are functional, i.e. reversible, or structural and persistent cannot be answered on the basis of the present protocol.

### **6.3 Hormonal and metabolic aspects and blood pressure (I, II)**

The hormonal changes and rapid growth that take place during puberty usually have an unfavourable effect on the metabolic balance in type 1 diabetes. Together with

psychological changes and poor compliance, these events form a challenging equation in the clinical care of adolescent patients with this disease.

The “dawn phenomenon”, i.e. increasing blood glucose levels towards the early morning hours has been clearly documented previously in adolescent patients with type 1 diabetes (184). As in healthy subjects, the highest circulating IGFBP-1 concentrations are seen during the night and morning, and these levels are associated with low insulin concentrations (185). The serum IGFBP-1 concentrations in non-diabetic adolescents decrease as puberty advances (14), but conversely, they may remain high in patients with type 1 diabetes in spite of exogenous insulin administration (186), as also demonstrated in the present series. Sustained growth hormone secretion has been implicated in the development of insulin resistance, poor metabolic control and the dawn phenomenon. Moreover, increased IGFBP-1 levels may reduce the local effects of IGF-I (13), and since the latter plays an important role in the feedback regulation of growth hormone secretion, a reduction in its effect could lead to increased secretion of growth hormone in pubertal subjects with type 1 diabetes (8), thereby maintaining a vicious circle.

Variables reflecting body composition and the serum lipid profile were higher in the girls with diabetes than in the control girls, and were even higher in the girls with poor metabolic control. Since increased serum triglyceride concentrations and a high waist to hip ratio were found in the EURODIAB Study to be risk factors for retinopathy (90) and persistent microalbuminuria (187) we cannot rule out the possibility that the girls in this particular study had a greater risk of microvascular complications.

The finding that the patients with type 1 diabetes had higher diastolic blood pressure than the controls is important in the light of recent reports which have demonstrated that diastolic blood pressure may be directly associated with early structural kidney changes in adolescents and young adult patients with AER in the normal range (43,44,188). Slightly increased systemic blood pressure may be a risk factor early in diabetes, but the mechanisms of the relationship between early kidney lesions and blood pressure remain to be defined, since persistently elevated AER has also been shown to precede any increase in systemic blood pressure (97).

## 6.4 Clinical implications

Although an intensive insulin regimen can prevent microvascular complications, an effect that can be seen even many years afterwards (69,100), one disadvantage may be an increase in weight relative to height among late pubertal and postpubertal girls. In the present series total body fat and the body mass index were higher in the girls with diabetes than in the control girls after puberty, while no such difference was seen among the boys. As overweight may lead to increased peripheral insulin resistance (189) and be associated with microvascular and macrovascular complications, adolescent patients should be advised on how to avoid weight gain, and this should especially apply to adolescent girls who are making efforts to improve their metabolic control.

Peripheral insulin levels were lower in the patients than in healthy adolescents, the lowest concentrations being observed in the prepubertal and postpubertal patients. This

may be a consequence of increased insulin clearance, since the highest insulin clearance rates are usually observed during early puberty and after puberty (189).

Although girls seem to have earlier signs of microvascular complications, presumably due to earlier pubertal maturation, boys were also shown to have some signs of subclinical neuropathy. It should therefore be emphasized that the differences between the genders may be minimal. Boys should not be overlooked in early and mid-puberty, since they are also prone to subclinical signs of peripheral nerve dysfunction, and possibly a higher risk for nephropathy later in the adulthood (45). The risk factors may also act differently in boys and girls during pubertal years (94).

Glycaemic control deteriorated during pubertal maturation, and there was a direct association between poor metabolic control and persistent microalbuminuria, peripheral nerve dysfunction and an unfavourable serum lipid profile in the present series. Consequently, increasing efforts should be directed towards better metabolic control during the late prepubertal years. The low serum free insulin and high IGFBP-1 levels already detectable in prepubertal patients nevertheless stress the impact of early interventions, and appropriate allowance should be made for the increasing insulin requirements during early pubertal maturation.

Although the mean daily insulin doses in the present series were relatively low, the low prevalence of microvascular complications suggests that glycaemic control was on average satisfactory enough to delay their development. On the other hand cross-sectional design does not allow speculations about what would have happened if the patients were followed for a couple of years. The advantage obtained with more intensive insulin therapy should outweigh the disadvantages resulting from the more frequent hypoglycaemic episodes (18,100). The daily lives of adolescent patients with type 1 diabetes nevertheless include factors that are beyond the scope of this study but which have a definite influence on therapeutic compliance and success during pubertal maturation.

## 7 Conclusions

1. Two percent of the adolescent patients in this series had retinopathy, as assessed by fundus photography.
2. The prevalence of persistent microalbuminuria was 6%, and all the affected patients were girls. Young girls with type 1 diabetes and an adverse metabolic control present a risk group for persistent microalbuminuria.
3. Girls with type 1 diabetes had higher total body fat at the end of their pubertal maturation than healthy control subjects, and they also had higher diastolic blood pressure and serum total cholesterol, LDL -cholesterol and triglycerides.
4. The prepubertal and postpubertal patients had lower free insulin levels and higher IGFBP-1 levels than the healthy controls, implying portal hypoinsulinaemia.
5. Adolescent patients with type 1 diabetes already demonstrated subclinical distal distal motor nerve dysfunction before puberty and progressive proximal nerve dysfunction during puberty. Mixed sensorimotor neuropathy was mainly observed in late puberty or after puberty. Distal symmetric polyneuropathy was associated with high long-term HbA1c and a long duration of diabetes.
6. The patients with distal diabetic polyneuropathy had lower HRV during the deep breathing than the other patients. There were no differences in the power spectrum analysis of HRV between the patients with and without neuropathy.
7. Adolescents with type 1 diabetes showed a blunted ANS reactivity, although cardiovascular integrity was rather well preserved with advancing pubertal maturation.

## 8 References

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