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Ambulatory blood pressure and heart rate in relation to kidney structure and metabolic control in adolescents with Type I diabetes

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Abstract

Aims/hypothesis. To evaluate the relationship between metabolic control, kidney function, ambulatory blood pressure and renal morphology in normoalbuminuric adolescents with Type I (insulin-dependent) diabetes mellitus.
Methods. Metabolic control, clearance of inulin and para-amino hippuric acid, 24 h ambulatory blood pressure and renal biopsies were studied in 41 patients who were 17.8 ± 0.5 (SEM) years of age and 10.7 ± 0.5 years after onset of diabetes.
Results. Glomerular filtration rate and filtration fraction were higher in diabetic patients than in healthy control subjects. At least one third of the patients had systolic and nocturnal diastolic blood pressures above the 90th centile. Basement membrane thickness was 512 ± 17 nm, volume fraction of mesangial matrix 10.7 ± 0.3 % and foot process width 415 ± 6 nm. Nocturnal mean arterial blood pressure, adjusted for body height and gender, correlated directly to the basement membrane thickness, the volume fraction of mesangial matrix and the foot process width. Multiple regression analysis showed that HbA_{1c}, nocturnal heart rate and body height account for 44 % of the variations in blood pressure. HbA_{1c}, nocturnal heart rate and body height explained 57 % of the variation in basal membrane thickness, and HbA_{1c}, nocturnal heart rate and duration of diabetes explained 43 % of Vv(matrix/glom). Actual renal function or urinary albumin excretion rate had no effect.
Conclusion/interpretation. Nocturnal heart rate and nocturnal blood pressure, especially the mean arterial blood pressure, seem to be related to glomerulopathy changes in patients in whom persistent microalbuminuria has not yet developed. These findings suggest that a disturbance in sympathovagal balance could have a pathogenic effect. [Diabetologia (2001) 44: 865–873]

Keywords Blood pressure monitoring, ambulatory, biopsy, adolescence, diabetes mellitus, insulin-dependent, kidney/glomerulus/pathology, heart rate.

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Abbreviations: AMBP, ambulatory blood pressure; MAP, mean arterial blood pressure; HR, heart rate; ERPF, effective renal plasma flow; FF, filtration fraction; UAE, urinary albumin excretion rate; Vv(mes/glom), mesangial volume fraction per glomerulus; Vv(matrix/glom), mesangial matrix volume fraction per glomerulus; Sv(pcap/glom), surface density of the peripheral capillary walls; Lv(slit pore/glom), length density of filtration slits; BMT, basal membrane thickness

Nephropathy is a threatening complication of Type I diabetes that leads to renal failure in many patients [1–4]. It is important to find those at risk of developing nephropathy as early as possible to prevent or postpone renal damage [5]. Nocturnal hypertension seems to be related to end organ damage [6–8] and we therefore need to find these patients and exclude white-coat hypertension [9].

We have recently described the relation between long-term kidney function, kidney morphology and metabolic control [10]. In this study, we investigated the association between 24-h blood pressure and heart rate measurements, renal function, kidney histopathology and long-term metabolic control in an

unselected group of adolescents and young adults with Type I diabetes. Our aim was to clarify these parameters and their interrelationships. None of the patients had persistent microalbuminuria. Our study gives information on the relations between 24-h blood pressure measurements and morphological data in Type I diabetes of short duration.

Subjects and methods

Patients. In our Paediatric Clinic at Huddinge University Hospital, all patients over 12 years of age who had had diabetes for more than 5 years, about to undergo their regular renal function test, were asked to take part in the kidney biopsy study. Of 61 patients, 45 participated while 16 declined or asked to have the biopsy done later. Of these 45 patients, 41 had 24-h ambulatory blood pressure measurements and are therefore presented here. Some data on 32 of these 41 patients, who have had at least 3 inulin-para-amino hippuric acid (PAH) investigations, have been reported elsewhere [10]. The 16 patients who did not participate, and the 4 with no ambulatory blood pressure measurements, had renal function, blood pressure, albuminuria and metabolic control similar to those who agreed to participate. None of the 41 patients had persistent microalbuminuria, defined as more than 15 μg/min in 2 of 3 urine samples taken at least 3 months apart. One patient had asymptomatic bacteriuria, 25% were smokers and 71% were in Tanner stage 5 (adult sex maturity). Casual blood pressure was measured in the right arm after the patients had rested for 30 min (Omron digital blood pressure monitor Model Hem-700C, Sjuma AB, Malmö, Boehringer Mannheim, Scandinavia AB, Bromma, Sweden), before the renal function test on the first day of the study. None were on antihypertensive treatment. Altogether, 2 patients had well-controlled hypothyroidism.

The investigations were performed over 3 days. On the first day, a renal function test was done. Overnight urine was collected on the first night to determine the albumin excretion rate. On the second day, a renal biopsy was taken. After the biopsy, the patients were kept supine for 24 h and the pulse rate and blood pressure were determined at regular intervals. Some patients had microscopic haematuria and 7/40 (18%) had subcapsular haematomas according to ultrasound but no clinical relevant complications occurred, apart from slight pain in the muscles overlying the biopsy site. The study was approved by the ethics committee of the Karolinska Institutet and was performed after informed consent had been obtained from the patients and parents.

Ambulatory blood pressure. Ambulatory blood pressure (AMBP) was recorded for 24 h with portable automatic Space Labs 90 207 equipment (SpaceLabs, Wokingham, England). The monitor was programmed for cuff insufflation every 20 min between 0700 h and 2200 h and every 30 min from 2200 h to 0700 h. The technique is described elsewhere [11–15]. In 24 patients, the recordings were started at about 1200 h on the day after the kidney biopsy when the patients were discharged from hospital. In 17 patients, these were done within 10 (mean 6) months of the kidney biopsy. They were asked to continue with the same lifestyle but to avoid sports. Diurnal and nocturnal blood pressures and heart rates were based on standardised daytime (0800 h to 2000 h) and nighttime (2400 h to 0600 h), as previously reported by Soergel et al. [16]. The time periods were corrected in patients whose "sleeping times" differed from the report of Soergel et al. One patient interrupted the 24 h registration, and therefore, only daytime measurements could be recorded. All patients had a mean of 92% successful readings (39/41 of them had more than 80% successful readings, one had 78% and one 71%).

The mean arterial blood pressure (MAP) was calculated as the diastolic blood pressure (BP) plus one third of the difference between the systolic and diastolic blood pressures. The 24-h MAP, the systolic, the diastolic, as well as the corresponding diurnal and nocturnal BPs, were calculated according to ABP PC Direct/Base Station Interface 90 219. Heart rate was recorded day night, and 24 h. We compared our blood pressure measurements with those of Soergel et al., based on 1141 healthy children and adolescents 5 to 21 years of age with a body height of 115 to 186 cm [16]. The heart rates of our patients were compared with their data from the same group of children and adolescents (personal communication).

Renal function tests. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined by the clearances of inulin and para-amino hippuric acid during water diuresis with a standard clearance technique [10, 17, 18]. The patients were given their ordinary insulin dose, followed by breakfast in the morning before the start of the renal function test. Filtration fraction (FF) was calculated as GFR/ERPF. Renal function was compared with that of 34 control children and young adults matched for age and gender evaluated in our Paediatric Nephrology Unit.

Urine samples. Timed overnight-collected urine was analysed for albumin by an automated immuno-nephelometric method (Behring Nephelometer Analyser; Behringwerken, Marburg, Germany) and the urine albumin excretion rate (UAE) was determined.

Metabolic control. Since 1980, total HbA_1 has been analysed by ion exchange chromatography, using Isolab's Fast Haemoglobin Test System (Quik-Step Fast Haemoglobin Test System; Isolab, Akron, Ohio., USA). The reference values were 5.5–8.5%. Since 1986, HbA_{1c} has been determined by the FPLC method (Fast Protein Liquid Chromatography, Pharmacia, Uppsala, Sweden) [19]. Normal reference values were 3.4–5.0%. To compare glycaemic control over time, HbA_1 data were converted to HbA_{1c}, using the equation $HbA_1 = 1.13 \cdot HbA_{1c} + 0.85$. The correlation coefficient between the two methods is 0.957 in our laboratory. Since 1980, we have checked the HbA_1 or HbA_{1c} value in each patient 3–4 times a year. Mean HbA_{1c} was calculated for each year and the mean of all years (mean HbA_{1c}) was determined as a measure of long-term metabolic control.

Renal biopsy. The biopsies were taken with ultrasound guidance (Acuson, Mountain View, Calif., USA), using an automatic biopsy device (Biopty Bard Urological; Covington, Ga., USA) and a 16-gauge needle (Manan Medical Products, Northbrook, Ill., USA) [10]. They were fixed in 4% paraformaldehyde in phosphate buffer. Small tissue blocks for electron microscopy, were postfixed in 2% glutaraldehyde and 4% paraformaldehyde in phosphate buffer and embedded in Polybed 812. Ultra-thin sections were stained with uranyl acetate and lead citrate.

Electron microscopic quantitation. The sampling of the glomerular profiles taken for ultra-thin sectioning was performed as previously described [10]. Altogether 4 to 5 glomeruli were analysed from each biopsy. We sampled 7 to 19 electron micro-

Table 1. Clinical data (means ± SEM or median and range) at time of kidney biopsy in 41 adolescents with diabetes

	All patients $n = 41$	Boys $n = 24$	Girls $n = 17$
Age at biopsy (years)	17.8 ± 0.5	17.1 ± 0.6	18.7 ± 0.7
Duration (years)	10.7 ± 0.5	10.0 ± 0.7	11.8 ± 0.7
Age at onset (years)	7.0 ± 0.6	7.2 ± 0.9	6.9 ± 0.7
BMI (kg/m^2)	22.4 ± 0.4	21.5 ± 0.6	23.6 ± 0.5
Body height (cm)	169.3 ± 1.3	172.5 ± 1.6	164.7 ± 1.4
UAE (μg/min)	6 (2–64)	6 (2–64)	6 (2–33)
HbA_{1c} at biopsy (%)	7.7 (5.4–14.8)	7.7 (5.6–13.4)	7.6 (5.4–14.8)
Mean HbA_{1c} (%)	8.0 (6.8–10.9)	7.9 (6.8–10.5)	8.0 (6.8–10.9)
Insulin dose (IU/kg)	0.95 ± 0.03	0.96 ± 0.04	0.93 ± 0.05
Casual blood pressure (mmHg)	122 ± 2/74 ± 2	125 ± 2/74 ± 2	118 ± 3/74 ± 2

Table 2. The 24 hour, diurnal and nocturnal blood pressures (mmHg) and heart rates (beats/min) in 41 adolescents with diabetes. Data are means ± SEM and percentage of patients having values above the 90th centile, according to the findings of Soergel et al. [16], (personal communication)

	$n = 41$	% of patients above 90th centile
24 h		
MAP	89 ± 1	30
Systolic	124 ± 1	45
Diastolic	71 ± 1	22
Heart rate	77 ± 2	12
Diurnal		
MAP	94 ± 1	20
Systolic	128 ± 1	29
Diastolic	77 ± 1	12
Heart rate	85 ± 2	15
Nocturnal		
MAP	79 ± 1	32
Systolic	113 ± 1	37
Diastolic	60 ± 1	30
Heart rate	65 ± 2	12

MAP, mean arterial blood pressure

graphs, covering about 40% of the area of each glomerular profile, in a systematic random manner by moving the specimen stage of the electron microscope (Philips 420, Eindhoven, The Netherlands) between predetermined points. The micrographs were analysed at a magnification of about 10 000, corrected using a grating grid (EF Fullan, 28 800 lines/inch). Glomerular profiles representing less than 7 sampled areas were excluded. The reference space, here called "glomerulus", was defined as a minimal convex polygon enclosing the glomerular tuft (i.e. tuft plus urinary space entrapped in the polygon) [20]. The definition of the mesangium and its demarcation from the peripheral capillary wall was used as previously described [20]. All the estimated relative structural quantities were expressed in relation to the reference space given above.

Mesangial, Vv (mes/glom), and mesangial matrix, Vv (matrix/glom), volume fractions per glomerulus. The Vv (mes/glom) and Vv (matrix/glom) were estimated by point-counting, using a superimposed lattice square grid with points 30 mm apart. Total points hitting the mesangial areas and the mesangial matrix substance were divided by the total points hitting the reference space. The mesangial matrix was defined as all extracellular material in the mesangial areas.

Using the same square lattice grid as above, the intersections (I) with the epithelial aspect of the basement membrane of the peripheral capillary walls were counted and the surface density of the peripheral capillary walls Sv(pcap/glom) was calculated according to the following formula:

$$Sv(pcap/glom) = 2 \cdot I/(P \cdot (2d/mag)) \ (\mu m^{-1}).$$

The number of their related filtration slits (Q), the length density of filtration slits (Lv(slit pore/glom)), was estimated using the following formula:

$$Lv(slit\ pore/glom) = 2 \cdot Q/(P \cdot (d^2/mag^2)) \ (\mu m^{-2}).$$

For both formulas, P is the total points in the reference space, d is the distance between each point of the grid and mag is the final magnification. The mean foot process width was estimated as the ratio of Sv(pcap/glom) and Lv(slit pore/glom) (nm). The basement membrane thickness (BMT), (nm), was estimated using the orthogonal intercept method of Jensen and coworkers [21]. None of our patients had any occluded glomeruli.

Statistical analyses. The Shapiro-Wilk W test for normality was used and all variables, except for HbA_{1c} and UAE, were normally distributed. Mean values ± standard error of mean (SEM) or median with range (mean HbA_{1c} and UAE) are given. UAE are log 10 transformed for calculations. The Student's t test was used to compare two groups. Single and multiple regression analyses were performed by the least square method. The residuals were normally distributed. Adjusted r^2 was used to adjust for the number of X-factors used. The α level for 2-sided tests was set at 0.05 that is a p value of less than 0.05 was considered significant. The statistical programme of JMP version 4.0 was used.

Results

The 24-h ambulatory blood pressure and heart rate measurements. Diurnal, nocturnal and 24-h BPs as well as the heart rates of our Type I diabetic patients are given in Table 2 and compared with the values accepted as norm of other investigators [16]. The cut-off point for elevation was chosen as blood pressure above the 90th centile related to the height and gender of the subjects [16]. At least one third of our patients had systolic and nocturnal diastolic BPs above the 90th centile. The boys had higher 24-h

Table 3. The estimated regression coefficients and corresponding *p* values of ambulatory blood pressure (BP, mmHg) and heart rate (beats/min) measurements, with or without adjustment for body height and gender, of importance for different morphological changes in 41 adolescents with diabetes[a]

	BMT (nm)				Vv(matrix/glom) (%)				Foot process width (nm)			
	Univariate model		Adjusted for gender and body height		Univariate model		Adjusted for gender and body height		Univariate model		Adjusted for gender and body height	
	estimate	*p*	estimate	*p*	estimate	*p*	estimate	*p*	estimate	*p*	estimate	*p*
24-h												
MAP	6	0.03	7	0.03	0.1	NS	0.1	NS	2	0.05	2	NS
Systolic BP	4	NS	5	0.03	0.0	NS	0.1	NS	2	0.03	1	NS
Diastolic BP	4	NS	4	NS	0.1	NS	0.1	NS	2	NS	2	NS
Heart rate	2	NS	2	NS	0.0	NS	0.0	NS	0	NS	0	NS
Diurnal												
MAP	5	NS	5	NS	0.0	NS	0.1	NS	2	NS	2	NS
Systolic BP	4	NS	5	0.03	0.0	NS	0.1	0.04	1	NS	1	NS
Diastolic BP	3	NS	3	NS	0.1	NS	0.1	NS	2	NS	1	NS
Heart rate	0	NS	0	NS	0.0	NS	0.0	NS	0	NS	0	NS
Nocturnal												
MAP	7	0.004	7	0.004	0.1	NS	0.1	0.01	2	0.02	2	0.03
Systolic BP	4	0.03	5	0.02	0.0	NS	0.1	NS	2	0.01	1	0.02
Diastolic BP	6	0.03	6	0.03	0.1	NS	0.1	0.05	2	NS	2	NS
Heart rate	4	0.01	5	0.007	0.1	0.02	0.0	NS	0	NS	1	NS

[a] A *p* value of less than 0.05 was considered significant
MAP, mean arterial blood pressure

(126 ± 8 versus 120 ± 8, *p* value = 0.021) and diurnal (131 ± 8 versus 125 ± 8, *p* value = 0.013) systolic blood pressure values than the girls. The girls had higher nocturnal heart rates than the boys (69 ± 13 versus 62 ± 8, *p* value = 0.036). A direct correlation between the nocturnal heart rate and the nocturnal diastolic BP was found ($r = 0.53$, $p = 0.0004$). Similar positive correlations were noted between the diurnal ($r = 0.34$, $p = 0.027$) and 24-h ($r = 0.43$, $p = 0.005$) diastolic blood pressures and the corresponding heart rates. No correlations were found between the duration of diabetes and the heart rate or the BPs.

Renal function. The GFR and filtration fraction (FF) at the time of biopsy were higher ($p < 0.001$) in the diabetic patients than in 34 healthy control subjects matched with them for age (133 ± 3 vs 116 ± 2 ml/min per 1.73 m^2 and 22.0 ± 0.5 vs 18.6 ± 0.4 %) while ERPF did not differ (617 ± 19 vs 633 ± 17 ml/min per 1.73 m^2). No correlations were found between the actual GFR, ERPF or FF and the BPs or heart rates.

UAE during the night before the renal function test varied between 2 and 64 (median 6) μg/min in 35 patients. Thus nine of 35 patients had microalbuminuria (> 15 μg/min) at this time while none had had persistent microalbuminuria. There were similar positive correlations between log UAE and 24 h ($r = 0.39$, $p = 0.023$), diurnal ($r = 0.39$, $p = 0.020$) and nocturnal ($r = 0.37$, $p = 0.033$) diastolic BP. The correlation persisted only for the diurnal diastolic BP, if the mean HbA_{1c} was included in a multiple regression analysis ($p = 0.040$).

Renal morphology. The BMT, Vv(mes/glom) and Vv(matrix/glom) were 512 ± 17 nm, 19.4 ± 0.5 % and 10.7 ± 0.3 %, respectively. The foot process width was 415 ± 6 nm. The Vv(matrix/glom) correlated significantly to the FF ($r = 0.35$, $p = 0.025$) but we found no other correlations between actual renal function and morphology.

Blood pressure and heart rate in relation to renal morphology. For the relations between BP or heart rate measurements and morphological changes, the highest significance was seen between nocturnal values, especially nocturnal MAP, and BMT (Table 3), Vv(matrix/glom) and foot process width.

Patients with nocturnal diastolic BP above the 90th centile ($n = 12$) had thicker BMT than those with nocturnal diastolic BP on the 90th centile or lower ($n = 28$); 569 ± 40 nm compared with 484 ± 16 nm ($p = 0.021$).

Blood pressure and heart rate in relation to metabolic control. A relation between the HbA_{1c} and AMBP was seen in 24-h MAP ($r = 0.37$, $p = 0.018$) and 24-h diastolic BP ($r = 0.32$, $p = 0.043$), in diurnal MAP ($r = 0.33$, $p = 0.038$) and in nocturnal MAP ($r = 0.40$, $p = 0.010$), (Table 4) and nocturnal diastolic BP ($r = 0.37$, $p = 0.017$). When log UAE was included in a multiple regression analysis, the relation between BP and HbA_{1c} was preserved only with nocturnal

Table 4. The estimated regression coefficients and corresponding 95 %-Confidence intervals of independent variables and possible confounders of importance for nocturnal mean arterial blood pressure (MAP) in 41 adolescents with diabetes[a]

Independent variables	Nocturnal MAP (mmHg)					
	Univariate model			Multiple model r^2 = 44 %		
	estimate	95 %-CI	*p* value	estimate	95 %-CI	*p* value
Mean HbA_{1c} (%)	2.4	0.7–4.2	0.010	2.1	0.6–3.6	0.008
Nocturnal HR (beats/min)	0.3	0.1–0.5	0.004	0.4	0.2–0.5	0.0002
Body height (cm)	0.1	–0.1–0.4	0.27	0.4	0.2–0.6	0.0007
Duration (years)	–0.1	–0.7–0.5	0.76			
Age (years)	0.2	–0.5–0.9	0.59			
Gender (boys = 0, girls = 1)	–0.9	–3.1–1.2	0.40			
Log UAE	4.7	–1.1–10.1	0.12			

[a]A *p* value of less than 0.05 was considered significant
HR, heart rate

Table 5. The estimated regression coefficients and corresponding 95 %-Confidence intervals of independent variables and possible confounders of importance for basal membrane thickness (BMT) in 41 adolescents with diabetes[a]

Independent variables	BMT (nm)					
	Univariate model			Multiple model r^2 = 57 %		
	estimate	95 %-CI	*p* value	estimate	95 %-CI	*p* value
Mean HbA_{1c} (%)	61	36–85	0.0001	54	33–75	0.0001
Nocturnal HR (beats/min)	4	1–7	0.01	3	0–5	0.04
Nocturnal HR · Mean HbA_{1c}				3	1–4	0.004
Nocturnal MAP (mmHg)	7	3–12	0.004			
Body height (cm)	0	–4–4	1	5	2–8	0.006
Duration (years)	3	–7–14	0.6			
Age (years)	6	–5–18	0.3			
Gender (boys = 0, girls = 1)	27	–41–95	0.4			
Filtration fraction (%)	10	–1–22	0.09			
Log UAE	52	–47–151	0.3			

[a]A *p* value of less than 0.05 was considered significant
HR, heart rate; MAP, mean arterial blood pressure

MAP ($p = 0.033$). No relation between metabolic control and heart rate was seen in a single correlation.

The relations between blood pressure, heart rate, metabolic control and renal morphology. Multiple linear regression analysis was used to analyse further the effects of various variables on nocturnal MAP as the outcome variable (Table 4). The mean HbA_{1c}, nocturnal heart rate, body height, duration of diabetes, age, gender and log UAE were used as independent variables. This analysis showed that 44 % (adjusted $r^2 = 0.44$) of the variation in nocturnal MAP was explained by the mean HbA_{1c}, nocturnal heart rate and body height while the duration, age, gender and log UAE were of no importance. According to this model, nocturnal MAP increases by 4 mmHg with each 10-beat increase in nocturnal heart rate, by 4 mmHg with each 10 cm increase in height and by 2 mmHg with each percent increase in mean HbA_{1c}. Similar results were found with the 24-h MAP and diurnal BPs as well as the nocturnal systolic and diastolic BPs as the outcome variables.

To analyse further the effects of various variables on BMT and Vv(matrix/glom) as the outcome variable, multiple regression analyses were used (Table 5, 6). These analyses showed that 57 % (adjusted $r^2 = 0.57$) of the variation in BMT was explained by the mean HbA_{1c}, nocturnal heart rate and body height. 43 % (adjusted $r^2 = 0.43$) of the variation in Vv(matrix/glom) was explained by the mean HbA_{1c}, nocturnal heart rate and duration of diabetes. It seems that heart rate rather than BP affects the morphology and that HbA_{1c} and heart rate interact. No other interaction effects on renal morphology were seen among the possible confounders in Tables 5 and 6.

Discussion

The present study is the first one on the relationship between early renal morphological changes and blood pressure, using accurate blood pressure monitoring in a group of adolescents with Type I diabetes before persistent microalbuminuria had developed.

Table 6. The estimated regression coefficients and corresponding 95 %-Confidence intervals of independent variables and possible confounders of importance for mesangial matrix volume fraction per glomerulus, Vv(matrix/glom), in 41 adolescents with diabetes[a]

Independent variables	Vv(matrix/glom) (%)					
	Univariate model			Multiple model r^2 = 43 %		
	estimate	95 %-CI	*p* value	estimate	95 %-CI	*p* value
Mean HbA_{1c} (%)	0.9	0.4–1.4	0.0009	0.7	0.3–1.2	0.003
Nocturnal HR (beats/min)	0.1	0.0–0.1	0.02	0.0	–0.5– 0.5	0.2
Nocturnal HR · Mean HbA_{1c}				0.0	0.0–0.1	0.02
Nocturnal MAP (mmHg)	0.1	0.0–0.2	0.07			
Body height (cm)	–0.1	–0.2–0.0	0.05			
Duration (years)	0.2	0.0–0.4	0.09	0.2	0.0–0.3	0.04
Age (years)	0.1	–0.1–0.3	0.3			
Gender (boys = 0, girls = 1)	0.8	0.2–1.5	0.009			
Filtration fraction (%)	0.3	0.0–0.5	0.03			
Log UAE	1.1	–0.7–3.0	0.2			

[a] A *p* value of less than 0.05 was considered significant
HR, heart rate; MAP, mean arterial blood pressure

The 24 h ambulatory blood pressure and heart rate measurements. The finding that 45 % of our patients had 24 h systolic BP above the 90th centile is in agreement with other studies of adolescents of similar age and diabetes duration [22, 23]. The higher systolic BP in the boys than in girls accords with the findings of other authors [9, 16] and could be because the boys were taller than the girls (172 ± 8 and 165 ± 6 cm, $p = 0.0014$). About one third of our patients also had higher (> 90th centile) nocturnal systolic and diastolic BPs, which accords with previous findings [9]. Higher ambulatory systolic and diastolic BPs (both day and night), compared to healthy control subjects, have been reported in children with diabetes of shorter duration [24] and in normoalbuminuric adults with longer duration of the disease [25].

The heart rates in our patients were within normal range, according to Soergel et al. Others report higher [25–27] or lower [6] heart rate in normoalbuminuric adolescents and adults with varying durations of disease. The neuropathy in diabetes could reduce parasympathetic activity [28, 29] or cause sympathetic predominance [29–32]. The differences in results regarding heart rate measurements could be explained by the findings of investigators [33] who noted a sequential increase in heart rate arising from cardiac parasympathetic damage, followed by a fall in heart rate, but not back to normal, because sympathetic damage had developed as well.

The direct correlation between diastolic BP and heart rate found in the present study has also been reported in other studies [22] and was confirmed by our multiple regression analyses, where heart rate was shown to be an important determinant of BP. This relation could be secondary to autonomic neuropathy [31], sodium and fluid retention [34–36] or a consequence of physiology where heart rate is a determinant of BP.

Some authors have reported a relation between duration of diabetes and BP or heart rate [26, 37, 38], but we did not find such a relation.

Renal function. The increases in GFR and FF noted in our patients agree with other studies [18, 39–42], and have been discussed in more detail in a previous paper [10]. The lack of correlation between actual GFR or FF and AMBP accords with a previous study [43]. Other investigators found that nocturnal diastolic BP was higher in hyperfiltrating adult patients with diabetes than in normofiltrating control subjects [36], while the normofiltrating patients had a BP in between.

The direct correlation between diastolic BP and UAE in our group of patients is in agreement with other studies [22]. Some authors investigating mixed normoalbuminuric and microalbuminuric patients have reported that systolic BP was related more to albumin excretion rate than to diastolic BP [6, 27]. This inconstancy in the effects of the systolic and diastolic BP suggests that diastolic BP is higher in normoalbuminuric patients of short duration and that increases in systolic BP come later with microalbuminuria and longer duration. The MAP can be used to reflect both the systolic and diastolic BP and other authors [7] have found the strongest correlation between MAP and UAE.

Renal morphology. The morphological findings are presented in more detail in a recent paper [10]. Consistent with our previous findings and the findings of others, there were strong positive correlations between BMT and Vv(matrix/glom) [10, 44, 45] and between log UAE and mean foot process width [10, 46].

Blood pressure and heart rate in relation to morphology. To our knowledge, no studies have been done on AMBP and heart rate in relation to renal morpholog-

ical changes. We therefore compared our results with studies of casual BP measurements.

The relation between BMT and nocturnal diastolic BP in our patients accords with the findings of other investigators, who studied a group of adolescents with diabetes for about the same duration as in our patients [47]. They found a correlation between the mean of four casual diastolic BPs and a factor called "peripheral capillary decrease" which included increased BMT. Other studies of microalbuminuric adolescents [48, 49] and adult patients [44, 50] have shown no correlation between morphological changes and casual BPs. Investigators studying 139 mostly adult diabetic patients, with overt diabetic nephropathy evaluated for pancreas transplantation, observed that the hypertensive patients had larger mesangial volumes than the normotensive ones [51]. Our findings of a correlation between nocturnal BPs and BMT, Vv(matrix/glom) and foot process width indicate the importance of 24-h AMBP measurements in detecting relationships between BP and renal morphology.

Another study reported impaired renal autoregulation in diabetic rats [52], which means that higher systemic blood pressure leads to higher intraglomerular pressure. This is also thought to occur in patients with diabetes [53] and could predispose them to develop nephropathy. Hence, the higher blood pressure that we found could cause elevated intraglomerular pressure. The higher FF in this and our previous study [10] could also be a sign of higher intraglomerular pressure, contributing to basal membrane thickening, foot process widening and an increase in mesangial matrix.

The relation between nephropathy and autonomic neuropathy has been discussed by several authors [29, 31, 54, 55], and could be due to the coexistence of two diabetic complications with a common background, i.e., poor metabolic control or genetic susceptibility. On the other hand, autonomic neuropathy could have a pathophysiological effect of its own and cause renal lesions through higher BP, renal vascular dilation and higher intraglomerular pressure [55–57]. It has been reported that Type I diabetic patients with autonomic neuropathy have a diminished circadian BP rhythm with elevated nocturnal BP [55, 56] and heart rate [31]. Patients with diabetes selected for the presence of autonomic neuropathy had higher nocturnal UAE than those without neuropathy [55]. This accords with other findings noting significant differences in sympathovagal balance in a comparison of normoalbuminuric patients with UAE above or below the median [31]. In our study, autonomic function was not investigated but the correlation between nocturnal heart rate and renal morphology indicates that autonomic neuropathy contributes to renal morphological changes.

Blood pressure and heart rate in relation to metabolic control. Our study suggests that metabolic control affects BP, especially diastolic BP and MAP. In a similar group of patients, a relation was found between poor metabolic control and higher 24 h diastolic BP [22]. In a mixed normoalbuminuric and microalbuminuric group of adult patients, HbA_{1c} correlated with both nocturnal systolic and diastolic BPs [27]. In a multiple regression analysis, UAE was identified as a main determinant of BP elevation, while HbA_{1c} was not [58]. We found similar results as regards diastolic BP, while nocturnal MAP correlated to HbA_{1c} whether or not the log UAE was included.

Final remarks. Our findings suggest that nocturnal BPs and nocturnal heart rates reflect end organ damage more than values during the entire 24-h period or during the daytime. One reason for the inaccuracy of the 24-h values could be that they depend on how many hours the patient sleeps during the particular night of measurements. A common view is that nocturnal hypertension is more associated with end organ damage because of the longer total period of high BP [59]. Another explanation could be that sleep is a more standardised activity than that during the daytime [37, 60–62].

Our patients had higher AMBP, although none had persistent microalbuminuria. We therefore believe that the rise in BP precedes persistent microalbuminuria. This is in accord with the findings of other authors who measured 24-h AMBP [7, 31, 63]. Studies of casual BP have suggested that microalbuminuria develops parallel to the rise in BP [64].

In conclusion, nocturnal BP, and especially nocturnal MAP, was found to be important for the glomerulopathy changes in normoalbuminuric adolescents with diabetes. However, in multiple regression analyses, nocturnal heart rate seems to be of greater importance than the BP for BMT and Vv(matrix/glom). On the other hand, heart rate is one of the determinants of BP. The relationship between BP, heart rate and renal morphological changes suggests that a disturbance in sympathovagal balance could have a pathogenic effect on the glomerulus itself. Because renal biopsy cannot be done routinely, early repeated 24-h AMBP could be a way to detect the subset of patients prone to develop nephropathy before microalbuminuria develops. Longitudinal studies of 24-h AMBP are therefore needed.

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