Increased Left Ventricular Mass in Normotensive Type 1 Diabetic Patients With Diabetic Nephropathy

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OBJECTIVE — Diabetic nephropathy increases the risk of premature cardiovascular disease and sudden death, particularly in type 1 diabetic patients. One possible mechanism for this risk may be left ventricular hypertrophy. In our study, we aimed to evaluate left ventricular structure and function in normotensive type 1 diabetic patients with and without nephropathy.

RESEARCH DESIGN AND METHODS — M-mode and Doppler echocardiography was performed in 17 type 1 diabetic patients with nephropathy (albuminuria [median (range)], 345 (135–2,846) mg/24 h) and compared with 34 normotensive, normoalbuminuric (10 [3–30] mg/24 h) type 1 diabetic patients matched for arterial blood pressure (mean ± SD) ([134/77] ± [13/7] vs. [129/78] ± [12/7] mmHg), age (40 ± 11 vs. 42 ± 10 years), duration of diabetes (28 ± 7 vs. 28 ± 6 years), and BMI (24.2 ± 4.2 vs. 24.6 ± 2.4 kg/m^2).

RESULTS — Left ventricular mass (LVM) index was significantly higher in patients with nephropathy compared with patients with normoalbuminuria (100.8 ± 10.3 vs. 88.2 ± 21.0 g/m^2, respectively; P = 0.02). Greater ventricular septum width was demonstrated in the nephropathic group compared with the control group (9.4 ± 1.0 vs. 8.2 ± 1.3 mm, respectively; P = 0.002). No significant difference in posterior wall thickness was apparent. The nephropathic group tended to have reduced diastolic function (E/A ratio, 1.2 ± 0.3 vs. 1.4 ± 0.4; P = 0.09). Fractional shortening was normal and about the same in the two groups. The groups did not differ with respect to serum creatinine or hemoglobin, while metabolic control (assessed by HbA_{1c}) and plasma renin and prorenin levels were elevated in the nephropathic group compared with the normoalbuminuric group.

CONCLUSIONS — A blood pressure–independent increase in LVM may contribute to the increased cardiac morbidity and mortality in normotensive type 1 diabetic patients with diabetic nephropathy. Glycemic abnormalities and activation of the renin-angiotensin system may lead to the ventricular enlargement.

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The excessive mortality associated with diabetic nephropathy is mainly due to end-stage renal failure and cardiovascular disease. The relative mortality from cardiovascular disease is, on average, increased 40-fold in type 1 diabetic patients with nephropathy compared with the general population (1). Abnormalities in well-established cardiovascular risk factors (e.g., dyslipidemia, arterial hypertension, smoking, glycemic control, and BMI) cannot alone account for this finding (2). Increased left ventricular mass (LVM) may contribute to the increased cardiovascular risk because left ventricular hypertrophy (LVH) is an ominous prognostic sign and an independent risk factor for sudden death, ventricular dysrhythmia, myocardial ischemia, coronary heart disease, and heart failure (3). LVH is often assumed to be little more than a marker for hypertension. In fact, the association between diastolic or systolic blood pressure and LVM is not always close (4,5).

The aim of our study was to evaluate putative mechanisms of the increased cardiac morbidity and mortality in normotensive type 1 diabetic patients with and without nephropathy by measuring LVM in a case-control design.

RESEARCH DESIGN AND METHODS

Patients

At Steno Diabetes Center, diabetic nephropathy is diagnosed clinically based on the following criteria: persistent albuminuria >300 mg/24 h in at least two out of three consecutive 24-h urine collections, presence of retinopathy, and no clinical or laboratory evidence of kidney or renal tract disease other than diabetic glomerulosclerosis. Glomerular filtration rate is measured every 12–18 months in all patients with diabetic nephropathy. During 1993, 242 type 1 diabetic patients had their glomerular filtration rate measured. They were all invited to participate in a case-control study (6,7), and 198 patients, all of whom were Caucasian, agreed. At the time of the original study, the World Health Organization's criterion for hypertension of 160/95 mmHg was generally accepted. We have chosen not to change the cutoff criterion for blood pressure in the present study; therefore, hypertension was considered to be present when arterial blood pressure was persistently ≥160/95 mmHg or when patients received blood pressure–lowering drugs. Of the 198 patients, 44 were not taking antihypertensive agents, nor had they done so in the past. This particular group is truly

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Abbreviations: EF, ejection fraction; FS, fractional shortening; I/D, insertion/deletion; IVRT, isovolumic relaxation time; LVDd, left ventricular end-diastolic diameter; LVDs, left ventricular end-systolic diameter; LVH, left ventricular hypertrophy; LVM, left ventricular mass; LVMI, left ventricular mass index; PA, peak atrial filling velocity; PE, peak early filling velocity; PWTd, posterior wall thickness in diastole; SVTd, ventricular septum thickness in diastole.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

representative of diabetic nephropathy, and it is well known that ~25% of type 1 diabetic patients with nephropathy are normotensive (8–10). We invited these patients to undergo echocardiography, and we evaluated their records of blood pressure for the last 6 months before the examination. We excluded patients with elevated blood pressure (n = 5) and patients with ischemic heart disease as evidenced by a history of angina, presence of abnormal Q wave in more than two leads on resting electrocardiogram, or impaired wall motion in any of 16 segments on echocardiography (n = 2). In addition, one patient could not be evaluated because of aortic valve regurgitation, and 19 patients did not wish to have an echocardiographic examination performed. Thus, 17 normotensive type 1 diabetic patients with diabetic nephropathy were included in this study. Thirty-four type 1 diabetic patients with persistent untreated normotension and normoalbuminuria (urinary albumin excretion <30 mg/day) were matched to the case subjects with respect to arterial blood pressure, sex, age, duration of diabetes, and BMI and served as a control group. The study was approved by the local ethical committee, and all patients gave fully informed consent.

Echocardiography

M-mode and pulsed Doppler echocardiography was performed according to the recommendations of the American Society of Echocardiography (11) using a Vingmed-CFM725 (Vingmed Sound, Horten, Norway) equipped with a 3.25-MHz transducer. M-mode recordings were guided by a two-dimensional image in the parasternal long axis.

The following M-mode parameters were measured: left ventricular end-diastolic diameter (LVDd) and end-systolic diameter (LVDs) and ventricular septum thickness (SVTd) and posterior wall thickness (PWTd) in diastole. LVM was calculated according to Penn's formula (12): $1.04 [(SVTd + LVDd + PWTd)^3 - (LVDd)^3] - 13.6$. Left ventricular mass index (LVMI) was calculated by dividing LVM by the body surface area. LVH was considered present if LVMI was >131 g/m^2 in men or >100 g/m^2 in women (13). Systolic function was assessed by calculation of fractional shortening (FS) of the left ventricle, using the formula (LVDd − LVDs)/LVDd, and by calculation of ejection fraction (EF), which was calculated by the formula $[(LVDd)^3 - (LVDs)^3]/(LVDd)^3$. Left ventricular wall motion was inspected in each of the 16 segments defined by the American Society of Echocardiography (14). Diastolic function was assessed by standard Doppler echocardiography performed in the apical four-chamber view. Left ventricular inflow signals were obtained in the pulse mode by placing the sample volume between the mitral leaflets and adjusting the position until the highest peaks of diastolic velocity were obtained. The peak early diastolic velocity (PE) and peak atrial systolic velocity (PA) and E/A were determined from transmitral flow velocity. The isovolumic relaxation time (IVRT) was measured from aortic valve closure to mitral valve opening. In 61 young (age <50 years) nondiabetic control subjects, Klein and Cohen (15) found an E/A of 1.9 ± 0.6 and an IVRT of 76 ± 11 ms (means ± SD). All measurements were averaged over five cycles. Echocardiography was performed by one experienced investigator (A.S.) blinded to clinical and laboratory data. The intraobserver error was <5%.

Clinical measurements

Arterial blood pressure was measured with patients in a sitting position after 10 min of rest, using a standard sphygmomanometer and appropriately sized cuff. Diastolic blood pressure was recorded at the disappearance of the fifth phase of Korotkoff's sounds. Baseline blood pressure was calculated as an average of all measurements performed during the last 6 months before echocardiographic examination (median [range], n = 2 [1–8]). Retinopathy was determined by fundus photography after pupillary dilatation and was graded as nil, simple, or proliferative diabetic retinopathy. Autonomic neuropathy was assessed by beat-to-beat variation. R-R intervals were recorded during deep breathing, and the difference between maximal and minimal heart rate was calculated (16).

Laboratory measurements

Urinary albumin concentration was measured using an enzyme immunoassay method (17), and urinary albumin excretion rate was expressed as the median of all 24-h urine collections obtained 1–6 months before echocardiography was performed. Urine concentration of sodium was measured using the flame photometric method. HbA_{1c} was measured by high-performance liquid chromatography (Bio-Rad DIAMAT, Richmond, CA) (normal range, 4.3–6.2%) and was expressed as the mean value of all measurements from the last 6 months before echocardiography was performed (n = 2 [1–4]). Serum creatinine and hemoglobin were measured by the standard laboratory technique. Plasma ACE levels were determined by an enzyme-linked immunosorbent assay method using a sandwich combination of monoclonal and polyclonal antibodies (18). Plasma renin and prorenin were measured by an immunoradiometric assay using a commercial kit (Nichols Institute, Wijchen, The Netherlands) (19). The assay was modified by shortening incubation to 6 h and performing the assay at 37°C. The interassay variations for prorenin (320 μU/ml) were 13%, and for renin (22.2 μU/ml) 15%.

Lymphocytes were isolated from peripheral blood and DNA prepared by standard techniques. Polymerase chain reaction was used to detect the two alleles of the ACE insertion/deletion (I/D) polymorphism, as described previously (20). Subjects were classified, according to the presence or absence of a 287–base pair insertion in intron 16 of the ACE gene, as homozygous for either II or DD, or heterozygous for ID.

Statistical analysis

Normally distributed variables are given as means ± SD. Urinary albumin excretion rate, serum creatinine, plasma ACE, plasma renin, and plasma prorenin were log-transformed before statistical analysis because of their positively skewed distribution and were given as medians (range). Comparison between groups was performed by an unpaired Student's t test or an analysis of variance. Noncontinuous variables were compared with a χ^2 test. A P value (two-tailed) <0.05 was considered statistically significant. All calculations were made with commercially available programs (Statgraphics; STSC, Rockville, MD).

RESULTS — The group of patients with nephropathy and those of the normoalbuminuric group were well matched regarding sex, age, duration of diabetes, BMI, and arterial blood pressure (Table 1).

Table 2 shows that LVMI was significantly higher in patients with nephropathy compared with normoalbuminuric patients (100.8 ± 10.3 vs. 88.2 ± 21.0 g/m^2, respectively; P = 0.02). Increased interventricular septum thickness was demonstrated in the nephropathic group compared with the control group (P = 0.002). No significant differences in posterior wall thickness or in left ventricular diameter were apparent.

Table 1—*Characteristics of 51 normotensive type 1 diabetic patients with and without diabetic nephropathy*

	Nephropathy	Normoalbuminuria	*P*
n	17	34	
Sex (M/F)	10/7	20/14	NS
Age (years)	40 ± 11	42 ± 10	NS
Duration of diabetes (years)	28 ± 7	28 ± 6	NS
BMI (kg/m²)	24.2 ± 4.2	24.6 ± 2.4	NS
HbA_{1c} (%)	9.2 ± 1.2	8.4 ± 1.0	<0.05
Retinopathy			<0.001
Proliferative	10 (59%)	2 (6%)	—
Simple	7 (41%)	23 (68%)	—
Nil	0 (0%)	9 (26%)	—
Urinary albumin (mg/24 h)	354 (135–2,846)	10 (3–30)	—
Urinary sodium (mmol/24 h)	169 (61–317)	134 (55–389)	NS
Serum creatinine (μmol/l)	82 (71–120)	80 (67–100)	NS
Hemoglobin (mmol/l)	8.4 ± 1.0	8.8 ± 0.8	NS
Systolic blood pressure (mmHg)	134 ± 13	129 ± 12	NS
Diastolic blood pressure (mmHg)	77 ± 7	78 ± 7	NS

Data are means ± SD, *n* (prevalence), or medians (range).

LVH was present in 3 (18%) of 17 nephropathic patients and in 2 (6%) of 34 patients in the normoalbuminuric group. None of the patients in our study had diastolic blood pressure >90 mmHg. Systolic blood pressure between 140 and 150 mmHg was observed in 6 of the 17 patients with nephropathy and in 7 of the 34 patients with normoalbuminuria. The average LVMI in these patients was 97.8 g/m² in the nephropathic group and 84.0 g/m² in the normoalbuminuric group, which is not significantly different from calculations based on the entire cohort. Furthermore, there was no correlation between either systolic or diastolic blood pressure and LVMI in subjects with and without diabetic nephropathy. The nephropathic group tended to have reduced diastolic function assessed by E/A, but this difference did not reach statistical significance ($P = 0.09$). Only four patients in each group had an E/A <1 (NS). No difference in IVRT was observed between the groups (NS). Systolic function, assessed by FS and EF, was normal and about the same in the two groups (Table 2). No subjects had valvular or wall motion abnormalities. Beat-to-beat variation and resting heart rate were similar in patients with nephropathy and those with normoalbuminuria (14 ± 7 vs. 18 ± 9 and 77 ± 10 vs. 74 ± 14 beats per minute, respectively [NS]).

No difference in serum creatinine, hemoglobin, or urinary sodium excretion was observed between patients with and without nephropathy, whereas metabolic control assessed by HbA_{1c} was higher in patients with diabetic nephropathy (Table 1). Furthermore, plasma levels of renin and prorenin were elevated in nephropathic versus normoalbuminuric type 1 diabetic patients (28.4 [7.7–114.8] and 603 [159–1320] vs. 16.8 [3.2–37.0] and 296 [85–1124] μU/ml, respectively; P <0.01 and <0.001).

No difference in ACE I/D genotype distribution was observed between type 1 diabetic patients with nephropathy and those with normoalbuminuria (6 [35%] DD, 7 [41%] ID, and 4 [24%] II vs. 10 [30%] DD, 18 [55%] ID, and 5 [15%] II, respectively; NS). Plasma ACE did not differ significantly between patients with nephropathy (479 [202–1134] ng/ml) and patients with normoalbuminuria (416 [55–1630] ng/ml).

CONCLUSIONS — In our cross-sectional case-control study of normotensive type 1 diabetic patients with and without nephropathy, we found increased LVMI in patients with nephropathy compared with patients with normoalbuminuria, particularly because of an increase of ventricular septal wall thickness. Furthermore, the nephropathic group did not have a significantly reduced diastolic function compared with the normoalbuminuric group, and systolic function was normal and about the same in the two groups. In patients with nephropathy, HbA_{1c}, plasma renin, and plasma prorenin were elevated compared with patients with normoalbuminuria.

Raev (21) has previously found an LVMI of 87.8 g/m² in 157 young, slim, normotensive type 1 diabetic patients. Even though this study included patients with diabetic complications, the mean LVMI is similar to that in our control group. Nielsen et al. (22) found a greater LVMI (137 g/m²) in 26 normoalbuminuric, normotensive type 2 diabetic patients. These patients differ from the patients in the present study by mean age (61 years) and BMI (29 kg/m²), both of which are much higher than in our present study and play a major role in LVH. Furthermore, these investigators studied insulin-resistant patients with type 2 diabetes, whereas we studied type 1 diabetic patients.

Table 2—*Left ventricular structure and function in 51 normotensive type 1 diabetic patients with and without diabetic nephropathy*

	Nephropathy	Normoalbuminuria	*P*
n	17	34	
LVDd (mm)	49.3 ± 3.4	50.2 ± 4.6	NS
LVDs (mm)	30.4 ± 3.9	30.6 ± 4.4	NS
SVTd (mm)	9.4 ± 1.0	8.2 ± 1.3	0.002
PWTd (mm)	9.0 ± 0.8	8.6 ± 1.2	NS
LVM (g)	184.9 ± 25.8	171.0 ± 51.1	NS
LVMI (g/m²)	100.8 ± 10.3	88.2 ± 21.0	<0.05
FS (%)	38.5 ± 5.5	39.8 ± 5.5	NS
EF (%)	76.2 ± 6.6	77.1 ± 5.5	NS
PE (cm/s)	85 ± 18	89 ± 18	NS
PA (cm/s)	70 ± 13	64 ± 10	NS
E/A	1.25 ± 0.31	1.42 ± 0.36	0.09
IVRT (ms)	81.0 ± 15.5	76.9 ± 16.8	NS

Data are means ± SD.

The coexistence of LVH and possible ischemic heart disease is a well-known phenomenon in the nondiabetic population (23). However, evidence suggests that ischemic heart disease is a consequence rather than cause of LVH (24). Lee et al. (25) investigated a cohort of more than 5,000 patients and found that increased wall thickness of the ventricular septum or of the left ventricular posterior wall was not associated with prevalent coronary heart disease. Consequently, our findings of an increased wall thickness could not be explained by such a mechanism.

The pathogenesis of increased LVMI is multifactorial, and risk factors can be divided into two major categories: hemodynamic and nonhemodynamic (3). The hemodynamic factors consist of blood pressure and volume overload. Several studies have demonstrated a correlation between arterial blood pressure and LVMI in hypertensive populations (4,5,26). However, the findings from studies of blood pressure during office visits or 24-h ambulatory blood pressure are not consistent regarding a relationship between blood pressure and LVMI (4,5,27,28). Blood pressure measured during office visits was nearly identical in our patients with and without diabetic nephropathy. Because we did not measure 24-h ambulatory blood pressure, we cannot exclude the possibility that nighttime blood pressure was higher in the albuminuric patients than in the normoalbuminuric subjects. However, in a study comparing two groups of patients similar to ours, no significant changes in the nighttime-to-daytime blood pressure ratio were demonstrated (29). It should be noted that increased LVMI has been demonstrated in normotensive subjects with polycystic kidney disease and normal renal function compared with control subjects (30).

We suggest that equal enlargement of the posterior and septal walls would be observed if increased pressure was the main mechanism and that differences in wall thickness may reflect local metabolic factors. Therefore, the present finding of increased ventricular septal wall thickness with unchanged thickness of the posterior wall would suggest that nonhemodynamic factors play a major role in pathogenesis.

LVH is known to be influenced by several nonhemodynamic factors that may also increase coronary risk; these include obesity and age (31), blood viscosity (32), salt intake (33), and insulin resistance (34). In our study, the two groups of type 1 diabetic patients were matched for age and BMI, and no difference of urinary sodium excretion was observed between the two groups. We did not directly measure blood viscosity, but we found no difference in hemoglobin between patients with nephropathy and those with normoalbuminuria. Sodium-lithium erythrocyte countertransport is enhanced in diabetic nephropathy (35,36), and association of this abnormality with LVH has been suggested (37).

In the last decade, microalbuminuria has received increased attention as a marker for cardiovascular and renal damage. Microalbuminuria indicates future development of clinical proteinuria, chronic renal failure, and premature cardiovascular mortality in type 1 diabetic patients (38). Redón et al. (39) reported a significant relationship between urinary albumin excretion and LVMI, independent of blood pressure, age, and sex, in nondiabetic patients with essential hypertension. The link between microalbuminuria and increased LVMI is poorly understood.

As in previous studies, patients with diabetic nephropathy had poorer metabolic control than patients with normoalbuminuria. Given that it reflects a persistently higher glucose level over several years, this metabolic abnormality might contribute to the development of increased LVMI in type 1 diabetic patients with diabetic nephropathy. Whether this effect is mediated indirectly through high glucose on the myocardium cannot be elucidated from the present study.

A local renin-angiotensin system is present in the heart. Angiotensin II has been implicated in cardiac hypertrophy. The central importance of Angiotensin II as a growth factor for the heart was initially suggested by the ability of ACE inhibitors to cause regression of cardiac hypertrophy in a variety of pathological conditions (40). Renin is known as a rate-limiting step in the conversion of angiotensinogen to Angiotensin II. In our study, both plasma prorenin and renin concentrations in the nephropathic group were significantly elevated compared with the normoalbuminuric group. Although we did not measure plasma renin activity, it remains plausible that an increased plasma renin concentration would stimulate the angiotensin-aldosterone system.

Beat-to-beat variation and resting heart rate were not significantly reduced in the normotensive nephropathic patients examined. Thus, the absence of severe autonomic dysfunction could help explain the lack of a significantly reduced diastolic function observed in this study.

The ACE I/D polymorphism has recently been found to be associated with LVH, and also with hypertrophic, ischemic, or idiopathic dilated cardiomyopathy, in some (41–43) but not all (44) studies. The ACE I/D polymorphism might exert its pathogenic effect through variation in plasma ACE concentration. As demonstrated by Rigat et al. (45), plasma ACE levels in DD subjects were twice that of II subjects, with ID subjects having intermediate levels, as also demonstrated in type 1 diabetic patients (6). In our study, no difference in either genotype distribution or plasma ACE levels was observed between the nephropathic and the normoalbuminuric groups, but numbers of patients were small.

We suggest that a blood pressure–independent increase in LVM may contribute to the increased cardiac morbidity in normotensive type 1 diabetic patients with diabetic nephropathy, and that glycemic abnormalities and activation of the renin-angiotensin system may lead to ventricular enlargement.

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