The Early Natural History of Nephropathy in Type 1 Diabetes

I. Study Design and Baseline Characteristics of the Study Participants

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This report describes the design and baseline demographic and clinical data in the study of the early natural history of diabetic nephropathy (DN) in type 1 diabetes carried out by the International Diabetic Nephropathy Study Group. The study enrolled 243 patients ages 10–40 years (16.8 ± 6.0, mean ± SD) with type 1 diabetes for 2–20 years (8.0 ± 4.2) at centers in the United States (Minneapolis), Canada (Montreal), and France (Paris). At baseline, all patients were normotensive, none had reduced glomerular filtration rate (GFR), and all but eight were normoalbuminuric (NA). All patients had baseline renal biopsies. During the study, patients will have multiple measurements of blood pressure (BP), renal function, albumin excretion rate (AER), glycemia, and other variables, with repeat renal biopsies planned at 5 years after baseline. The 31.3% of the approached patients who agreed to participate were similar in age, diabetes duration, HbA_{1c}, AER, and sex to those refusing participation. Age, diabetes duration, HbA_{1c}, and AER were similar among the three centers, but systolic BP, GFR, renal plasma flow (RPF), and filtration fraction were lower in the Paris center. The 153 patients with hyperfiltration (GFR >130 ml · min^{-1} · 1.73 m^{-2}) had greater RPF than those with normal GFR. The eight microalbuminuric patients tended to have longer duration of diabetes but were otherwise similar to the NA patients. The role of these and other variables in determining the development rate of the early lesions of DN over the 5 years between biopsies is the central issue under study. *Diabetes* 51: 1572–1579, 2002

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AER, albumin excretion rate; AP, adjusted parameter; BP, blood pressure; CV, coefficient of variation; DBP, diastolic blood pressure; DN, diabetic nephropathy; ESRD, end-stage renal disease; FF, filtration fraction; GFR, glomerular filtration rate; HPLC, high-performance liquid chromatography; IDNSG, International Diabetic Nephropathy Study Group; MA, microalbuminuric; NA, normoalbuminuric; PAH, para-amino hippurate; RBF, renal blood flow; SBP, systolic blood pressure; UUN, urinary urea nitrogen; Vv(Mes/glom), fractional mesangial volume.

Diabetic nephropathy (DN), the leading cause of end-stage renal disease (ESRD) in the Western world, is responsible for >40% of all new cases of ESRD in the United States (1) and develops in ~25% of patients with type 1 diabetes (2). Although poor glycemic control is an important risk factor, glycemia does not fully explain why only a subset of diabetic patients progress to ESRD (3).

A number of studies have demonstrated that the renal histopathologic abnormalities of DN, especially the glomerular lesions, are closely related to the clinical expression of the renal disease, i.e., increasing albumin excretion rate (AER), hypertension, and declining glomerular filtration rate (GFR) (4,5). The structural abnormality most closely related to renal functional changes in type 1 diabetes is mesangial expansion, although other glomerular as well as vascular and interstitial lesions also appear to contribute (3,4). Other variables posited as important in determining risk include genetic background (6,7), renal hemodynamics (8), sex (9), and age of onset (10).

Through much of the natural history of DN, renal disease develops without detectable clinical expression, with normal AER and blood pressure (BP) and with GFR normal to elevated (3). However, during this period, which can last from 10 to 30 years or more, the renal lesions of diabetes may progress and may overlap in severity with those of microalbuminuric (MA) patients and approach the severity of patients with overt DN (3,5).

Once overt DN is established, current therapeutic strategies, including improved glycemic control and effective antihypertensive therapy, tend to slow but are usually unable to arrest progression of renal disease (11). Therefore, methods for early identification of diabetic patients at risk for development of DN are needed. Understanding the influence of age, diabetes duration, sex, puberty, glycemia, systemic BP, glomerular permeability to protein, hyperfiltration, lipids, genetic suceptibility, and other variables on the development of the early lesions of DN may provide this information. This is important, since it is a logical assumption that patients not developing the early lesions of DN will not progress to more advanced lesions and, hence, will not develop overt DN (3–5).

In this report, we describe a longitudinal study of the natural history of DN being carried out by the Interna-

tional Diabetic Nephropathy Study Group (IDNSG) in a group consisting almost entirely of normoalbuminuric (NA) patients. Given that the anticipated clinical event rate (progression from normoalbuminuria to microalbuminuria or proteinuria) over the 5 years of this study was low, the study was designed with the end point of progression of the key structural changes of DN, especially mesangial expansion (3,4). Thus, using two renal biopsies performed 5 years apart in a large cohort of type 1 diabetic patients, changes in renal structure will be assessed with regard to measures of glycemia, BP, GFR, renal blood flow (RBF), and other variables to identify the factors associated with the earliest changes in renal structure in DN.

RESEARCH DESIGN AND METHODS

Participating institutions. This study enrolled participants in three university centers: *1*) Department of Pediatrics, Montreal Children's Hospital, McGill University Faculty of Medicine, with affiliations at the Montreal General Hospital, Center Hospitalier (Universitaire de Sherbrooke, Sherbrooke, Canada), the Ottawa Civic Hospital (Ottawa, Canada), and the Children's Hospital of Eastern Ontario (Ottawa); *2*) Department of Pediatrics, University of Minnesota Medical School (Minneapolis, MN), with affiliations at St. Paul Children's Hospital (St. Paul, MN) and the International Diabetes Center (Minneapolis, MN); and *3*) Hôpital Robert Debré (Paris, France), with affiliations at Hôpital Saint-Louis (Paris) and INSERM Unité 192 at the Hôpital Necker-Enfants Malades (Paris). The Data Coordinating Center is in the Department of Epidemiology and Biostatistics at McGill University. Core Laboratories for AER, GFR, RBF, plasma lipids, and renal morphometry are at the University of Minnesota. Together, these constitute the IDNSG. All clinics associated with IDNSG represent either university-based practices or private practice groups affiliated with medical schools and teaching hospitals providing care to children or adults with type 1 diabetes.

Recruitment. Eligible patients were identified in each center and were informed by their treating diabetologist about this research study. Study personnel also attended the diabetes clinics and introduced the study to the patients. Interested patients met with the local study coordinator, who provided a complete description of the project and a discussion of potential risks. Informed consent was obtained from all patients; for children (under 18 years of age), consent was obtained from the children and their parents. This study was approved by the committees for the use of human subjects in research at the three study centers and at each of the eight affiliated institutions from which patients were recruited.

Inclusion criteria. Type 1 diabetes was defined as age of onset before the 31st birthday and insulin dependence within 1 year of diagnosis. All patients were 10–40 years of age at entry, had type 1 diabetes for 2–20 years, and had onset of diabetes before age 31.

Exclusion criteria. *1*) Median AER >100 μg/min in three consecutive timed overnight urine collections; *2*) GFR (see below) <90 ml · min^{-1} · 1.73 m^{-2}; *3*) hypertension, defined as above the 95th percentile of published age-appropriate pediatric and adult normative standards available in 1990 (12,13), when these studies were initiated; *4*) solitary kidney, evidence of unilateral renal disease, or other kidney disease; *5*) significant psychiatric or physical chronic disease; *6*) pregnancy—in patients who became pregnant during the study, kidney function tests and renal biopsies were postponed until at least 3 months after the pregnancy.

Schedule of studies. Patients were seen at baseline and quarterly at each study center for measures of BP, AER, HbA_{1c}, and protein intake. GFR, RPF, 24-h BP monitoring, plasma lipids, and anthropomorphic measurements were obtained annually.

Specific studies

General data. Demographic data and present and past health status were recorded. At each quarterly visit, subjects underwent a review of physiologic systems and physical examination including Tanner staging. Also obtained was the patient's birth weight, gestational age at birth, and infant feeding history. Blood was obtained for DNA isolation from leukocytes. A family medical history was obtained, including information on hypertension, coronary artery disease, stroke, or other cardiovascular problem in relatives. Blood specimens for lipid profiles and isolation of leukocyte DNA were obtained from the patients and their biologic parents, and BP measurements were obtained on the parents.

Renal biopsy. All biopsies were performed by the senior nephrologists at each of the study centers. All patients had normal BP, coagulation studies and platelet counts, and precise renal localization by ultrasound. Standard percutaneous renal biopsy techniques were used including ultrasound guidance and the Franklin modified Vim-Silverman needle; when requested, patients received sedation. Patients were requested to undergo a second biopsy if the first biopsy did not provide an adequate number of glomeruli (a minimal of two nonsclerosed glomeruli) for morphometric measures.

Kidney function studies: GFR, RPF, and AER. Constant infusion techniques were used. Radioactive or nonradioactive iothalomate clearances were used for 90% of the subjects; nonradioactive inulin clearance was used for the remainder. In Montreal, 91 patients had nonradioactive and 25 had radioactive iothalomate studies. In Minneapolis, 69 patients had nonradioactive and 7 had radioactive iothalomate GFRs; 7 had inulin GFRs. In Paris, 25 patients had nonradioactive iothalomate, and 16 had inulin GFRs. The correlation between the radioactive and nonradioactive iothalomate methods performed simultaneously in patients was $r = 0.76$ ($P = 0.0001$), with no difference on paired t testing between the two methods ($P = 0.53$). Bland-Altman plots indicated no trend or deviation, and only 7 of 116 iothalomate GFR determinations were outside the 95% normal range. The correlation between the nonradioactive iothalomate and inulin methods performed simultaneously in patients was $r = 0.89$ ($P = 0.0001$). Bland-Altman plots indicated no trend or deviation, and only 3 of 41 determinations were outside the 95% normal range. However, the GFR estimate was significantly higher for the inulin method in these simultaneous studies, but the bias was constant over the range of GFRs ($P = 0.80$). Thus, values for inulin clearance were corrected for iothalomate method by subtracting the mean difference between the two methods. After the baseline studies, all subsequent GFR estimates used the nonradioactive iothalomate method.

RPF was estimated by para-amino hippurate (PAH) clearance during the GFR tests. After water loading, four timed urine and blood collections were taken. Iothalomate or inulin and PAH clearances were measured using high-performance liquid chromatography (HPLC). Inulin was measured by HPLC or using I^{125} isotope counting. When the coefficient of variation (CV) for the four collection periods was <15%, values for all four periods were used. When the CV was >15%, the highest and lowest values were discarded and the mean of the remaining values was used. However, if these two values were highly discordant and were in two different categories (normal, reduced, or increased GFR), then the data were not used. GFR and RPF are expressed as ml · min^{-1} · 1.73 m^{-2}.

AER (μg/min) was measured every 3 months in timed overnight urine collections using a sensitive fluorescence immunoassay (14). The standard curve for this assay is linear (range, 0.5 to 20.0 μg/ml albumin; $r = 0.99$). The intra-assay and interassay variations were 6.0 and 6.7%, respectively. Patients with AER <20 μg/min in at least two of three consecutive urine samples during the first year of the study were classified as NA. Patients with AER ≥20 but <200 μg/min in at least two of three of these consecutive samples were defined as having persistent MA (15).

BP. BP was measured at baseline by trained observers using a Dinamap Vital Signs Monitor (model 1846 5X) in a quiet setting after the patient was seated for 5 min. Three readings were taken 1 min apart; the average of the second and third readings was used. Hypertensive patients were not included in the study, and no patient reported here was receiving antihypertensive medication. Twenty-four-hour ambulatory BP monitoring is the subject of a separate report.

Kidney size. Kidney size was estimated by ultrasound in 199 patients by a single reader at the Minneapolis center. Normal values for kidney length in children up to age 18 (16) and for adults (17) were as previously published. The children's values were normalized for their body surface area (16).

Glycemia. HbA_{1c} was measured in each local laboratory using standard HPLC methods including the Diamat glycosylated hemoglobin analyzer (Bio-Rad, Hercules, CA) and the same lots of columns, resins, and reagents (18).

Dietary protein. Dietary protein intake was estimated quarterly by measuring 24-h urinary urea nitrogen (UUN). UUN and urinary creatinine were measured on the Beckman ASTRA System (Beckman Instruments, Brea, CA) with urease-containing reagent and the Jaffe rate reaction between creatinine and the picrate ions, respectively. Protein intake was estimated by a published formula (19). The correlation between 24-h true urinary nitrogen and UUN in 26 study subjects was $r = 0.98$ ($P = 0.00001$). Only urine collections of 20- to 28-h duration containing between 9 and 31 mg · kg · day^{-1} of urinary creatinine were accepted.

Power calculations and planned statistical analyses. The sample size was originally determined from cross-sectional data and was reassessed using data from an interim analysis of the final and initial fractional mesangial volume [Vv(Mes/glom)] data among the first 40 patients in this study who completed both biopsies. These data indicated that the mean Vv(Mes/glom) rate of change, r, over the 5-year period was 0.017 (SD, 0.0584; range, −0.017 to 0.19). The sample size was determined on the basis of the primary method of analysis, namely linear regression. The simple linear regression analysis of

TABLE 1
Patient recruitment

	All	Montreal	Minneapolis	Paris
Approached (*n*)	825	449	294	82
Refused participation (*n*)	499	287	185	27
Excluded (*n*)	66	40	21	5
Biopsied				
n	260	122	88	50
%	31.5	27.2	29.9	61.0
Included in baseline study (*n*)	243	117	85	41

the rate of change of Vv(Mes/glom) in on the initial Vv(Mes/glom) showed that 15% of its variance is explained by the initial Vv(Mes/glom). We assumed that, as the final study will also use age, sex, and disease duration to explain the variation, we could expect that at least 25% of the variance of rate of change would be explained by these factors. Thus, the residual SD on which inference regarding the study factors will be based is expected to be <0.0506. Using this adjusted variance estimate, a two-sided significance level of 5%, and powers of 80–90%, a total study size of 250 patients would allow the detection of differences in the Vv(Mes/glom) rate of change of 0.02 to 0.03. These differences correspond to roughly one-half of the SD.

The primary data analysis will focus on one of the principal outcome variables, namely the annual rate of change (r) of Vv(Mes/glom), which we denote by $r = (MV_F - MV_I)/t$, where MV_F and MV_I are the final and initial Vv(Mes/glom) values, respectively, and t is the time spanned between the two biopsies. The first step will assess, by multiple linear regression, the variability of r explained by background factors such as the initial Vv(Mes/glom), duration of disease as of first biopsy, age at onset, sex, etc. The second step will attempt to explain part of the remaining variability from the various explanatory factors under study, namely glycemic control, blood pressure, renal hemodynamics, etc. A common aspect of these variables is that they are all measured several times within the interval spanned by the two biopsies. In that respect, it will be necessary to distinguish between three types of variables: *1*) variables that remain fixed within the study (e.g., sex, age of onset); *2*) variables that are expected to remain relatively stable (although they could vary randomly) during the study period (e.g., HbA_{1c}); and *3*) variables that are expected to show some systematic trend (increase or decrease) as part of the course of the disease and thus during the study period (e.g., blood pressure, renal hemodynamics). These may be both predictors and outcome variables.

For variables of the second type, three measures will be used to assess their relationship to the outcome r, the annual rate of change of Vv(Mes/glom): *1*) the mean for the first 3–12 months, to indicate the value at initial biopsy; *2*) the mean over the whole study period, to indicate the overall value; and *3*) yearly means, to identify possible trends in the association. Multiple linear regression will be used.

For variables of the third type, the same analyses as for above will be used initially. In addition, simple linear regression methods will be used within each individual to compute an estimated slope of change in time in the explanatory factor and its approximate (because of correlated observations within each subject) SE. The standardized slope will then be used as an independent predictor of the outcome r. Here again, multiple linear regression will be used.

Statistical analyses in the current report. Standard univariate methods of data analysis were used. Comparisons of means were done with *t* test and ANOVA. Comparisons of proportions were performed using the χ^2 test. The reliability of measures taken on the same subject was assessed by the paired *t* test and the correlation coefficient. Adjustments for age, duration, and other factors, when performed, are noted.

TABLE 2
Comparison of participants with patients refusing participation

	Participants	Refused participation	*P*
n	243	499	
Age at entry (years)	16.8 ± 6.0	17.6 ± 5.6	0.068
Age at diabetes onset (years)	8.8 ± 4.8	8.8 ± 5.3	0.921
Diabetes duration (years)	8.0 ± 4.2	8.8 ± 4.5	0.01
HbA_{1c} (%)	8.7 ± 1.5*	8.7 ± 2.2†	0.7345
AER (μg/min)	7.6 ± 12.5‡	11.7 ± 16.7§	0.286
Female (%)	51	46	0.1402
Male (%)	49	54	
Caucasian (%)	96	97‖	0.4127

Data are means ± SD. *n = 241; †n = 400; ‡n = 228; §n = 17; ‖n = 426.

RESULTS

Recruitment. Eight hundred twenty-five patients were identified as potential participants and recruited for the study. Of these, 499 refused participation, 66 were excluded, and 260 (31.5%) agreed to participate and had baseline renal biopsies (Table 1). These represented 27.2% of the eligible patients in Montreal, 29.9% in Minneapolis, and 61.0% in Paris. Eight of the 260 patients undergoing baseline biopsy did not have adequate tissue for study (see also below), and 9 patients did not meet all the inclusion criteria described above (1 was hypertensive, 3 had AER >100 μg/min, 2 did not meet the baseline GFR criteria, and 3 did not meet the age, age at onset, or diabetes duration criteria), leaving 243 patients who make up the cohort of this study. The majority of the participating patients (69%) came from families with one or both college-educated parents. There were no center differences for this variable, other than a lower proportion of mothers of Paris center patients (27%) with postsecondary school education compared with Montreal (52%) or Minneapolis (69%) (χ^2 = 16.9, $P < 0.001$).

Comparison of participants and patients refusing study participation. Data were available on the 499 patients who declined participation. There were no significant differences between the participants and those refusing participation for age at recruitment or age of diabetes onset, HbA_{1c}, AER, sex, or race. Participants had slightly shorter diabetes duration (Table 2).

Renal biopsy success rate and complications. Successful renal biopsies (defined as the availability of at least two glomeruli for electron microscopy studies) were obtained in 243 of 260 (93.5%) initial biopsy attempts. Of the 17 unsuccessful first biopsies, 14 had inadequate tissue for study, and the tissue was not properly fixed in 3. Biopsies were repeated in 11 participants and were successful in 9.

There were 25 biopsy complications in 22 patients. These included eight (3.1%) with transient gross hematuria without urinary obstruction, five (1.9%) with mild postbiopsy discomfort, five (1.9%) with perirenal or subcapsular hematoma with pain (1.9%), one (0.4%) with postbiopsy pain without documented hematoma, two (0.8%) with bladder obstruction with clots requiring bladder catheter-

TABLE 3
Additional baseline clinical characteristics of participants

Variable	n	Mean ± SD	Range
GFR (ml · min^{-1} · 1.73 m^{-2})	237	142 ± 28	85.0–226.0
RPF (ml · min^{-1} · 1.73 m^{-2})	197	660 ± 174	175–1156
FF	197	0.2 ± 0.06	0.10–0.70
SBP (mmHg)	242	113.5 ± 11.1	85.5–145.0
DBP (mmHg)	242	62.8 ± 8.9	44.5–89.5
Mean arterial pressure (mmHg)	242	79.7 ± 8.6	59.2–104.8

Abbreviations used: GFR = glomerular filtration rate; RPF = renal plasma flow; FF = filtration fraction; SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure

ization, and one (0.4%) with repeated episodes of gross hematuria requiring angiographic embolization of a small bleeding renal artery branch. There were three adverse reactions to prebiopsy sedation (1.2%), two with brief hysterical reactions and one instance of respiratory depression requiring a brief period of intubation. No patient suffered any permanent injury from the renal biopsy procedure.

First-year compliance. Patients successfully performed 88% of the quarterly tasks required for compliance during the first year (80% of the requested urine samples, 92% of the HbA_{1c} blood samples, and 91% of the BP recordings). There were no significant center differences in compliance.

Baseline clinical characteristics of the participants. Participants' age at entry, diabetes duration, and HbA_{1c} values are presented in Table 2. Eighteen (4 women) were Tanner stage 1, 99 (53 women) were Tanner stage 2–4, and 126 (68 women) were Tanner stage 5.

Because children as young as age 10 years were included, there were wide variations in height (128–195 cm), weight (27–100 kg), and BMI (13.9–33.3 kg/m^2). GFR and RPF were high, but filtration fraction (FF) (0.2 ± 0.06) was normal (Table 3). Kidney length (n = 199) was within normal limits in 173 patients (87%), increased in 21 patients (10.5%), and decreased in 5 patients (2.5%). Overall, kidney length was increased ($P < 0.0001$) by an average of 0.3 cm (3%).

Obesity was defined as BMI above the 95th percentile for age and sex. Forty-four patients were obese, 23 men and 21 women, 23 from Montreal, 16 from Minnesota, and 5 from Paris. There were no differences in obesity in relation to sex, age, diabetes duration, HbA_{1c}, diastolic BP (DBP), GFR, RPF, or FF. Obese patients had higher systolic BP (SBP) than nonobese patients (120 ± 11 vs. 112 ± 11 mmHg, respectively; $P = 0.0003$) when adjusted for age.

Comparison of baseline characteristics by center. Age at biopsy, age at onset, and diabetes duration were similar in the centers (Table 4). There were no significant center differences in baseline height, weight, BMI, HbA_{1c}, or AER. There were center differences in BP. SBP at baseline was higher in Montreal than in Minneapolis patients and was also higher in the Montreal and Minneapolis patients compared with the Paris patients. DBP was comparable in Minneapolis and Montreal and higher in both centers than in Paris. The center differences in BP persisted after adjusting for age and diabetes duration. There were also center differences in renal function, with higher GFR in Montreal or Minneapolis than in Paris. RPF was higher in Montreal than in Minneapolis or Paris. FF was lower in the Paris center than in Minneapolis or Montreal. These differences persisted after adjustment for protein intake. Kidney length was greater in the Paris center.

Multiple linear regression analysis with GFR as the dependent variable and BMI, age, diabetes duration, sex, HbA_{1c}, SBP, DBP, and center as the independent variables found that 23% of the variability of GFR was explained by these independent variables. There were significant effects of female sex (adjusted parameter [AP] in ml · min^{-1} · 1.73 m^{-2} = −10.5, $P = 0.0037$) and center (Minneapolis AP = 22.9, $P = 0.0002$; Montreal AP = 27.3, $P = 0.0001$). There was a trend toward an effect of HbA_{1c} (AP = 2.1, $P = 0.06$). The model applied to RPF found 16% of the variability to be associated with these variables. There were similar female sex (AP in ml · min^{-1} · 1.73 m^{-2} = −55.4, $P = 0.04$) and Montreal center (AP = 73.0, $P = 0.0341$) effects and a trend for a BMI effect (AP = 7.8, $P = 0.054$). The model applied to FF found 14% of the variability to be associated with these independent variables. Significant effects were seen for BMI (AP in kg/m^2 = −0.003, $P = 0.044$), HbA_{1c} (AP in % = 0.0083, $P = 0.007$), and center (Minneapolis AP = 0.0428, $P = 0.0008$; Montreal AP = 0.03, $P = 0.02$).

Hyperfiltration (GFR >130 ml · min^{-1} · 1.73 m^{-2}) at baseline was present in 65% of patients, whereas 35% had GFR ≤130 ml · min^{-1} · 1.73 m^{-2} (Table 5). HbA_{1c} was similar in the patients with and without hyperfiltration.

TABLE 4
Comparison of baseline characteristics by center

Variable	Minneapolis	Montreal	Paris	P
Age at biopsy (years)	16.3 ± 7.2 (85)	17.3 ± 5.7 (117)	16.4 ± 3.3 (41)	0.475
Age at diabetes onset (years)	8.6 ± 5.8 (85)	9.0 ± 4.4 (117)	8.6 ± 3.3 (41)	0.846
Diabetes duration (years)	7.7 ± 4.2 (85)	8.3 ± 4.4 (117)	7.8 ± 3.7 (41)	0.560
HbA_{1c} (%)	8.7 ± 1.3 (83)	8.6 ± 1.7 (117)	8.9 ± 1.3 (41)	0.487
AER (μg/min)	7.9 ± 7.1 (72)	7.0 ± 13.1 (116)	9.0 ± 17.7 (40)	0.693
SBP (mmHg)	112.0 ± 11.6 (84)	117.1 ± 9.3 (117)	106.3 ± 10.9 (41)	0.0001
DBP (mmHg)	63.4 ± 9.8 (84)	63.5 ± 8.7 (117)	59.9 ± 6.6 (41)	0.06
GFR (ml · min^{-1} · 1.73 m^{-2})	143.1 ± 26.6 (81)	149.1 ± 25.6 (115)	119.6 ± 22.9 (41)	0.0001
RPF (ml · min^{-1} · 1.73 m^{-2})	622 ± 148.5 (67)	708 ± 194.8 (89)	619 ± 141.4 (41)	0.0021
FF	0.24 ± 0.06 (67)	0.22 ± 0.07 (89)	0.20 ± 0.04 (41)	0.0031
Kidney length (cm/1.73 m^2)	11.9 ± 1.5 (90)	11.2 ± 1.0 (107)	12.2 ± 1.2 (7)	0.001

Data are means ± SD (n).

TABLE 5
Comparison of participants with normal GFR and participants with hyperfiltration

	Normal GFR (≤130 ml · min^{-1} · 1.73 m^{-2})	Hyperfiltration (>130 ml · min^{-1} · 1.73 m^{-2})	*P*
HbA_{1c} (baseline) (%)	8.6 ± 1.5 (84)	8.7 ± 1.5 (152)	0.706
GFR (ml · min^{-1} · 1.73 m^{-2})	113.7 ± 11.4 (84)	157.4 ± 20.6 (153)	—
RPF (ml · min^{-1} · 1.73 m^{-2})	555 ± 133 (70)	718 ± 168 (127)	0.0001
FF	0.22 ± 0.07 (70)	0.23 ± 0.06 (127)	0.171

Data are means ± SD (*n*).

The hyperfiltration group had higher RPF (Table 5), but there was no difference in FF.

Dietary protein intake at baseline, available in 214 patients, was 1.24 ± 0.49 g · kg^{-1} · day^{-1}, and there were no center differences ($P = 0.48$). The center effects on renal function (GFR, RPF, and FF) persisted after adjustment for protein intake.

Microalbuminuria. Baseline AER correlated weakly with baseline HbA_{1c} ($r = 0.18$, $P = 0.0052$) and tended to increase with age ($r = 0.13$, $P = 0.049$) and diabetes duration ($r = 0.10$, $P = 0.13$). Persistent microalbuminuria (as defined by at least two of three consecutive measures in the MA range during the first year) was present in eight (4%) of the participants. The MA patients tended to have longer diabetes duration ($P = 0.052$) (Table 6).

DISCUSSION

The early development of DN occurs without overt clinical signs or symptoms (3). By the time elevations in AER, hypertension, or reduced GFR are present, renal structural changes are well established and may be quite advanced (3,5). Therefore, careful longitudinal evaluation of renal biopsy specimens from asymptomatic patients is required to study the development rate of early renal changes of DN. Moreover, because renal structural measures vary among normal individuals (20), the starting point—the structure of the kidney just before the onset of diabetes—cannot be precisely known. For these reasons, this IDNSG study was designed to define the natural history of early DN based on two renal biopsies obtained 5 years apart. Changes in renal morphology during the course of the disease will be correlated with glycemia, blood pressure, kidney function, AER, pubertal stage, and other variables. Moreover, to account for the fact that the initial biopsy is not taken at the onset of diabetes, these correlations will be adjusted for duration of disease. The present article presents the study design and provides baseline information regarding the study cohort.

TABLE 6
Comparison of participants with normoalbuminuria and persistent microalbuminuria during the first year

	Normo-albuminuria	Micro-albuminuria	*P*
n	213	8	
Age at diabetes onset (years)	8.9 ± 4.6	8.3 ± 4.8	0.707
Diabetes duration (years)	7.9 ± 4.3	10.9 ± 5.3	0.052
AER (μg/min)	4.5 (0.3–18.7)	31.4 (21.4–77.5)	—
Baseline HbA_{1c} (%)	8.6 ± 1.5	9.4 ± 1.4	0.124
Baseline SBP (mmHg)	114 ± 11	114 ± 11	0.957
Baseline DBP (mmHg)	62 ± 9	67 ± 10	0.097

Data are means ± SD or median (range). AER data are based on the median of the AER determinations used to classify patients as normoalbuminuric or microalbuminuric.

This study design is based on the simple notion that all patients who ultimately develop sufficiently advanced diabetic glomerulopathy lesions will have clinical findings of DN (4). Thus, although the severity of lesions present when proteinuria is found varies from patient to patient, there is a level of lesions beyond which all patients have overt DN (4). Change in mesangial fractional volume [Vv(Mes/glom)], the single structural parameter most closely associated with the functional abnormalities of DN (3,4), was selected as the primary end point for these studies. Moreover, NA long-standing type 1 diabetic patients with DN who progressed to microalbuminuria or proteinuria had greater Vv(Mes/glom) at baseline evaluation than patients who did not progress over 5–17 years of follow-up (21).

It is not proven that the selected primary end point, change in Vv(Mes/glom) over 5 years, is a precise predictor of DN risk, and this is clearly a limitation of these studies. Nonetheless, considering the facts presented above, it is argued that this structural end point is a reasonable selection, given the almost insurmountable impracticality of functional end points in a natural history study beginning at the earliest stages of this disorder.

The study confronted important ethical considerations, since the protocol involves the performance of kidney biopsies in a cohort of type 1 diabetic patients without detectable renal functional abnormalities at baseline. This is a natural history study with no treatment component, children as young as 10 years were included, and, at most, only 30% of the recruited patients would be expected to develop serious kidney disease. Approval for these studies was given by 11 separate institutional review boards on the following justification. Diagnostic renal biopsies are routinely performed for disorders carrying much lower risks of ESRD than type 1 diabetes (22,23). Clinically indicated native kidney biopsies are largely performed for diagnosis and prognosis and, in the majority of instances, do not affect treatment strategies (22,23). Confirmation that important renal structural changes are not present should give assurance to diabetic patients and their families; such findings are expected in at least 65% of cases, and this is similar to the service provided to patients with persistent microscopic hematuria (22,23). Finally, given current technologies of ultrasound renal localization and biopsy guidance (24,25), and given that only experienced nephrologists would perform the biopsies, the anticipated complication rate is low.

A possible concern is whether this cohort of participants is representative of type 1 diabetic patients. It is possible that more difficult diabetes management cases are

referred to the medical school and medical school–affiliated clinics participating in this study. However, it was possible to compare the patients recruited into this study with patients refusing participation, and there were no significant differences with regard to age, sex, and glycemia, whereas diabetes duration was slightly longer in those refusing participation. Moreover, study subjects were selected so as to exclude patients with all but the earliest findings of diabetic renal complications at baseline (only 8 of 243 had microalbuminuria, all were normotensive, and all had normal to high GFR values), reducing any tendency toward more difficult and complicated patients. In any case, the primary goal of this study is to assess the determinants of changes in kidney structure over time, which may be less affected by the issue of representability.

There are few investigations of the natural history of diabetic nephropathology in type 1 diabetic patients before renal functional abnormalities are evident. Renal biopsy studies of patients with shorter diabetes duration have included much smaller numbers of subjects (<50) and have largely been cross-sectional in design (26–30) or have included MA patients (31–33), whereas our patients were nearly all NA. Longitudinal studies at earlier stages of DN have included relatively small numbers of MA patients followed for <5 years and usually in the context of a treatment trial (34,35). The influence on early DN of potentially important variables such as age of onset, pubertal status, and other determinants could not be well examined in previous studies because of design limitations, small patient numbers, and/or short intervals between renal biopsies. Nonetheless, those studies and the present study indicate that research biopsies are not a major deterrent to a high rate of participation by children and young adults in studies of DN.

Significant biopsy complications occurred in 14 patients, 4 required intervention, and there was no permanent injury consequent to the baseline renal biopsy procedure. Only one patient discontinued participation after the initial biopsy. Patient compliance with study procedures and protocols during the first year was high and comparable to other large multicenter studies. Thus, the biopsy procedure did not appear to alienate the patients from this study.

Approximately one-third of eligible patients who were contacted agreed to participate in these studies. This was less than the 80% (36 of 45) participation reported in Europe (29). The recruitment rate was nearly twice as high in the Paris center than the other two centers. Renal biopsies in the studies of Berg et al. (29) were requested from patients who had previously agreed to serial measures of renal function; thus, some preselection may have occurred. Our patients' compliance rate with the research tasks in the first year of the present study was high, exceeding 85% overall, and there were no center differences. However, it remains unclear whether longer-term study compliance will be the same in centers with higher versus lower initial recruitment rates. The early experience in this study provides some indication of the population of diabetic patients needed as a recruitment base for studies of comparable design.

Despite the center differences in recruitment rates, there were no center differences in baseline age, diabetes duration, sex distribution, HbA_{1c}, or AER. However, there were significant center differences for other important variables. Thus, SBP was higher in Montreal than Minneapolis, and both SBP and DBP were higher in North America compared with the Paris center. Some of these findings may be explained by lower norms for BP in young people in France versus the United States (36). Italian studies (37), on the other hand, have reported higher childhood BP values than in U.S. subjects but lower values in late adolescence compared with Northern European norms (38). Whether these represent genetic or environmental variables, or both, is unknown. Insofar as systemic BP may play a direct or indirect role in modulating DN risk (39), the role of this hemodynamic factor in the pathogenetics of important DN lesions may be revealed by this longitudinal study.

There were also strong center differences in renal function, GFR, and FF, which were lowest in the Paris center, whereas RPF was higher in the Montreal center. Based on multiple regression analyses, these differences appear to be unrelated to systemic BP or dietary protein intake. The exact explanation for these center differences is unknown. It is not likely that these findings represent methodologic differences, since renal function measurement protocols were similar in the three centers and values were adjusted for methodologic differences. The center differences could represent genetic differences in responses to the diabetic state, such as tendencies to renal hypertrophy, physiologic responses to glycemia, or other as yet undescribed environmental influences. However, there were no center differences for glycemia in our study. There were nonsignificant trends for GFR or RPF to be directly related to HbA_{1c}, whereas the direct relationship of FF to HbA_{1c}, as previously reported by Berg et al. (29), was highly statistically significant. This could represent influences of glycemia on glomerular arteriolar resistances (40), changes in glomerular filtration surface (41), changes in glomerular capillary wall hydraulic conductivity, or combinations of these and other factors. Hopefully, longitudinal studies including analyses of rates of DN lesion development and renal functional variables will provide further insights. Interestingly, female sex was associated with relatively lower GFR and RPF compared with male sex, but sex was unrelated to FF. FF was also inversely related to BMI. These phenomena were not explained by protein intake. Male sex is a well-known risk factor for nephropathy in type 2 diabetic patients (42), but it is not generally agreed that male sex increases DN risk in type 1 diabetes (9,43–45).

Almost two-thirds of the patients in this study had hyperfiltration, defined as GFR >130 ml $\cdot$ min^{-1} $\cdot$ 1.73 m^{-2}, and this, as expected, was associated with increased RPF but not with increased FF. The presence of hyperfiltration has been associated with increased nephropathy risk (46–48), although not all investigators have confirmed this (49), and AER and BP may play more important roles (50). The investigation of the relationship of hyperfiltration to the rate of development of DN lesions will thus be an important outcome of these studies.

AER correlated weakly with HbA_{1c} and diabetes duration, as has been previously noted (2,51–55). Only 8 of the 220 patients with sufficient AER measurements early in

this study to define their status had microalbuminuria. The reason for the somewhat lower prevalence of persistent microalbuminuria in our patient population (3.6%) than in most previously reported studies using a similar microalbuminuria definition (2,52,54–56) is not known. In part, the low microalbuminuria incidence may have reflected the selection criterion that rejected patients from this study with AER exceeding 100 μg/min. However, only two patients were rejected. Microalbuminuria may be uncommon in prepubertal children (54,57,58), who made up a large portion of our study subjects. Microalbuminuria is relatively uncommon in the first decade of type 1 diabetes (45,54), and mean diabetes duration in our study was <10 years. Finally, a few patients with hypertension at baseline were excluded, and this could lower the prevalence of microalbuminuria (59). Of substantial concern is the marked variability in the prevalence of microalbuminuria among type 1 diabetic patients, varying from 3.7 to 30% in prior studies (2,51–53,54–58,60,61). A number of complex variables could account for these differences among studies, including study location, the background genetic populations, selection criteria, microalbuminuria definition, and other factors that cannot be easily extracted from the published literature. The present longitudinal study of renal structure and function in this carefully selected and studied patient population should provide clarity to these issues. However, as revealed here, important center differences may exist, and these may have been underestimated in their potential influence on the outcomes of epidemiologic and natural history studies. The role of these and other variables in this study in determining the development rate of the lesions of DN over the 5 years between biopsies is the central issue under study by the IDNSG.

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Susan Kupcho performed the urinary albumin measurements. Jean Bucksa headed the clinical research laboratory. Joyce Stein prepared this manuscript. Trudy Strand, Marlys Nolander, Patricia Moynihan, Vicky Siefert, and Michele Watrin performed the coordinator duties in Minnesota; Brigitte Maruca assisted in this capacity in Montreal; and Christine Delcroix, MD, and Dominique Simon, MD, performed the coordinator duties in Paris. Moira Mills coordinated the Montreal laboratory efforts. Hélène Beaufils and Veronique Beaudoin performed many of the clinical studies in Paris.

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APPENDIX

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