Screening for Subclinical Complications in Young Type 1 Diabetic Patients: Experience Acquired in Brussels

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Abstract

Clinical studies conducted since the 1970s by the pediatric diabetology group of the Free University of Brussels have demonstrated that screening for subclinical retinopathy, neuropathy and nephropathy should be started at puberty and at least 3 years after the diabetes diagnosis. The goal is to detect early abnormalities responsible for subclinical disorders that can be reversed by improved metabolic control, thus preventing the occurrence of irreversible potentially incapacitating lesions. A 1974 retinal fluorescein angiography study showed that the development of microaneurysms, which are irreversible lesions, could be preceded by fluorescein leakage due to disruption of the blood-retinal barrier. Risk factors for early retinopathy include: duration of diabetes, age at diagnosis (with younger children having longer times to retinopathy), puberty and sex (with onset one year earlier in girls than in boys), long-term bad metabolic control over several years, high cholesterol levels and excessive body mass index (BMI). On the other hand, rapid improvement of diabetic control may worsen diabetic retinopathy (1985). Minimal EEG abnormalities were found in relationship to frequent and severe hypoglycemic comas and/or convulsions and retinopathy (1979). Desynchronization of action potentials in distal nerve fibers preceded conduction velocity slowing (1981). A single high glycated hemoglobin value was associated with peroneal motor nerve conduction slowing (1985), which was not observed in the femoral nerve (1987). Sympathetic skin response (1996) and statistical analysis of heart rate variability (2001) could have some interest for the diagnosis of early diabetic autonomic neuropathy. Early microproteinuria is of mixed origin, being both glomerular (microalbumin) and tubular (β2-microglobulin). Exercise testing to exhaustion did not provide additional information than the basal excretion (1976). Microtransferrinuria (1984) and urinary acid glycosaminoglycans output (2001) could also be predictive markers of glomerular dysfunction. Physical training reduced exercise-related proteinuria by half (1988). High levels of serum lipoprotein (a) were not associated with the presence of subclinical complications (1996). On the other hand, ultra sensitive C-reactive protein could be an interesting indicator for the risk of developing early complications (2002). Poor metabolic control was associated with higher levels of triglycerides, total cholesterol, LDL cholesterol and apolipoprotein B (1990). Decreased glutathione peroxidase, glutathione reductase and vitamin C levels, denoting moderate oxidative stress, were found (1996), although there was no evidence of increased LDL cholesterol peroxidation (1998). Erythrocytes exhibited increased glycolytic activity and neutrophils decreased migration in relationship with metabolic control (1992). The degree of metabolic control influenced serum triiodothyronine (levels (1985), magnesium concentrations (1999) and infection by Helicobacter pylori (1997). Insulin therapy could activate the complement pathway if intermediate and long-acting insulin preparations without protamine sulphate are used (1992) and provoke higher BMI in adolescents on 4 insulin injections (1988). Well-being was inversely related to glycated hemoglobin levels (1997).


Key words: type 1 diabetes, diabetic complications, diabetic children, retinopathy, neuropathy, nephropathy, lipoproteins, lipoprotein (a), C-reactive protein, oxidative stress, glycolytic activity, triiodothyronine, Helicobacter pylori, complement, obesity, well-being.

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Introduction

Role of glycemic control
The principal aims of therapeutic management of the child, adolescent and adult with type 1 diabetes are to allow good quality of life (1,2) and to avoid long-term complications (retinopathy, neuropathy, nephropathy, etc.) by maintaining blood glucose concentrations close to the normal range (3-5), even if it is possible that some complications - namely nephropathy - could be influenced by genetic factors (6-8).

Repeated determinations of glycated hemoglobin levels (HbA1c) provide a good criterion of overall control. They must be under 7% (4), if the upper normal limit is about 6%, which is possible, in our experience, even in diabetic children and adolescents (9-12). The number of daily insulin injections, 2 or 4, by itself does not necessarily give better results, but the 4-injection regimen allows greater freedom, taking into account that the proper insulin adjustment is difficult before adolescence. Successful glycemic control in young patients depends mainly on the quality and intensity of diabetes education. Any dogmatism must be avoided. Details on our way of treating diabetic children and adolescents have been published elsewhere (13-18). The mean HbA1c levels of our diabetic children and adolescents are among the lowest in a comparison with major studies of glycemic control in diabetic children (19) and in the international comparisons by the Hvidere Study Group on Childhood Diabetes (20-23). In unselected patients, we obtain a mean HbA1c of 7-140 percent of normal mean being 100%, i.e. 7 percent if the normal mean is 5% (9-11).

Subclinical complications
Clinical studies conducted since the 1970s by our team (9,24) have demonstrated that screening for subclinical retinopathy, neuropathy and nephropathy should be started at puberty and three years after diagnosis (Figure 1), as also shown by the Berlin group (25), with the goal of detecting early abnormalities responsible for functional disorders that can be reversed by improved metabolic control, thus preventing the occurrence of potentially irreversible incapacitating lesions (26-28). This motivates both the patient and the multidisciplinary diabetes team in order to obtain good HbA1c levels.

Effect of puberty
If diabetic complications are very rare before puberty, except if metabolic control is very bad as shown in cases of Mauriac syndrome (29), the years of insufficient glycemic control before puberty influence the development of microvascular complications (30,31). The prepubertal diabetes duration remains a significant predictor of retinopathy in young adults. The effect of time on the risk of retinopathy and microalbuminuria is not uniform, with an increasing delay in the onset of complications in those with a longer prepubertal duration (32). In a longitudinal study, we have shown earlier that the younger the child was when he or she became diabetic, the longer the retinopathy-free period (33). Moreover, girls acquired their first retinal lesions one year earlier than boys, although the age at onset of diabetes did not differ between boys and girls (34), maybe because puberty appears earlier in girls than in boys. Hormonal changes could be involved in the development of complications (35). Insulin resistance during puberty, related to BMI and adiposity among others, could be a factor favoring complications (36).

Personal experience
The purpose of this paper is to summarize briefly our clinical studies, begun more than 30 years ago, on the screening for subclinical complications in young type 1 diabetic people. It is not an extensive review of the scientific literature. Of course, the cited articles have a bibliography brought up to date at the moment of their publication.

Only few general or review papers on complications in diabetic children have been published during the last years (37-44). Recommended screening procedures for diabetic complications in children have been published by the International Society for Pediatric and Adolescent Diabetes (ISPAD) (45).
Retinopathy

Contribution of fluorescein angiography

Used for the first time by Novotny and Alvis in 1961 (46), the retinal fluorescein angiography is a considerable development. This allows detection of early abnormalities that are undetectable by regular ophthalmoscopy; it enables the study of the vascular walls and the perturbations of the dynamic circulation. The principle of angiofluororetinography is simple. After an intravenous injection of fluorescein (10 ml of a 10% sodium solution), the retina is lit up by an electronic flash (47-49) crossing a blue filter, giving an intense light, the wavelength being more or less 4,900 Å, which corresponds to the absorption band of fluorescein. A photograph is taken at the same time behind a yellow filter that only allows the passage of the light emitted by the fluorescein, eliminating all interference. The photographic sequence entails one per second for 10 or 15 seconds. Two complementary photographs are taken 15 to 30 minutes after the fluorescein injection. In practice, for many ophthalmologists the method for obtaining the best photographs remains unclear and subsequent interpretation of these pictures is difficult. Paradoxically, fluorescein angiography (a sensitive method) is prescribed most often for patients who already show retinal lesions by normal ophthalmoscopy (a less sensitive method) so that the initial abnormalities of diabetic retinopathy may not be seen for several years (37,48,49).

Among 114 diabetics whose disease was diagnosed before the age of 14, with the duration of diabetes ranging from 1 month to 19 years (mean: 6 years), retinopathy was diagnosed in 21 patients (18%) by regular ophthalmoscopy with green filter, whereas fluorescein angiography evidenced abnormalities in 39 patients (34%) (49). Compared with regular ophthalmoscopy, fluorescein angiography doubles the diagnosis of incipient retinopathy.

Characterization of early stages of diabetic retinopathy: fluorescein leaks

Since 1974, the late Daniel Toussaint and myself have shown that microaneurysms could be preceded by a functional abnormality characterized by the appearance of fluorescein leaks secondary to an increased permeability of the pigment epithelium and retinal capillaries (28,47-54) (Figure 2 and Figure 3a). These leakages are different to those seen later in the disease in the (pre)proliferative stage (Figure 3b). Even though our first studies were published in French (not in English) (47,48), in 1978, Arnall Patz, from the Wilner Ophthalmological Institute of the John Hopkins Hospital, in fairness, wrote in the New England Journal of Medicine that: "Drs. Dorcy and Toussaint have pioneered in the use of fluorescein angiography in the study of diabetic retinopathy and have defined early changes observed in patients with juvenile-onset diabetes... Their observations of capillary leakage as a result of vascular incompetence before specific morphologic lesions occur represent an important contribution" (55). However, nearly three decades after our first publication (47), it is difficult to persuade some diabetologists or ophthalmologists of the importance of fluorescein leaks as an incipient functional abnormality.

In 1991, we published a longitudinal study of 161 type 1 diabetic children and adolescents to investigate, by fluorescein...
angiography, the nature of the initial vascular changes in childhood diabetes, their frequency and their occurrence (56). The criteria for inclusion were to have had at least one normal angiogram no more than 3 years before the occurrence of the first observed angiographic changes or to have one normal eye. The different types of significant retinal abnormalities, isolated or not, as well as the mean duration of diabetes and age, are shown in Table 1. Although capillary nonperfusion was rarely an initial lesion, occurring after a longer duration of diabetes and at a later age than the other abnormalities, no significant difference could be found between the various types of lesions for either the patient’s age at onset or the duration of diabetes. The type of initial abnormality was also unrelated to sex, age at onset of diabetes or long-term glycemic control evaluated by mean values of glycated hemoglobin from either the onset of follow-up or from 1977 onwards, when the method became available. Retinopathy was not found in children <12 years of age and was detected only after 3 years of diabetes. However, we published the case of a boy with Mauriac syndrome who had become diabetic at 19 months of age and showed retinopathy at 11 years of age (29). The longest retinopathy-free period was 16 years, which confirms our previous cross-sectional and longitudinal study (34) (Figure 4). The mean interval between the onset of retinopathy in the two eyes was 1.2 years with a maximum of 6 years. The conclusion of this study was that if microaneurysms, isolated or associated with other abnormalities, are the most frequently observed lesion (65% of the eyes), leakages will be seen in 52% of the eyes and in the absence of other lesions, in 18% of cases.

We also observed, in a 14-years-old adolescent, a rare manifestation of capillary permeability involving the optic disc capillaries, resulting in transient leakage at the level of the optic nerves (Figure 5), consecutive to rapid improvement in the degree of control as shown by Daneman et al (58).

**Classification of diabetic retinopathy**

Since 1979 (49) we proposed a new classification of diabetic retinopathy (Figure 6) taking into account the existence of early fluorescein leaks, which have been quantified by Cunha-Vaz et al. using fluorophotometry (59). Haut et al. (60), reviewing the literature, consider that only fluorescein angiography can take into consideration the functional...
evaluation of the retinal circulation with unquestionable pathogenic correlation and adapted treatment: edematous diabetic retinopathy corresponds to capillary dilatation and ischemic diabetic retinopathy corresponds to capillary occlusion. Macular diseases are classified according to these same criteria.

Comparative data from the literature on the use of fluorescein angiography in diabetic children and adolescents have been recently published by Salardi et al. (61).

Several reasons can explain why the initial leakages pass unnoticed by several authors: 1. angiofluorographies should be carried out before the appearance of detectable lesions by ophthalmoscopy or by fundus photography; 2. the dose of fluorescein must be sufficient; 3. angiofluorographic investigation demands meticulousness as well as subsequent excellent quality of the photographic development and impression at a magnification of 13x13 cm; 4. the number of exposures must be numerous, including late-phase photographs. Even if digital retinal imaging is very fashionable, until now its accuracy has proved unsatisfactory.

The ISPAD recommendations (45) for diabetic retinopathy screening are: "Early retinopathy is asymptomatic but may be detected by sensitive methods (e.g. fundus photography or fluorescein angiography) in a large proportion of young people with diabetes duration of more than 10 years. Fluorescein angiography is not performed in many pediatric centers but is a sensitive method of detecting early functional vascular abnormalities of the retina which are potentially reversible by improvements in metabolic control".

Mechanisms of increased vascular permeability

Whereas some of the mechanisms that lead to proliferation in the late stages of retinopathy have been explained, the early processes, which launch the onset of the disease, are still obscure. In 1994, Wardle (62) reviewed the factors involved in increased vascular permeability in diabetics, namely hyperglycemia leading to increased production of diacylglycerol and thence protein kinase C, non-enzymatic glycation generating free radicals and lipid peroxides, sorbitol formation, loss of endothelial cell surface heparin sulphates and the activation of arachidonate derivatives that affect endothelial cell contractibility. Schmetterer and Woltzt (63) have analyzed other mechanisms: C-peptide could prevent increased retinal blood flow; impaired blood rheology contributes to altered retinal blood flow; the altered endothelin-1 system could be linked to perturbations in the retinal response to hypoxia; vascular endothelial growth factor (VEGF) contributes to the retinal perfusion abnormalities; the exact role of the angiotensin-renin system in the regulation of retinal blood flow is still not clear; recently, it has been shown that not only vascular cells but also Müller cells of the retina are affected and they express endothelin as well as nitric oxide; etc.

The blood-retinal barrier (BRB) acts at two levels: pigmented epithelium (external barrier) and endothelial cells of the retinal capillaries. These two cell types are united by tight junctions that prevent the passage of substances (including fluorescein) in the intercellular spaces. Tight junctions are comprised of at least seven proteins. Occludin is a 65-kDa protein specific to cells that contain tight junctions and is thought to span the plasma membrane, conferring the cell-to-cell interaction of tight junctions. Antonetti et al. (64) have suggested that VEGF decreases retinal endothelial cell occluding content, which accounts for at least some of the loss of BRB integrity and the increased vascular permeability.

Factors related to diabetic retinopathy

We conducted several studies to determine the relationship between some clinical and biological factors and diabetic retinopathy. Ancient studies have been summarized elsewhere (53,65). Residual endogenous insulin secretion, measured by C-peptide immunoreactivity, was significantly higher in patients without retinal abnormalities. In this group, however, the duration of diabetes was shorter.

The effect of the duration of diabetes has been found constantly since our first study in 1977 (34,48,49,56).

The effect of age at onset, before or after puberty, plays an important role. In patients whose diabetes started before age 11 (i.e. before puberty) the mean duration of diabetes before the occurrence of retinopathy in the first affected eye was 8.5 years, whereas in patients whose diabetes began later, it was 5.2 years (p <0.001) (24,33). Moreover, there was a linear negative correlation between the duration of diabetes free of
retinopathy and the age at onset of diabetes \( r = -0.72; p < 0.001 \). In the patients who became diabetic before puberty, the "good" control subgroup (mean age at onset of diabetes = 5.5 years) had their first retinal abnormality after a mean duration of diabetes of 11.7 yr while in the "poor" control subgroup (mean age at onset of diabetes = 6.9 years), this duration was only 7.6 years \( p < 0.001 \).

In our studies of 1977 and 1979 \((48,49)\), insufficient or poor long-term metabolic control, evaluated according to clinical estimates, increased the frequency of retinopathy. However, in 1977, Malone et al. \((66)\) did not observe a relationship between frequency of retinopathy and the degree of control, because of bad control criteria \((67)\). After the introduction of glycated hemoglobin as an objective marker of glycemic control, we found no relationship between diabetic retinopathy and total glycated hemoglobin repeatedly measured during a test period of one year \((68)\). Correlations between retinopathy and glycated hemoglobin appears when measured during several years \((34,69,70)\).

We did not find any relationship between the prevalence of retinopathy and the presence of HLA-DR3 and/or DR4 antigens \((34,69)\).

In 1999, we illustrated the major role of metabolic control in diabetic retinopathy with the case report of two homozygous twins becoming diabetic at age 8 yr \((5)\). They share the HLA-DQ genotype which is associated with the highest risk of type 1 diabetes in Belgium: A1*0301-B1*0302/A1*0501-B1*0201. Their personal medical history, blood pressure, lipoprotein levels and way of life were very similar, except that one brother had always been very compliant, which resulted in good metabolic control of his diabetes (hemoglobin A1c always <115% of normal values, the upper limit being 100%). His brother, in contrast, had, since shortly after the onset of diabetes, shown a poor metabolic control (hemoglobin A1c from 130% to 150% of normal values). The twin with poor metabolic control had the first signs of retinopathy, as determined by fluorescein angiography, at age 17, three years before his brother and had a higher level of severity of retinopathy than his brother all through the follow-up. He had a major proliferative retinopathy with bilateral retinal vessel changes at age 31, after 23 years of diabetes (Figure 7a); at the same time, his brother only showed moderate background retinopathy (Figure 7b). At age 32, the twin with poor metabolic control had a slightly reduced peroneal motor nerve conduction velocity. Neither of the 2 brothers had abnormal microalbuminuria. In conclusion, by suppressing the differential genetic influence on diabetic retinopathy evolution and reducing to a minimum external and personal differences, this naturally occurring twin study points out the major importance of metabolic control on the development of diabetic retinopathy.

In 1991, we published a longitudinal study \((56)\), begun in the 1970s \((48)\), in order to detect the initial retinal abnormalities at fluorescein angiography in 161 type 1 diabetic children and adolescents. Sixty-nine of them developed an incipient retinopathy. In the year 2000, 32 subjects (15 females and 17 males), among these 69 patients who developed incipient retinopathy, were always treated by our team \((71)\). Their mean age was 33 years and their mean diabetes duration was 26 years. Some potential risk factors (glycated hemoglobin, total cholesterol, blood pressure, BMI, insulin dose, frequency of home blood glucose monitoring and of clinic attendance, smoking tobacco, presence of other complications), measured during the whole follow-up, were analyzed in relationship of the evolution of retinopathy to the proliferative stage, using fluorescein angiography. Proliferative retinopathy was diagnosed in 6 patients \((19\%)\), 3 males and 3 females. Its occurrence was significantly related to poor glycemic control during the preceding years, cumulated glycated hemoglobin being 143% vs 120% \((100\% = \text{the upper normal limit}) (p = 0.049)\), to cumulated cholesterol levels above 200 mg/dl \((p = 0.014)\), to a higher BMI, 27 kg/m² vs 22 \((p = 0.035)\), to the presence of other complications \((p = 0.029)\). In conclusion, our data suggest that the risk factors for developing proliferative retinopathy are long-term bad metabolic control, which is well known and an elevated BMI, which is novel, as noticed by Zhang et al. \((72)\). They confirm the importance of maintaining a glycated hemoglobin level always < 120% of the upper normal limit, as we have shown in homozygous diabetic twins \((5)\).

In an as-yet unpublished longitudinal study, we tried to determine the risk factors associated with the progression of non-proliferative diabetic retinopathy, in extension of our previous longitudinal study on the first microangiographic abnormalities in diabetic children \((56)\). Appearance of
retinopathy was significantly correlated with cumulated glycated hemoglobin levels >125% of the upper normal limit, cumulated mean cholesterol level >220 mg/dl, BMI >27 kg/m². Worsening of retinopathy, observed over a time period of 3 years, was significantly correlated with raised glycated hemoglobin levels. The results prove the deleterious effect of bad glycemic control during 3 years.

Neuropathy

Electro-encephalogram (EEG)

In 1979, we published a study on EEG in diabetic children (73). Abnormal EEG patterns were found in 15 of 61 patients (25%). Among the abnormal EEG patterns, 6 were diffuse (non-rhythmic slowing) and 9 were paroxysmal (spike-and-wave in 5, spikes in 1, sharp waves in 1, bursts of delta waves in 2). A significant positive correlation was found between minor EEG abnormalities and long-term degree of diabetic control according to clinical estimates, but no definite increase was noted in relation to the duration of diabetes. Eighty per cent of the patients having more than 5 severe hypoglycemic attacks showed evidence of abnormal EEG, suggesting that hypoglycemic coma or convulsions are closely related to EEG abnormalities (minor hypoglycemic episodes had no effect on the EEG). With the sensitive technique of fluorescein angiography, we demonstrated a clear correlation between incipient retinal angiopathy and EEG abnormalities, perhaps because both retinal and brain capillaries have tight junctions that become leaky in the presence of chronic hyperglycemia. In conclusion, the factors that most positively relate to pathologic electrocerebral activity in diabetic children are frequent and severe hypoglycemic attacks, comas and/or convulsions and vascular changes in the retina. In 1995, Hauser et al. (74) demonstrated that the level of HbA1c influences the EEG and that improvement of glucose metabolism is an important factor in avoiding EEG abnormalities in young diabetic patients. In 1996, Bjorgaas et al. (75), in a controlled blind study, have shown that episodes of severe hypoglycemia may affect frontocentral function slightly in some diabetic children. Putative mechanisms of cerebral abnormalities in diabetes have been reviewed by Bissels et al. (76).

Afferent nerve action potential and cerebral somatosensory evoked potentials

Clinical neuropathy is rare in children and adolescents with satisfactory glycemic control. Sensitive tests can detect subclinical neurological abnormalities, the natural history of these being unclear (45). In 1981, in a pilot study, we measured the afferent conduction of nerve impulses from digits to the cortical sensory area (77). The recording of the afferent nerve impulse volley at different levels of the peripheral and central nervous system (CNS) is particularly rewarding in the precise determination of mild, subclinical abnormalities. The cerebral somatosensory evoked potential affords ultimately critical data allowing an evaluation of the propagation of the same volley in the CNS, namely the spinal cord and intracerebral pathway. In diabetic patients without clinical neuropathy, subclinical neuropathy, characterized by desynchronization of action potentials in the median nerve, precedes conduction velocity (CV) slowing. A reduction of the maximal CV is not the sole feature indicating a dysfunction of the peripheral nerve. Additional evidence of a pathologic process can be demonstrated by late components of the nerve action potential (NAP) (Figure 8). Late components result from small demyelinated segments of the nerve, not sufficiently important to slow maximal CV (Figure 9). In the patient illustrated in Figure 8b, the
13.3-ms latency of the last deflection points to an additional delay. A demyelinated segment of 3.7 cm, corresponding to approximately 18 internodes, either clustered or randomly distributed along the nerve, should be postulated to account for such an abnormality. There is a distal predominance of these abnormalities. By contrast, the central CV is normal.

If the recording of the afferent nerve impulse volley at different levels is particularly sensitive in the determination of subclinical alterations, it cannot be proposed as a routine diagnostic tool. Indeed, this technique is time-consuming, unpleasant for the patient and requires sophisticated electronic equipment. Less sensitive methods, but less invasive, can be used to evidence peripheral sensory nerve dysfunction (78).

Peroneal and femoral motor nerve conduction velocity

In 1985, we published a study in which we have investigated incipient diabetic motor neuropathy (79). Peroneal motor nerve conduction velocity (PMNCV) was measured in 61 diabetic children and adolescents (age range: 7-22 yr; diabetes duration: 1-15 yr) whose type 1 diabetes became clinically apparent before the age of 14 years. They had no neurological symptoms. PMNCV in diabetic patients (48.3±5.6 m/s) was significantly lower than in controls (56.5±5.5 m/s), 23 diabetics (36%) having a value more than 2 SD below the mean for normals. Moreover, late components, indicating low CV in some fibers, were detected in 5 patients with normal CV. This attests a subclinical neuropathy even in those patients in whom the PMNCV is normal. It must be emphasized that the PMNCV is in fact the CV in the fastest fibers and thus, when normal, gives no clue to the function of the nerve trunk considered as a whole. There was a highly significant negative correlation between PMNCV and HbA1C levels concomitant with PMNCV measurement (r=-0.43; p<0.001) or mean annual HbA1C concentrations preceding PMNCV (r=-0.42; p<0.001). The relationship between PMNCV and the clinical score of diabetic control since the onset of the disease was also significant. Age, duration of diabetes and HLR-DR antigens were unrelated to PMNCV. EEG abnormalities and retinopathy, whose pathogenesis is different, were not necessarily associated with subclinical neuropathy. Being easy and sensitive, PMNCV determination provides the pediatric diabetologist and the patient himself with an important motivation to improve diabetic control.

In order to compare the prevalence of diabetic neuropathy in proximal and distal peripheral nerves, femoral and peroneal motor conduction was also evaluated in 61 diabetic children, adolescents and young adults (age range: 6-24 years; diabetes duration: 1-17 years) whose type 1 diabetes had become clinically apparent before the age of 14 years (80). They had no neurological symptoms. Femoral motor nerve conduction velocity (FMNCV) in diabetic patients (63.8±10.4 m/sec) was not significantly different from FMNCV in control subjects (65.6±7.1 m/sec). However, 13% of the patients have a value more than 2 SD below the mean for normals. By contrast, PMNCV in diabetic patients (50.2±6.9 m/sec) was significantly lower than in controls (54.1±3.5 m/sec), 31% of the patients having a value more than 2 SD below the mean for normals, which confirms our previous study (79). Peroneal nerve abnormality was negatively correlated with HbA1C levels, while femoral nerve abnormality was positively correlated with the presence of retinopathy. This discrepancy is not fully understood. Age and duration of diabetes were unrelated to femoral or peroneal motor nerve conduction velocity. Our data emphasize the occurrence of subclinical proximal neuropathy in diabetic children and adolescents. However, for a routine diagnostic tool PMNCV determination must be chosen. Moreover, very recently, Carrington et al. (81) have concluded that PMNCV is the best predictor of foot ulceration.

Sympathetic skin response

The sympathetic skin response (SSR) is a transient reflex change in the electrical potential of the skin that can be elicited by a variety of stimuli. The SSR is generated by the sweat glands and can be measured with surface electrodes connected to a standard electromyogram instrument. During our study on the evolution of subclinical complications (70), the SSR test was performed on 108 diabetic subjects with a mean age of 21 years (range: 8-37 years) and a mean duration of diabetes of 14 years (range: 6-31 years) (24). A palmar electrode was used to record the SSR, with stimulating electrode placed at the level of the supra-orbital nerve. Abnormal palmar amplitude is <500 µV. Slowing of palmar amplitude was detected in 16 patients, i.e. 15%, while the prevalence of subclinical retinopathy, motor-sensory neuropathy and nephropathy was, respectively, 49%, 45% and 11%. As a multiplicity of factors may intrude upon the measurement of this highly complex neurological reflex, the use of SSR testing could not to be a reliable and consistent index of the autonomic dysfunction (82).

Heart rate variability: statistical and spectral analysis

Since the 1970s, a lot of tests have been proposed for the diagnosis of diabetic neuropathy, namely 5 simple non-invasive cardio-vascular reflex tests (Valsalva maneuver, heart rate response to deep breathing, heart rate response to standing up, blood pressure response to standing up, blood pressure response to sustained handgrip) (83). In 2001, we carried out a preliminary study in order to determine whether the double, statistical and spectral analysis of the heart rate variability (HRV), a refined testing, could be used to detect cardiac autonomic neuropathy (CAN) in young adult diabetic patients, who already present ocular, renal or neurological complications (84). The study included 8 type 1 diabetic
patients with a median age of 29 years and median illness duration of 18 years. Retinopathy (fluorescein angiography) was diagnosed in 7 of them, peripheral neuropathy (conduction velocities in sensory and motor nerves) in 5 and nephropathy (microalbuminuria) in 3. Five time domain and 3 frequency domain HRV indices were determined from 24-h Holter recordings and then compared to reference values. In the 8 patients, CAN was assessed at different degrees of severity (Figure 10). Abnormalities were characterized by a slowing in activity of both vagal and sympathetic nervous system, with, however, a sympathetic-vagal balance in favor of the sympathetic nervous system. The severity of CAN was associated with increasing age and diabetes duration, as well as with the number and severity of the other complications. The statistical and spectral analysis of HRV seems to be an efficient tool in the evaluation of cardiac autonomic function. As it has been demonstrated that diabetic complications may appear from puberty and after 3 years of illness duration, it would be useful to apply this method in young diabetic patients from adolescence (85, 86).

Nephropathy

Glomerular hyperfiltration

Diabetic nephropathy and end-stage renal failure have been a major cause of mortality amongst young adults with type 1 diabetes. In the 1970s, the cumulative incidence of nephropathy, defined as persistent proteinuria, was <40% after 40 years of type 1 diabetes (87). The incidence increases sharply 10 years after onset of diabetes but is low after 35 years. In recent decades, a decrease in clinical nephropathy in some countries probably reflects improvements in diabetes management and glycemic control (45).

In patients with type 1 diabetes, microalbuminuria (MA), defined as an albumin excretion rate between 20-200 µg/min, precedes persistent proteinuria and is an accepted early sign of nephropathy and glomerular damage (88). However, MA can be preceded by glomerular hyperfiltration. Glomerular hyperfiltration in diabetes mellitus was first described by a Belgian man, P. Cambier (89). Soper et al. (90) have shown that poor glycemic control directly correlates with hyperfiltration and renal hyperperfusion in early type 1 diabetes. In our experience, glomerular filtration rate, determined by \(^{131}\text{I}-\text{EDTA}\) at the onset of diabetes, is >145 ml/min/1.73 m in 75% of diabetic children under 15 years of age (91). Later, there is normalization when good HbA1c is obtained.

Markers of early glomerular and tubular dysfunction during exercise

In 1975, Mogensen and Vittinghus (92) proposed a light physical exercise stress test (100W) to elicit and screen for the early presence of albuminuria in diabetic adults. In 1976, in a preliminary study (93), our group did not find exercise until exhaustion to be a stimulating effect on proteinuria in diabetic children under 16 years of age. In order to verify our previous results on a greater number of young patients, we determined whether protein excretion during intense physical exercise is an earlier sign of renal dysfunction in diabetic adolescents than the basal measurements (94). Urinary creatinine, total proteins, albumin and 62-microglobulin were studied before, immediately after and 30 minutes after exercise until exhaustion on a bicycle ergometer in a group of 21 adolescent diabetic boys (Albusitix negative) and in a comparable control group. The controls, as well as the diabetic subjects, ranged from 13 to 25 years of age and the duration of diabetes varied from 3 to 14 years. Among the 21 diabetic subjects, 11 had an incipient retinopathy diagnosed by fluorescein angiography. Urinary output of creatinine was similar in diabetic and in nondiabetic groups and did not vary during exercise. At rest, the urinary output of total proteins, albumin and 62-microglobulin was significantly higher in diabetic subjects than in controls (Figure 11). These data suggest that the subclinical proteinuria of diabetes is of mixed origin, being

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Figure 10. Spectral analysis of HRV. Illustration of the sympathetic-vagal imbalance. In a: minimal CAN, in b: severe CAN.
both glomerular and tubular. An exercise test leading to exhaustion did not give any additional information other than the basal excretion. There was no difference between diabetic subjects with early retinal and those free from any retinopathy (Figure 11). From these results, it may be concluded that exhaustive physical exercise does not provoke, in diabetic patients, an enhanced dysfunction of the kidney, while moderate loads induce a slight increase in postexercise proteinuria in diabetic patients, which is not observed in a healthy population (95).

We also investigated the potential benefit of exercise training on the renal handling of plasma proteins in a young diabetic population (11 to 18 years old; diabetes duration: 1-13 years) who participated in a 15-day sport camp with a daily exercise period of about 6 h of various physical activities (96). Under the influence of training, both albumin and β2-microglobulin excretion were reduced by half for the same load of exercise (Figure 12). Whether this effect is beneficial for diabetic adolescents remains an open question.

**Urinary excretion of transferrin and acid glycosaminoglycans**

It has been proposed that the determination of urinary transferrin is more sensitive than the determination of albumin for early detection of glomerular dysfunction (97). Earlier (in a pilot study published in 1984), we had shown that urinary transferrin excretion is higher than normal even in patients with normal MA (98). Transferrin is a protein very similar to albumin in molecular weight (MW) (90,000 vs 69,000) and shape, but with a higher isoelectric point. In general, proteins with high isoelectric points are filtered more easily through the glomerular barrier. Abnormal transferrin or MA excretion was unrelated to incipient retinopathy diagnosed by fluorescein angiography or subclinical neuropathy revealed by slowing of peroneal motor nerve conduction velocity. On the other hand, urinary excretion of α2-HS-glycoprotein (MW: 49,000) and immunoglobulin G (MW: 160,000) was normal.

**Figure 11.** Urinary output of total proteins (a), albumin (b) and β2-microglobulin (c) in controls (C) and in diabetic subjects (D), at rest (R) and after exercise until exhaustion (E). At rest, the urinary output of total proteins, albumin and β2-microglobulin was significantly higher in diabetic subjects than in controls. There was no difference between diabetic patients with subclinical retinopathy (RET+) and those free from all retinopathy (RET0) (from ref. 94).

**Figure 12.** Urinary excretion of albumin (a) and β2-microglobulin (b) at rest (R) and 15 minutes after physical exercise (E), before (TRAIN−) and after training (TRAIN+) on a regular basis, six hours per day during 15 days, in 21 adolescents (from ref. 96).
Recently we tried to confirm, with a better methodology, whether increased urinary transferrin excretion (UTE) could be found in patients with normal urinary albumin, in a larger population (99). The study included 105 patients (64 boys, 41 girls). The median age was 16 years (5-42) and the median duration of diabetes was 8 years (1-32). They had a mean HbA1c level of 7.4% (upper normal limit: 6.1%). Blood pressure was normal (<14/9 cm Hg). Urine was collected overnight and kept at 4°C until albumin and transferrin determination (1-2 days). Albumin was determined by immunoturbidimetry and transferrin by nephelometry. Normal values were measured in 27 healthy subjects: albumin<31 mg/g creatinine; UTE<0.002 mg/g creatinine. Slightly elevated MA was diagnosed in 7 patients (6.7%) and a higher UTE in 4 patients (3.8%), after a minimum duration of diabetes of 6 years. Only 2 subjects (1.9%) had concomitantly increased MA and UTE. Taken together, there was no correlation between urine albumin and UTE (p=0.77). The conclusion was that, in this unselected population of young diabetic subjects with more or less good glycemic control, the prevalence of abnormal MA or UTE is low. There is no relation between urine albumin and UTE. UTE contributes less than MA as a marker of early renal dysfunction in young diabetic patients. It is interesting to note that the prevalence of subclinical nephropathy is lower than that observed 10 years ago (9).

Acid glycosaminoglycans (GAG) are incorporated within the glomerular membrane. Their negative charges prevent the transfer of macromolecules through the membrane. In adult diabetic patients, it has been shown that the synthesis of GAG is reduced, which could contribute to impairment in the glomerular anionic filtration barrier. Therefore we tried to answer the following question: would GAG excretion be an earlier marker of glomerular dysfunction than microalbuminuria (100)? The study included 101 patients (60 boys, 41 girls). Their mean age was 17 years and the mean duration of diabetes was 8 years. They had a mean HbA1c level of 7.4% (upper normal limit: 6.1%). Urine was collected overnight and kept at 4°C until albumin and GAG determination (1-2 days). Albumin was determined by an immunological technique (nephelometry) while GAG was assayed by an ELISA system using the diethyl ethylene blue method (colorimetry). Normal values are: albumin<20 µg/min; GAG<25 µg/min. Slightly elevated microalbuminuria (mean: 35 µg/min) was diagnosed in 5 patients (5%) and a higher GAG excretion rate (mean: 47 µg.min-1) in 6 other patients (6%). Taken together, there was no correlation between albumin and GAG urine excretion (p=0.16). In conclusion, in this unselected population of young diabetic subjects with more or less good glycemic control, the prevalence of abnormal microalbumin or GAG urine excretion is low. There is no relationship between albumin and GAG excretion. The independent predictive value of GAG as an early marker of glomerular dysfunction should be confirmed in a prospective study.

In the literature, other markers of both glomerular and tubular dysfunction have been studied: 1. glomerular: fibronectin, laminin P1, type IV collagen, etc; 2. tubular: retinal binding protein, α1-microglobulin, Tamm-Horsfall protein, N-acetyl-β-D-glucosaminidase, etc (100). There is not yet consensus as to which will emerge as being widely accepted for use. Kordonouri et al. (102) have confirmed our findings of 1982 (94), i.e. that in diabetic children and adolescents both glomerular and tubular dysfunction may be present. Therefore, the sole measurement of MA is not satisfactory, even if it is proposed in the ISPAD guidelines (45). Schultz et al. (103) have suggested that a rise in albumin-to-creatinine ratio within the normal range may be a risk marker for diabetic nephropathy. HbA1c is a determinant of risk for MA, but pubertal factors have a greater effect on rates of progression of urine albumin excretion during adolescence. It has been shown that, in patients with microalbuminuria, the risk of progression to overt proteinuria can be reduced by improving glycemic control only if the HbA1c is maintained below 8.5%. Moreover, below that value, the risk declines as the level of HbA1c decreases (104). Diabetic nephropathy is limited to a subset of patients with long-standing poorly controlled diabetes and an apparent hereditary predisposition. Sodium-hydrogen exchange appears to detect a subset of diabetic patients prone to develop renal damage (105). Several observational follow-up studies have found that the D allele of the insertion/deletion polymorphism of the ACE gene is associated with an increased risk of renal function loss, even during ACE inhibition (106). Losartan could have similar renoprotective effects in diabetic patients with ACE II and DD genotypes.

Predictive Markers for Diabetic Triopathy

Lipoprotein (a)

Ten years ago, the role of lipoprotein (Lp) (a) as a genetically determined marker to predict complications in diabetic adults was controversial (107). This protein is composed of apolipoprotein (a) covalently linked to apoprotein B-100. The homology of structure of Lp(a) to plasminogen was evoked to provide a link between the clotting and lipoprotein system. In 1993, Coupey et al. (108) have shown that only pubertal and postpubertal young diabetic patients had higher serum Lp(a) levels than control subjects. In 1996, we published a study (109) on an eventual relationship between Lp(a) and three main complications (retinopathy, neuropathy, nephropathy) diagnosed with sensitive methods even at a subclinical level in 106 young type 1 diabetic patients, between 1 and 30 years of age. The patients were subdivided according to puberty and to the presence or not of subclinical complications (no complications [n=32]: retinopathy at fluorescein angiography [n=28]; neuropathy diagnosed by reduced peroneal motor nerve conduction velocity [n=30]; nephropathy determined by
presence of microalbuminuria [n=15]. Lp(a) concentrations were not significantly increased in the whole group of diabetic patients. There was no difference between girls and boys or between the prediabetic children and the others. There were no significant correlations between the markers of metabolic control (Hba1c or fructosamine) and Lp(a). Nevertheless, if the diabetic patients were divided into two groups according to the levels of Hba1c (≥7.6 or ≥7.6%), Lp(a) tends to be higher in the poorly controlled, but not at any significant degree. On the other hand, significant increases of total cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-c) and apolipoprotein B levels were observed in poorly controlled patients. Lp(a) concentrations were significantly lower in patients with subclinical neuropathy or nephropathy than in patients without these complications, but not in patients with retinopathy versus no retinopathy. These results were confirmed by categorical analysis (i.e. Lp(a)≥30 vs >30 mg/dl). In conclusion Lp(a) levels are not significantly increased in poorly controlled young type I diabetic patients. High levels of Lp(a), in young diabetic patients, are not markers for subclinical complications (retinopathy, neuropathy and nephropathy). On the contrary, low Lp(a) levels were found in subjects with subclinical neuropathy or nephropathy. Recently Hernandez et al. (110) have observed that LDL cholesterol (positively) and triglycerides (negatively) were independently related to Lp(a) concentration in diabetic patients, whatever the Lp(a) phenotype. These results indicate that Lp(a) concentrations depend on lipid profiles and suggest that treatment of diabetic dyslipidemia may also affect Lp(a) concentrations. In a review of the literature published in 2003 (111), it is stated that the contribution of Lp(a) to the enhanced risk of vascular disease in the diabetic population is not yet clearly defined.

Ultra sensitive CRP

Observations support the theory that chronic low-grade inflammation is involved in the initiation or progression of atherosclerosis. A feature of inflammatory activity is the increase in plasma concentration of acute-phase proteins produced by the liver such as the C-reactive protein (CRP). Ridker et al. (112) have conducted a prospective study among 28,263 apparently healthy post-menopausal women over a mean follow-up period of 3 years to assess the risk of cardiovascular events associated with the baseline levels of 12 markers of inflammation and also homocysteine and lipids. The conclusion is that the addition of the measurements of ultra sensitive CRP (US-CRP) and of total cholesterol and HDL-cholesterol is the best method to identify persons at high risk for future cardiovascular events. Little information exists in young type I diabetic patients. In type I diabetic patients, Schalkwijk et al. (113), have found a correlation between C-reactive protein and markers of endothelial dysfunction suggesting a relation between activation of the endothelium and chronic inflammation. There are no data in diabetic children and adolescents on a possible relationship between US-CRP and complications.

Therefore we initiated a study in order to investigate the impact of the diabetic state (Hba1c, blood lipids, subclinical complications, etc.) on the levels of US-CRP. US-CRP was determined in 126 young type I diabetic patients (55 girls and 71 boys), excluding the youngest that are certainly free of subclinical complications and in 52 healthy controls (114). The patients were divided into 2 groups according to the presence of subclinical complications: retinopathy (fluorescein angiography); neuropathy (conduction velocities); nephropathy (microalbuminuria). 81 subjects (group A) were free of complications (mean age ± SD: 16±6 years; diabetes duration: 7±4 years); 45 had at least 1 subclinical complication (mean age ± SD: 27±7 years; diabetes duration: 19±8 years). US-CRP was measured by a nephelometric assay. Blood lipids and Hba1c (HPLC) were also determined. Circulating levels of US-CRP were significantly higher in patients than in controls (2.6±4 mg/l vs 0.6±0.6; p<0.001). This difference persisted when comparing normal subjects with those of group A (2.0±3.1; p<0.01) and of group B (3.6±5.1; p<0.001) (Figure 13). Group B patients also had significantly higher levels of lipids (except for HDL-cholesterol) than those of group A (Figure 13). US-CRP levels were significantly correlated to diabetes

Figure 13. Mean values of US-CRP, TC, HDL-c, LDL-c, TG in controls and in diabetic patients without or with subclinical complications.
duration (in group A only), total cholesterol (TC), TC/HDL-C ratio, LDL-C, triglycerides (TG), but not with HDL-C, HbA1c, or blood glucose. The multivariate regression analysis showed that US-CRP concentration and the TC/HDL-C ratio interact independently on the risk for subclinical complications. In conclusion, mean plasma concentration of US-CRP is increased nearly 3-fold in diabetic patients without subclinical complications and 5-fold in those with subclinical complications in parallel with higher levels of TC, LDL-C and TG. US-CRP seems to be an interesting indicator of the risk for developing early complications.

Other Subclinical Complications

(Apolipoproteins abnormalities)

Many studies have demonstrated that the atherosclerotic process begins in childhood in association with high blood cholesterol levels (115). Lipid levels show a strong familial aggregation that has both a genetic and environmental component. In the 1980s, data on lipoprotein abnormalities and their relationship with glycemic control were controversial. To clarify this question, we began a study (116) in which plasma levels of triglycerides (TG), total cholesterol (TC), HDL- and LDL-cholesterol, apolipoproteins (Apo) A1 and B were measured in 120 young patients aged from 4 to 32 years (mean ± 1 SD: 17±6 years) whose diabetes had been present for a mean period of 10±6 years (range: less than one year to 25 years). The results obtained were analyzed in relation to total glycated hemoglobin (N: 6.8±0.6%) and plasma fructosamine (N: 1.9±0.2 mmol/l) levels. The patients were divided into 3 groups according to their HbA1 level: group 1: <9%; group 2: 9% <HbA1 <11%; group 3: ≥11%. The most significant increases of TG, TC, LDL-C and ApoB levels were observed in group 3, i.e. in patients whose diabetes was the most poorly controlled (HbA1: 12.9±1.3%; fructosamine: 4.6±0.9 mmol/l). These parameters were significantly correlated with HbA1 (p<0.01) and even more significantly with fructosamine (p<0.001). No significant difference in HDL-C and ApoA1 levels was found in the 3 groups of patients. HDL2-C and HDL3-C were unrelated to metabolic control (117). Thus, TG, TC, LDL-C and ApoB are increased in young diabetics whose HbA1 and fructosamine levels exceed reference values by more than 5 standard deviations. Consequently, blood glucose should be maximized and dietary counseling should be provided namely in Belgium where the fat intake is too high and the polyunsaturated-to-saturated fat ratio too low at 0.45 (18). An Australian group has shown that a modest increase in the monounsaturated fat content of an adolescent diet has the potential to improve glycemic control and lipid profile (118). Evidence that postprandial lipoproteins are themselves atherogenic is expanding and with this new information, a new emphasis must be placed on measures to normalize both fasting and postprandial apo B-containing lipoprotein abnormalities in order to prevent the vascular complications of diabetes (119). Of course, hyperlipidemia is in part genetically determined and it has been demonstrated that both mean parental total cholesterol and HbA1c were significant determinants of the child’s total cholesterol (120).

Oxidative stress

Endothelial dysfunction, a forerunner of diabetic angiopathy, is present early in the course of childhood diabetes. The mechanisms of such endothelial dysfunction are not clear, but oxidative stress has been proposed as one of the putative mechanisms of vascular injury in diabetes. It results from the balance between the different antioxidant defenses, non-enzymatic (vitamins C, E or A, free radical scavengers) and enzymes (red cell superoxide dismutase [SOD], red cell glutathione peroxidase [GPX], glutathione reductase [GRD]) and the free radical production (121). Recent studies have pointed out the key role of superoxide production in the endothelial cells at the mitochondrial level during hyperglycemia in the activation of the pathways involved in the pathogenesis of diabetic complications. These include increased polyol pathway flux, increased advanced glycation end product formation, activation of protein kinase C and increased hexosamine pathway flux (122). Superoxide overproduction is accompanied by increased nitric oxide generation, which in turn damages DNA. This leads to activation of the nuclear enzyme poly(ADP-ribose) polymerase, which depletes the intracellular content of its substrate NAD, slowing the rate of glycolysis, electron transport and ATP formation.

In a first study (123-5), some biological parameters involved in cell defense against oxygen radicals (plasma vitamins C and E, GPX, GRD and SOD) were measured in single blood samples from 119 diabetic infants, adolescents and young adults. Data were studied in relation to residual insulin secretion determined by C peptide level of metabolic control appreciated by glycated hemoglobin, lipid abnormalities and subclinical complications (retinopathy by fluorescein angiography, neuropathy by peroneal nerve conduction velocity and nephropathy by microalbuminuria). There was no change in antioxidant parameters with residual insulin secretion. Patients with poor glycemic control and high plasma lipids had higher levels of plasma vitamin E. Patients with subclinical nephropathy had lower plasma vitamin C levels and those with neuropathy showed lower GPX activity. Plasma vitamin C concentrations and GRD activities were negatively correlated with the age of the patients and with duration of diabetes. We concluded that higher transport capacity of vitamin E probably explains the elevated levels of vitamin E observed in patients with high lipid levels. The lower levels of vitamin C in the presence of nephropathy may be due to an increased renal excretion of this vitamin, as also shown by Hirch et al (126). The lower GPX activity associated with subclinical nephropathy confirms the animal studies having
demonstrated that oxidative stress can reduce Na\+K\+-ATPase activity (127). The reduction of GPX, GRD activities and vitamin C levels confirms the existence of an oxidative stress in young type 1 diabetic subjects. Another pediatric study has shown that systemic oxidative stress is present upon early onset of diabetes and is increased by early adulthood (128).

As oxidized LDL and autoantibodies to oxidized LDL seem to play an important role in the atherosogenic process and, as studies in young type 1 diabetic patients are scarce, it was decided to evaluate autoantibodies against oxidized LDL (o-LAB) and antioxidant status in relationship with levels of HbA1c and lipids and with the presence of subclinical complications (retinopathy by fluorescein angiography, neuropathy by peroneal nerve conduction velocity and nephropathy by microalbuminuria) (129). The study included 110 young type 1 diabetic patients, with a median age of 15 years and a median diabetes duration of 5 years. The mean ± SEM of HbA1c levels was 7.1±0.2 % (upper normal limit: 6.1 %). Subclinical complications were detected in 26 patients. Total antioxidant status (TAS), vitamin A or E was not decreased in the patients and no significant differences were noted between the different subgroups of patients classified according to their subclinical complications. HbA1c levels were not related to autoantibodies. Autoantibodies against LDL-lipoproteins decreased with age and diabetes duration, as reported in healthy non diabetic subjects. The decrease of o-LAB could be associated with the onset of the atherosclerotic process if we consider that o-LAB antibodies are an accompanying immunological expression of lipid peroxidation. The titers of circulating o-LAB antibodies reflect the balance between the amount of antibodies generated and released into the circulation and the consumption of these antibodies. In conclusion, in diabetic patients with a more or less good diabetic control, increased lipid peroxidation or reduced lipid antioxidant defense could not be demonstrated, even for the patients with subclinical complications. Recently, Varvarovska et al. (130) have shown reduced plasma antioxidant capacity in diabetic children, but their mean HbA1c level was high: 9.2 % (upper normal limit: 5.8 %).

To fight against the oxidative stress, antioxidant drugs, in particular vitamins C and E have failed to demonstrate any effect (122). In the last few years, lipolic acid has been found to reduce neuropathic symptoms (127). Statins, ACE inhibitors and angiotensin 1 inhibitors can also reduce intracellular oxidative stress generation. Finally, the best way to avoid increased superoxide production is to maintain blood glucose values close to the normal range.

**Metabolic disruptions in red and white cells**

Metabolic and structural modifications of the red blood cell may play a role in the pathogenesis of diabetic microangiopathy. Impaired deformability of the diabetic erythrocyte affecting the blood flow at the capillary level, increased glycation of membrane proteins and hemoglobin, decreased intra-erythrocytic adenosine triphosphate (ATP) level have been implicated as causative factors in the development of vascular complications. Ditzel (131) pointed out the importance of the alterations of oxygen delivery to the tissues in the disease: in the acidic patient, the acidosis and hypophosphatemia interfere with the red cell 2,3 diphosphoglycerate (2,3 DPG) generation. The resulting increase in hemoglobin-oxygen affinity together with an excessive formation of glycated hemoglobin could be, in part, responsible for tissue hypoxia. Data concerning diabetic red cell glycolysis activity, ATP and 2,3 DPG levels are rather fragmentary and mainly focused on adult diabetes. Therefore, we published in 1992 a paper, the aim of which being to study this metabolic pathway in red cells from type 1 diabetic children and adolescents (132). Erythrocytes from young type 1 diabetic patients (n=11), incubated in their plasma in anaerobic conditions, exhibited higher glucose consumption than cells from controls (n=11). This increased metabolic activity is believed to reflect erythrocyte alterations dependent on the degree of metabolic control, as glucose consumption was significantly correlated to glycated hemoglobin (HbA1) and to glucose levels (p<0.05 and p<0.01, respectively). Red cell hexokinase (HK) and pyruvate kinase (PK) activities were similar in both groups whereas phosphofructokinase (PFK) activity was slightly higher in patients' cells (p<0.05). No difference was found between patients and controls for red cell ATP and 2,3 DPG levels. However, the concentrations of these glycolytic products seem also closely related to the glucose homeostasis in diabetes. Indeed, within the diabetic group, ATP levels showed a negative relationship with glucose level (p<0.05) and 2,3 DPG a positive relationship with HbA1 (p<0.05). In conclusion, higher glycolytic activity is present in young diabetic red cells. This activity as well as ATP and 2,3 DPG levels are related to the degree of short- or long-term diabetic control. These findings stress the importance of a careful metabolic control to avoid hematological disturbances.

In the literature, some diabetic patients have been shown to present with disturbances to one or more functions of their polymorphonuclear neutrophils (PMN). Abnormalities of adherence, chemotaxis, phagocytosis, nitroblue tetrazolium (NBT) reduction, superoxide anion formation, chemiluminescence and bacterial killing have all been described and may play a role in the decreased host resistance to infections which is observed in poorly controlled diabetic patients. The published data are, however, equivocal; for instance, the PMN dysfunction has been attributed to serum and/or cellular abnormalities. These discrepancies may be due to at least 2 factors: 1. the variability of the methods used; 2. the variable and sometimes poorly defined clinical status of the patients under consideration. The aims of our work (133) were to compare PMN functions in type 1 diabetic children with
controls and to determine the role of glycation. Twenty unselected young type 1 diabetic patients aged between 13 and 25 years, with a diabetes duration of 2-16 years, were involved in the study. The following PMN functions were analyzed: random and induced migration, saphylococcus aureus and baker’s yeast phagocytosis, saphylococcus aureus killing, spontaneous and stimulated NBT reduction, myeloperoxidase score. The effect of the glycation of normal serum on baker’s yeast was measured. No significant difference between patients and controls was observed in the PMN functions studied. When diabetic serum was used in the tests, random and induced PMN migrations correlated negatively with the HbA1 level. However, there was a positive correlation between baker’s yeast phagocytosis and HbA1 level. The nature of the relevant glycated proteins, which stimulate phagocytosis, is unknown. In conclusion, the PMN dysfunction, which occurs in type 1 diabetes, is a result of both inhibiting and stimulating phenomena and is related to glycated hemoglobin levels.

**Low T3 syndrome**

As type 1 diabetes is frequently associated with thyroid autoimmunity (134), in 1985 we published a study on thyroid function (thyroid stimulating hormone, TSH; triiodothyronine, T3; reverse T3, rT3; thyroxine, T4; free T4, FT4) in relationship with the presence of thyroid antibodies (antithyroglobulin and antithyroid microsomes antibodies) as well as with the degree of metabolic control (HbA1c; upper normal limit: 6.5 %) (135). The serum levels of thyroid hormones and TSH were compared in 64 type 1 diabetic children and adolescents (13.8±4.2 years; diabetes duration: 5.6±3.9 years) without ketosis and in 28 age matched normal subjects (13.9±4.9 years). In diabetic children, HbA1c was 8.1±2.5 %. Only T3 levels were significantly different in the diabetic patients (2.38±0.41 nmol/l) than in controls (2.64±0.52 nmol/l) (p<0.01) confirming the existence of the ‘low T3 syndrome’ in diabetic children as already described in diabetic adults with poor metabolic control. A negative correlation was found between T3 and blood glucose as well as glycated hemoglobin suggesting that short-term hyperglycemia could regulate T3 concentration. Low T3 level is secondary to an impaired peripheral T3 production from T4. This conversion is catalyzed by the enzyme T4-5’- deiodinase; the activity of this enzyme has been shown to be reduced by hyperglycemia in diabetic rats. Thyroid function was not different in diabetic children with and without thyroid antibodies. We conclude that serum T3 level is influenced by the degree of metabolic control and that thyroid function in diabetic children should be assessed by the measurement of the serum concentration of T4, FT4 and TSH. The negative correlation between HbA1c and T3 has been confirmed by Radetti et al. (136).

Recently, Kordonouri et al. (137) found that 10% of diabetic patients, with a median age of 13 years, had elevated titers of antibodies to thyroperoxidase - anti-TPO - (more in girls than in boys) and 6% had antibodies to thyroglobulin. Because 50% of children with diabetes and significant titers of anti-TPO develop thyroid problems within 3-4 years, examinations of thyroid antibodies should be performed yearly.

**Magnesium and HbA1c**

Pediatric studies on magnesium depletion in type 1 diabetic patients are scarce and there are no data on erythrocyte magnesium content (EMC), which is important since 99% of total magnesium (Mg) is intracellular. Moreover, in the pediatric studies there are no data on the relationships between hypomagnesemia and HbA1c levels or subclinical complications. Therefore, we have carried out a study in order to answer to these questions (138).

Serum Mg levels, EMC, magnesuria and HbA1c were determined in 118 type 1 diabetic subjects (105 boys and 83 girls) aged 19±8 years (mean ± SD) with a diabetes duration of 11±8 years and in 96 controls. Mg was measured by colorimetric calmagite kits and HbA1c using an HPLC method. We searched for subclinical retinopathy (fluorescein angiography), neuropathy (conduction velocities in the limbs), nephropathy (microalbuminuria and β2-microglobulinuria) in patients aged >12 years with a diabetes duration >3 years.

The mean ± SD Mg serum concentration was 1.8±0.2 mg/dl in the diabetic population and 2.0±0.2 mg/dl in the controls (p<0.001). The mean EMC was 5.0±0.5 mg/dl in the patients, vs 5.3±0.2 mg/dl in the controls (p<0.001). In 14% of the patients, serum Mg levels were less than -2 SD below the normal mean, while 6% of the diabetic subjects had EMC less than -2 SD below the normal mean. In diabetic patients, serum Mg levels were positively correlated with EMC (r=0.19; p<0.01) and negatively with age (r=−0.24; p<0.01), duration of diabetes (r=−0.19; p<0.05), HbA1c (r=0.16; p<0.05). EMC levels were negatively correlated to magnesuria (r=−0.31; p<0.05), which is related to microalbuminuria (r=0.24; p<0.05) and β2-microglobulinuria (r=0.26; p<0.05). In the 74 diabetic patients with one or more subclinical complications, serum Mg levels were significantly lower than in the patients without complications (1.8±0.2 mg/dl vs 1.9±0.2 mg/dl; p<0.01). It has been shown that low plasma magnesium concentration was associated with development and progression of retinopathy (139).

In conclusion, lower serum Mg levels and EMC are found in type 1 young diabetic patients. Hypomagnesemia is related to age, duration of diabetes, bad glycemic control and presence of subclinical complications. An increased renal magnesium clearance during hyperglycemia has been shown (140). Mg depletion should be searched for, even in the pediatric population and supplementation with Mg should be considered.

**Helicobacter pylori (HP) and HbA1c**

Many studies have been published to elucidate the prevalence of HP infection in childhood (141). The prevalence of HP in children living in developed countries is low (15-25%) compared
to the prevalence in children living in developing countries (40-60%). In 1996, Oldenburg et al. (142) have found that the age-adjusted seroprevalence of HP (IgG and IgA) in type 1 and type 2 diabetic patients was higher than in control subjects in several age groups.

In a preliminary study summarized in 1997 (143), we studied the prevalence of HP-seropositivity in 278 diabetic children with a mean age of 18 years and a mean duration of diabetes of 10 years, 69% being European Caucasians (EC) and 31% mainly Mughrabin Caucasians (MC). Anti-HP IgG (Elisa Cobas Core) were detected in 18% of the patients (32% in the MC group and 11% in the EC group). The age of seropositive children was lower in the MC group than in the EC group. Less than half of seropositive subjects have abdominal complaints. We have also evidenced that HP-positive diabetic children, adolescents and young adults had higher HbA1c levels than our total diabetic population (139% vs 120% of normal values, the upper normal limit being 100%). In 43 out of the 49 seropositive patients, it was possible to verify the presence of HP in the stomach by a 13C-urea breath test and an upper gastrointestinal endoscopy with biopsies for histology and HP-culture. An active infection has been proven in 81% of the seropositive subjects. In conclusion, HP-positive patients have a significantly higher HbA1c level than HP-negative ones. One third of young MC is HP-seropositive, mainly before the age of 18 years, which is 3 times more than in EC, probably due to a lower mean socio-economic status. In a Turkish study, anti-HP IgG was positive in 56% of diabetic children and in 31% of controls (144).

In another study we examined the relationships between HbA1c levels and eradication of HP infection. (145). A total of 47 patients (age: 18±6 years; diabetes duration: 9±5 years) with HP infection, proven by histology, culture and 13C-urea breath test (UBT), were included in the study during a 6 month period after a bi-therapy based on a bacterial antibiogram. HP eradication was checked with UBT 2 months after the end of treatment. HbA1c levels, measured by an HPLC method at diagnosis, 2 and 6 months after treatment, were expressed as % of normal values, the upper normal limit being 100%. Eradication of HP infection was obtained in 32/47 patients (68%). Age and diabetes duration were not significantly different in the eradicated and non-eradicated groups, nor was the ratio immigrants/non immigrants. HbA1c levels were significantly higher in HP-non-eradicated patients than in HP-eradicated subjects at diagnosis (147% vs 136%), 2 months after treatment (147% vs 137%) and 6 months after treatment (145% vs 135%). However, treatment of HP infection whether successful or not, did not modify HbA1c levels after 2 and 6 months. In conclusion, eradication of HP infection is less efficient in type 1 diabetic subjects with the poorest glycemic control and eradication of HP has no influence on HbA1c levels during the following 6 months. Similarly, in type 1 diabetic adults, de Luis et al. (146) don't observe improvement in HbA1c levels 6 months after treatment for HP infection. Ojetti et al. (147) have reported that 38% of type 1 diabetic patients, compared with 5% in controls, were re-infected with HP one year after successful eradication. Better metabolic control in diabetic patients in whom HP has been eradicated compared with re-infected subjects was observed.

Complications Related to Treatment

**Complication activation by zinc insulins**

More than 15 years ago, we determined the effect of the switch-over from porcine (Actrapid MC and Monotard MC to semi-synthetic human insulin (Actrapid HM and Protaphane HM) in a prospective study comparing complement evaluation (CH50, C3, C3d/C3, C4) as well as other immunological factors (insulin antibodies, autoantibodies, etc), metabolic control (HbA1, lipids, etc) and clinical data (insulin dose/kg, number of hypoglycemic episodes, etc) (148). Forty-six type 1 diabetic children and adolescents (mean age ± SD: 14.3±3.8 years) participated in the trial. The duration of diabetes ranged from 1.0 to 16.9 years (mean ± SD: 7.3±3.7). The study protocol consisted of a 9 month period during which, at monthly intervals, the subjects were assessed clinically and blood samples taken for measurement of biological data. After 3 months, porcine insulins were switched to human insulins. The main results were as follows:

1. The insulin dose/kg was increased after the switch-over (porcine insulin: 0.90±0.03 U/kg; human insulin: 0.98±0.03 U/kg; p<0.001). This can perhaps be explained by the different bioavailabilities of Monotard MC and Protaphane HM.

2. The objective degree of metabolic control (HbA1c), as well as the HDL-cholesterol level and the apolipoproteins A1/B ratio, were not statistically different before and after the switch-over.

3. IgG insulin antibody binding was not statistically different on transfer from porcine insulin to semi-synthetic insulin.

4. Mean level of total IgE and IgG specific insulin antibodies, determined before and after the switch-over, were not different.

5. Autoantibodies and antinuclear factor were unchanged.

The prevalence of immune complexes was decreased by half (p<0.05) after 6 months on human insulin.

7. The patients treated by porcine insulins had an increased complement activity (CH50) (p<0.001) and an increased C3 activation (p<0.001) as compared to controls while mean C4 remained unchanged. Moreover, there was an increased mean value of C3 breakdown product C3d and of the catabolic index C3d/C3 (p<0.001). After the switch-over to human insulins, CH50, C3 and C3d/C3 ratio decreased to the values observed in healthy controls.

In conclusion, the main feature of this prospective study was
the demonstration of an abnormal in vivo complement metabolism in diabetic children and adolescents treated with porcine insulins Actrapid MC and Monotard MC and its correction through the use of semisynthetic human insulins Actrapid HM and Protaphane HM.

The prospective study was prolonged until 2 years in 45 diabetic children and adolescents in order to follow the evolution of the insulin antibodies (149). IgG insulin antibodies were detected in 21 children out of 45 (47%) at a mean level of 0.96 mIU/ml (0.77-1.15). A significant and important decrease of both the number of patients having insulin antibodies and the level of insulin antibodies was observed 18 months after the switch-over. After 24 months, IgG insulin antibodies were only present in 5 patients (11%) at a mean level reduced by half (0.53 mIU/ml; p<0.05). Four patients out of the five (80%) had HLA DR3/4 antigens while this phenotype was only prevalent in 26% of the initial patient cohort. The prevalence of autoantibodies and of antinuclear factors showed no significant variations. The practical implication of these findings was that the use of human insulins could be of interest even in patients previously treated by porcine insulins because of the decrease of insulin antibodies.

In order to understand the abnormal in vivo complement metabolism in type 1 diabetic children treated with monoclonal porcine insulin Monotard MC and its correction after switch-over to human insulin Protaphane HM (148), we have investigated the ability of different kinds of insulin preparations to induce complement activation in vitro (150). Freshly collected serum samples from healthy blood donors were incubated with commercial fast and intermediate or long-acting (by protamine sulphate [PS] or zinc) insulin preparations for 2 hours at 37 degrees C. The C3 activity of the supernatants was measured by turbidimetry as a marker of C3 complement fraction consumption. Only long-acting preparations of insulins without PS were associated with highly significant increased levels of C3 activity, whatever the source of insulin, animal or human. Moreover, addition of exogenous PS was able to inhibit the C3 conversion. This effect was dose-dependent and peaked at the concentration of commercial NPH insulin preparations. The mechanism by which PS inhibits complement activation in vitro could be related to its ability to interfere with the physical nature of the solid surfaces presented by the insulin crystals. Indeed, insulin crystals were rapidly cleared (<5 min) in the incubated serum when small doses of PS were added. The complement activating capacity of long-acting insulin without protamine was dose-dependent, equivalent to the known complement activator Zymosan and abolished in the presence of EDTA (Figure 14). In conclusion, the study has documented the ability of some protracted insulin preparations to activate the complement system in vitro if they are devoided of PS. On the other hand, short-acting and NPH insulins are not complement activators. The practical conclusion is to avoid the use of zinc insulins which are complement activators. Moreover, NPH and zinc insulins differ in their ability to form stable mixtures with neutral insulin solutions, since only NPH insulin can be mixed with regular insulin without changing the specific course of effect of regular insulin and the variability of resorption from the subcutaneous deposit is higher for zinc insulins than for NPH.

[LysB28,ProB29]-human insulin analogue is not a complement activator in vitro (151).

Overweight in adolescents on 4 daily insulin injections

In a preliminary study published more than 15 years ago, when the basal-bolus regimen was made easier thanks to the invention of the pen injector (Novopen), we have evaluated the use of the Novopen in 23 type 1 diabetic adolescents and young adults between 14 and 28 years of age (152). All patients were diabetic before age 15 and the duration of diabetes varied from 5 to 23 years. All patients were previously treated with a conventional regimen of Actrapid HM and Monotard HM or Protaphane HM twice daily (0.98+0.24 U/kg/day). The patients used Novopen to inject Actrapid HM in a bolus regimen with Ultratard HM as basal insulin, administered before bedtime. The mean duration of the Novopen experience was 8.1 months. During the first 4 months after transfer to Novopen, the total daily dosage of insulin was higher than one U/kg; afterwards the insulin needs decreased to 0.8 U/kg (at 12 months). On the other hand, the weight/height ratio increased significantly from the 4th month. After one year, the

![Figure 14.](image-url)
mean increase was 14%. The mean level of HbA1c was unchanged after the transfer to Novopen. The patients’ self-evaluation of the therapy was documented by asking them to fill in a questionnaire: 91% of the patients considered the use of Novopen more pleasant than the previous injection therapy and 87% reported a greater freedom regarding diet.

Later in more large studies we have confirmed that HbA1c levels were unrelated to the number of insulin injections (9-11). We have observed that after the age of 13 years, BMI was significantly higher in girls and in adolescents on four daily injections, as a result of greater dietary freedom (11). The Hvidere Study Group on Childhood Diabetes has confirmed our findings showing that the increase in daily insulin injections was not associated with changes in the average HbA1c levels of the participating centers (20-23) and that adolescent girls, but to a lesser extent also boys, on 4 or more injections had significantly higher BMI than girls on twice-daily insulin (20).

The adjustment of insulin dosage is more complicated in the basal-bolus regimen because dose alteration cannot be done only according to sliding scales based on the glycemia immediately preceding the insulin injection. Insulin dose alteration must be retrospective according to previous experiences, prospective according to physical activity and programmed meals, with only a "touch" of compensatory adaptation according to the present glucose level (9-18).

The proper use of this system gives more freedom for sports and meals, but young patients rarely succeed in following it. On the other hand, the proper use of the two-injection regimen, in countries where the meal schedule allows correct allocation of diet, may lead to "intensive conventional therapy" and good metabolic control.

**Well-being indices and HbA1c**

Therapeutic constraints should not decrease the quality of life and well-being of patients. Therefore, we carried out a study in order to evaluate by a questionnaire the well-being of our autonomous diabetic adolescents and young adults in relationship with their HbA1c levels and other characteristics (1). A total of 100 unselected subjects (73 men and 44 women), with a mean age of 21 years (14-38) and a mean diabetes duration of 12 years (0-26), were included in the study over a 3-month period. Mean age at onset of diabetes was 10 years. Twenty-five percent of the patients were of Moroccan origin. All the patients were autonomous for self-management and treatment. Their socioeconomic status was not different from that of the normal population. The mean annual HbA1c level in the 100 diabetic patients was 7.3 (4.7-11.7). Well-being was measured using a questionnaire developed by a working group of the World Health Organization, International Diabetes Federation and St Vincent Declaration (153). The questionnaire included 4 subscales labeled depression, anxiety, energy and positive well-being. The measurement of all 4 subscales involved 22 items and allowed an estimation of general well-being. General well-being in women was not as good as in men due to a greater tendency toward depression. Well-being was better in patients with a professional activity than in the others. Patients' age, duration of diabetes, number of insulin injections, frequency of home blood glucose monitoring, presence of 1 or 2 subclinical complications, had no effect on well-being. On the other hand, well-being was negatively correlated with the HbA1c levels: the higher the HbA1c, the higher the anxiety and the depression and the lower the energy and the positive well-being (Figure 15). In conclusion, well-being was mainly associated with HbA1c levels; it improved with better glycemic control.

**Conclusion**

Successful treatment is reflected in good HbA1c associated with less severe hypoglycemic events, a better quality of life, lack of long-term complications and is not necessarily exportable without adjustment to the local way of life and the socio-economic status. No dogmatism! Only the objective result is important.

Our "recipes" have been summarized (9-11,15,18,154):

- Critical mass of patients; friendly contacts and personalized long-term follow-up until adulthood, at the age where clinical complications are possible which gives the so-called pediatric diabetologist more motivation to require good control.
Chronic diabetic complications

- High frequency of home blood glucose monitoring, of HbA1c measurements and of long-term consultations; screening for subclinical complications by sensitive methods from puberty in order to increase the motivation of both the patient and the doctor (see below)

- Two daily insulin injections in children <15 years: easy and effective; an individualized mixture of insulins in a syringe gives better results, in terms of HbA1c, than the use of premixed insulins with a pen injector; the proportion of carbohydrates of the mid-morning snack must be more important than that for breakfast

- Basal-bolus regimen in adolescents: increased flexibility in daily life and dietary freedom, but more complicated; no simplistic sliding scales; insulin dose alteration must be triple: 1. retrospective, according to numerous previous experiments, in order to enjoy more freedom for meals, sports, etc; 2. prospective according to programmed changes in meals and sports; 3. with only a "touch" of compensatory adaptation according to the present glycaemia. This needs psychological maturity, otherwise the multiple injection system leads to anarchy and obesity, mainly in adolescent girls

- Fast-acting analogs in the basal-bolus regimen: no systematic replacement of rapid-acting insulins if the time period between 2 injections exceeds 3 or 4 hours; in our experience, the fast-acting analogs are recommended under well defined circumstances; 1. to correct hyperglycaemia rapidly; 2. to allow to eat something between the main meals; 3. to allow a snack at 4 o'clock if the period between lunch and dinner exceeds 6-8 h, i.e. the length of action of the rapid-acting insulins; 4. if the patient sleeps in, in order to avoid the cumulation of the activities of the rapid-acting insulins injected before late breakfast and before lunch, the analog can replace the rapid-acting insulin before breakfast; 5. if dinner is near bedtime, in order to avoid the cumulation of the activities of the rapid-acting insulin injected before dinner and of the intermediate-acting insulin injected at bedtime, the analog can replace the rapid-acting insulin before late dinner, reducing the risk of nocturnal hypoglycaemia.

In 1998, Rosilio et al. (19), summarized the main pediatric studies on glycemic control since 1981 (Figure 16), showing that the best results, in terms of HbA1c, were obtained by our team in Brussels (11), with 2 or 4 insulin injections. The classic twice-daily insulin regimen is appropriate for Belgian children and adolescents until the end of the secondary school, according to Belgian customs, namely those concerning the meal timetable. In the most recent publications (155-159) on HbA1c in relation to the use of multiple injection regimens or of insulin pumps, the results are poorer than what we have published since 10 years with 2 (children) or 4 injections (adolescents) (9,11).

The motivation of both the patient and the doctor is fundamental in order to obtain for life good glycemic control. After age 13 and 3 years of diabetes, we perform every year: retinal fluorescein angiography, measurements of motor and sensitive conduction velocities (which are different to a painful electromyography) and dosage of microalbuminuria and 82-microglobulinuria. The majority of my colleagues use only the dosage of microalbuminuria and the observation of the eye fundus at regular ophthalmoscopy. It is important to able to say to the patient, for example, "You have no complaint, but, as you can see on this photograph, there are 2 leakages of fluorescein in your left eye; it is reversible if you improve your Hba1c; otherwise, that will become an irreversible lesion leading later to overt complications". The same message applies to the slowing of conduction velocity or the presence of abnormal microalbuminuria. For many ophthalmologists, the most important operational outcome for retinal screening is the detection of severe lesions that must be treated by laser therapy in order to avoid blindness and, therefore, they don't understand why to use fluorescein angiography if fundus at regular ophthalmoscopy or through digital non mydriatic retinal imaging is normal (160). Tele-ophthalmology via stereoscopic digital imaging has even been proposed (161). A virtual doctor for a real diabetic patient.

At the onset of diabetes, we look for eventual EEG abnormalities; therefore in the case of convulsions during severe

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Figure 16. Comparison of glycated hemoglobin levels in major pediatric studies. HbA1c is expressed in % of normal mean being 100% (from ref. 19)
hypoglycemia we are able to exclude epilepsy. Every year, we check for lipoprotein abnormalities, US-CPD, raise, autoimmune disorders (namely thyroid antibodies and antithyroid (IgG and IgA) antibodies plus eventually anti-endomysial IgA antibodies), serum and erythrocyte magnesium content, anti-HP IgG, complement consumption, as well as other classical measurements (urea, creatinine, etc). Of course we search for hypertension, lipodystrophies, necrobiosis lipoidica diabeticorum, etc. We have never met impaired growth, except in cases of Mauriac syndrome (29) or limited joint mobility (162), probably because of the good mean HbA1c of our patients. Diabetes-related foot problems usually occur in older people with neuropathy and vascular complications (45).

The most important message to give to diabetic children, adolescents and young adults is that potentially disabling complications are not due to diabetes per se (if true, they should be ineluctable), but to long-term hyperglycemia and are therefore avoidable. We have a marker to claim that complications are not running: to obtain a glycated hemoglobin level under 7% (4). The Hvidøre studies (20-23), performed in developed countries without financial restrictions, have shown that treatment of childhood diabetes is in general inadequate and that levels of HbA1c are very different. Diabetes treatment teams should individually explore the reasons for failure without any prejudice or bias (9,11,15,18). Education and multidisciplinary team concepts for pediatric and adolescent diabetes mellitus (163-165), as well as educational vacation camps (166) must be evaluated objectively. Quality of care and patient well-being should be compared across diabetology teams with the goal of optimizing both these parameters (167).

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