

Original Article

Salt-sensitive blood pressure—an intermediate phenotype predisposing to diabetic nephropathy?

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Abstract

Background. Family studies point to important genetic determinants of diabetic nephropathy (DN). Blood pressure (BP) is higher in offspring of patients with type 2 diabetes and DN, but the pathomechanisms involved have not been elucidated.

Methods. We examined the salt sensitivity of BP after 5 days equilibration on a low (20 mmol/day) vs high salt diet (220 mmol/day) in three matched groups of 15 subjects each: (i) control individuals; (ii) offspring of type 2 diabetic parents without DN (DN[−]); and (iii) offspring of type 2 diabetic parents with DN (DN⁺). Ambulatory BP and hormones involved in sodium homeostasis [plasma renin activity (PRA), aldosterone and atrial natriuretic peptide (ANP)] as well as the tetrahydrocortisol + 5-allotetrahydrocortisol/tetrahydrocortisone (THF + 5 α THF)/THE ratio in the urine as an index of 11 β -hydroxysteroid dehydrogenase type 2 (11 β HSD2) activity were analysed.

Results. In offspring of DN⁺ patients on a high salt diet, systolic and diastolic BP was 137/82 \pm 10/8 mmHg vs 125/77 \pm 12/8 mmHg in offspring of DN[−] patients ($P < 0.01$ for systolic BP). The salt-induced difference in mean BP between high and low salt diet was 5.2 \pm 3.3 mmHg in offspring of DN⁺ patients vs 0.7 \pm 4.7 mmHg in offspring of DN[−] patients ($P < 0.002$). The proportion of 'salt-sensitive' individuals was 67% in offspring of DN⁺ patients vs 20% in offspring of DN[−] patients ($P < 0.05$). In all groups, a high salt diet caused a comparable decrease of PRA and *p*-aldosterone accompanied by an increase in ANP. The urinary (THF + 5 α THF)/THE ratio was 1.23 \pm 0.36 in salt-sensitive individuals and 0.99 \pm 0.33

($P < 0.03$) in salt-resistant subjects, consistent with increased activity of 11 β HSD2.

Conclusions. BP is more salt sensitive in offspring of type 2 diabetic patients with diabetic nephropathy. The salt sensitivity of BP may be an intermediate phenotype in individuals with a high risk of future diabetic nephropathy.

Keywords: diabetic nephropathy; hypertension; intermediate phenotype; salt sensitivity

Introduction

Diabetic nephropathy (DN) is observed in up to 30% of patients who suffer from type 2 diabetes for >10 years [1–3]. Familial clustering [4,5] and a high frequency of cardiovascular accidents in first-degree relatives of patients with type 2 diabetes and DN [6] also point to strong independent genetic determination. We and others observed higher rates of albumin excretion in (pre-diabetic) offspring of type 2 patients with DN [7,8]. We also observed that blood pressure (BP) was higher in the offspring of patients with type 2 diabetes with DN as opposed to offspring of patients with type 2 diabetes without DN [7]. These findings raised the issue of whether BP was linked to the risk of development and/or progression of DN. Such a link between BP and glomerular disease is not unique to diabetic renal disease. Schmid *et al.* found a higher prevalence of hypertension in parents of patients with glomerulonephritis as compared with matched non-glomerulonephritis individuals [9]. Furthermore, Fagerudd *et al.* measured ambulatory BP in parents whose offspring had type 1 diabetes either with or without DN [10]. Blood pressure by ambulatory BP monitoring was significantly higher in parents whose offspring suffered from DN, arguing for a link between

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a genetic predisposition to hypertension on the one hand and to glomerular disease on the other hand.

The enzyme 11 β -hydroxysteroid dehydrogenase type 2 (11 β HSD2) is a potential candidate for abnormal BP regulation in hypertension and renal diseases [11]. In mineralocorticoid target tissues, 11 β HSD2 inactivates cortisol into cortisone, thus protecting the non-specific mineralocorticoid receptor from excess activation by glucocorticoids. The enzyme is expressed in the cortical collecting duct of the kidney.

In an effort to define potential intermediate phenotypes predisposing to nephropathy in type 2 diabetes more clearly, we assessed (i) salt sensitivity of BP and (ii) hormonal responses to low salt and high salt diets in non-diabetic normotensive offspring of patients with type 2 diabetes, i.e. patients who either had developed or failed to develop DN after at least 10 years duration of diabetes.

Subjects and methods

Description of study cohort

We identified a group of 30 type 2 diabetic patients [12] with known duration of diabetes of >10 years who were followed in a tertiary care centre (Regional Outpatient Clinic for Diabetics in Zabrze, Poland) and who had healthy offspring available for the study. Mean age was 63 ± 5 years and known duration of diabetes 13 ± 2 years.

Fifteen diabetic parents had DN: five were on renal replacement therapy and 10 had proteinuria (>0.5 g/24 h). The presence of non-diabetic renal disease was excluded by clinical evaluation (phase contrast microscopy of urinary sediment, renal ultrasonography and clinical work-up).

A further 15 diabetic parents had a similar duration of diabetes. They had normoalbuminuria (<30 mg/24 h on three occasions during the 6 months preceding the study).

Exclusion criteria for the offspring were casual BP $>140/90$ mmHg under resting conditions and fasting plasma glucose >110 mg/dl. Furthermore, smokers and female subjects using oral contraceptives were excluded. We studied a total of 15 offspring of type 2 diabetic patients without nephropathy (DN-) and 15 offspring of type 2 diabetic patients with nephropathy (DN+). Staff members ($n=15$) without a family history of diabetes mellitus, coronary artery disease or hypertension and satisfying the above criteria were selected as controls. Clinical characteristics of the examined subjects are given in Table 1.

All subjects gave written informed consent. The protocol was approved by the ethics Committee of the Silesian Medical Academy in Katowice. The study was executed in the Department of Internal Medicine and Diabetology Silesian Medical Academy (Zabrze, Poland), and was conducted in accordance with the guidelines proposed in the Declaration of Helsinki.

All subjects underwent an identical protocol consisting of two 5 day study periods in sequential order, i.e. a low followed by a high salt diet on an out-patient basis. They received a pre-cooked diet with defined NaCl content. Daily 24 h urine collections were used to monitor urinary sodium excretion throughout the study, to assess protein intake by urea nitrogen excretion and to measure

Table 1. Anthropometric and blood chemistry data of the examined groups

	Controls ($n=15$)	Offspring of type 2 diabetic patients	
		DN- ($n=15$)	DN+ ($n=15$)
Age (years)	36 ± 7	38 ± 8	38 ± 5
Gender (M/F)	2/13	4/11	7/8
Fasting glycaemia (mg/dl)	79 ± 8	79 ± 8	81 ± 5
HbA1c (%)	5.4 ± 0.4	5.4 ± 0.4	5.4 ± 0.3
BMI (kg/m ²)	22.5 ± 3.1	25.1 ± 3.44^a	27.2 ± 4.22^b
S-creatinine (mg/dl)	0.86 ± 0.18	0.93 ± 0.21	0.90 ± 0.23
S-cholesterol (mg/dl)	202 ± 48.8	210 ± 35.3	218 ± 37.2
S-triglycerides (mg/dl)	125 ± 53.6	131 ± 50.1	128 ± 76.5
LVMI (g/m ²)	97.7 ± 12.5	93.7 ± 13.1	107 ± 21.4
Kidney length (mm)	108 ± 5.64	108 ± 6.71	117 ± 8.74

^a $P < 0.05$ vs the control group.

^b $P < 0.01$ vs the control group.

LVMI = left ventricular mass index; BMI = body mass index.

the urinary tetrahydrocortisol + 5-allotetrahydrocortisol/tetrahydrocortisone (THF + 5 α THF)/THE ratio at the end of the low salt period. High salt intake was achieved by adding slow-release sodium chloride tablets providing a daily NaCl intake of 220 mmol/day. Ambulatory BP was recorded on the fifth day of each study period. Blood was taken at 8.00 a.m. in the fasting state after 30 min in the supine position for biochemical and hormonal measurements, as well as body weight measured on day 0 and day 5 of each study period.

Analytical methods

Continuous BP recordings were obtained using Medilog DX System (Oxford, UK). Night-time pressure was defined as BP between 24:00 and 06:00 h. Salt sensitivity of BP was evaluated according to Sharma *et al.* [13]. According to his proposal, BP was categorized as salt sensitive when the increment of 24 h mean arterial pressure (MAP) on a high salt diet exceeded 3 mmHg compared with the period on a low sodium diet.

Blood and urine chemistries were measured using an autoanalyser technique; haemoglobin A1c (HbA1c) using the high-performance liquid chromatography method on a BioRad Variant analyser (Bio-Rad Laboratories, Hercules, CA); haematocrit by centrifugation; plasma renin activity (PRA), aldosterone, immunoreactive insulin and atrial natriuretic peptide (ANP), and urinary albumin concentrations using radioimmunoassays. The urinary steroid metabolites THF + 5 α THF and THE were assessed by gas chromatography as described previously [11,14].

Statistics

Data are given as means \pm SD (if normally distributed) or median and range (if not normally distributed). The zero hypothesis was rejected at $P < 0.05$ by non-parametric analysis using the Wilcoxon's or Mann-Whitney tests or by analysis of variance (ANOVA) using the SAS statistical

Table 2. Ancillary measurements during low and high salt periods

	Controls		Offspring DN–		Offspring DN+	
	Low salt	High salt	Low salt	High salt	Low salt	High salt
Body weight (kg)	62 ± 11 ^b	64 ± 11	68 ± 13 ^b	70 ± 13	79 ± 16 ^b	80 ± 16
Haematocrit (%)	42 ± 4 ^b	39 ± 4	43 ± 4 ^b	38 ± 3	42 ± 4 ^b	39 ± 3
S-Na (mmol/l)	141 ± 2	141 ± 2	141 ± 3	142 ± 2	142 ± 3	143 ± 4
S-K (mmol/l)	4.5 ± 0.3	4.2 ± 0.5	4.6 ± 0.4	4.5 ± 0.3	4.4 ± 0.4	4.5 ± 0.4
UV _{Na} (mmol/day)	14 ± 6 ^b	241 ± 27	16 ± 6 ^b	269 ± 48	16 ± 9 ^b	264 ± 29
UV _K (mmol/day)	49 ± 26	39 ± 15	50 ± 23 ^a	55 ± 22	71 ± 18	60 ± 25
Protein intake (g/24 h)	38 ± 8	40 ± 11	51 ± 15	41 ± 15	54 ± 15	45 ± 12
Fasting insulin (mIU/l)	7.7 (3.0–24.3)	10.2 (2.2–37.1)	12 (1.7–31.7)	15.1 (0.7–33.4)	14.0 ^c (4.4–41.1)	17.6 (0.2–32.1)
Albuminuria (mg/24 h)	21.0 (5.9–59.4)	23.4 (6.9–50.6)	13.6 (6.6–52.2)	18.9 (10.8–36.1)	18.1 (7.3–36.3)	21.4 (7.2–70.5)

Results are in means ± SD or in median (range), *n* = 15 per group.

^a*P* < 0.05 low vs high salt intake.

^b*P* < 0.01 low vs high salt intake.

^c*P* < 0.05 vs control on low salt.

software program (SAS, Cary, NC). Bonferroni correction for repeated measures was made as indicated.

Results

Baseline data (Table 1)

Offspring of type 2 diabetic DN+ and DN– were well matched with respect to age, gender, fasting glycaemia and HbA1c. The latter parameters proved the absence of diabetes in the subjects investigated. As a potential reflection of a more marked predisposition to the metabolic syndrome, the body mass index was significantly higher in offspring of DN+ patients than in offspring of DN– patients or in controls. Normal renal function was verified by normal serum creatinine values and estimated glomerular filtration rate (data not shown).

Ancillary measurements during low and high salt periods (Table 2)

A significant increase in body weight and a significant decrease in haematocrit in all three study groups documented salt and volume retention during the high salt period. Good compliance was reflected by urinary sodium excretion rates closely corresponding to the target sodium intakes. Urinary albumin excretion was similar in all groups.

Blood pressure (Figures 1 and 2, and Table 3)

The pre-defined primary end-point of the study was the change of 24 h MAP in response to the switch over from low to high salt intake. As illustrated in Figure 1, there was a marked and significant (*P* < 0.002) difference between offspring of DN+ patients and offspring of DN– patients or control subjects, respectively.

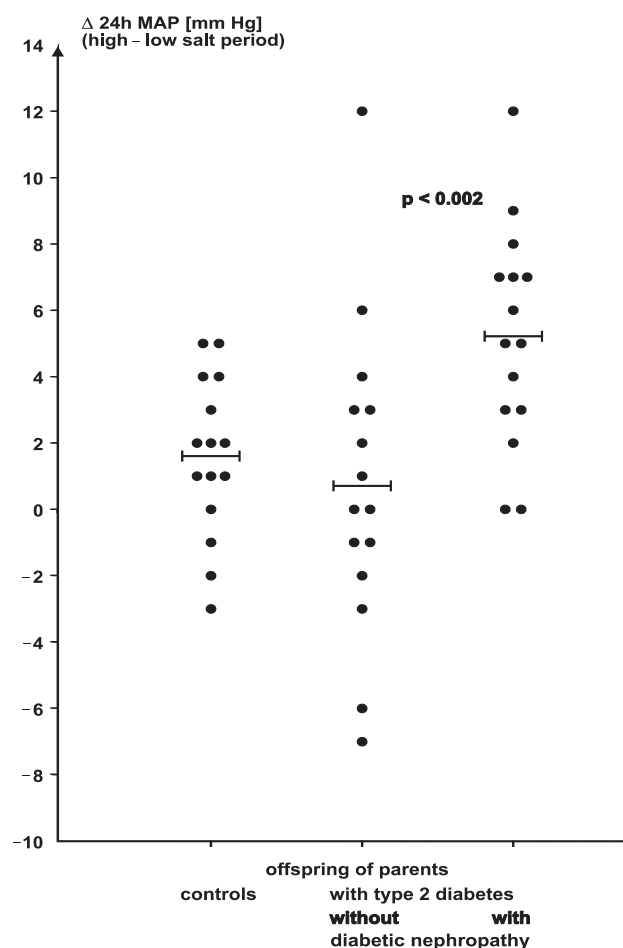


Fig. 1. Difference of the average mean arterial pressure (Δ MAP) by ambulatory 24 h measurement on low compared with high dietary intake of sodium between examined offspring groups. Controls = offspring of non-diabetic patients; DN– = offspring of diabetic patients without diabetic nephropathy; DN+ = offspring of diabetic patients with nephropathy. Taking Δ MAP as a continuous variable, differences between DN+ vs DN– or controls were highly significant (*P* < 0.002).

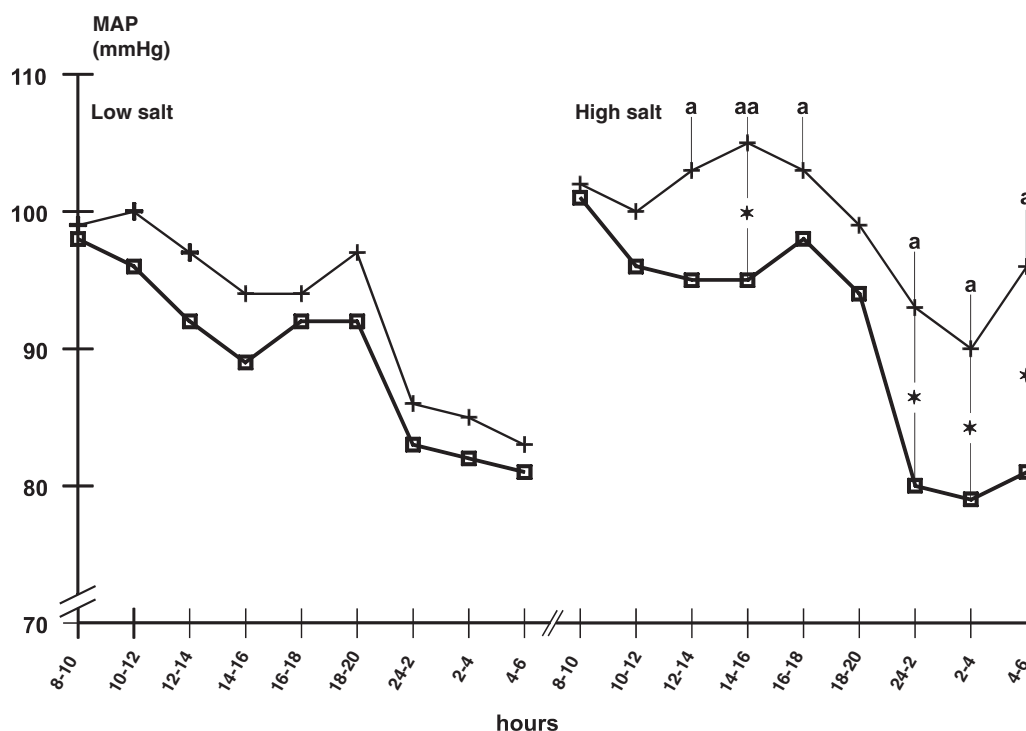


Fig. 2. Mean circadian blood pressure profile on low and high dietary salt intake in offspring of diabetic patients without diabetic nephropathy (open squares) and with diabetic nephropathy (+). ^a $P < 0.05$ and ^{aa} $P < 0.01$ high vs low salt, * $P < 0.01$ DN+ vs DN-.

Table 3. Systolic and diastolic blood pressure during low and high salt intake

		Low salt period			High salt period		
		Daytime	Night-time	24 h	Daytime	Night-time	24 h
Systolic BP (mmHg)	Control	123 ± 9	111 ± 9 ^b	120 ± 9	125 ± 9 ^b	110 ± 6 ^b	122 ± 9 ^a
	DN-	123 ± 11	115 ± 16	120 ± 12	125 ± 12 ^b	113 ± 16 ^a	121 ± 12 ^b
	DN+	129 ± 13	121 ± 15	128 ± 13	137 ± 10 ^d	128 ± 16	135 ± 11 ^d
Diastolic BP (mmHg)	Control	76 ± 6	65 ± 3	74 ± 5	76 ± 6 ^a	67 ± 4 ^b	74 ± 5
	DN-	77 ± 9	69 ± 11	75 ± 9	77 ± 8	67 ± 14	75 ± 8
	DN+	77 ± 8	66 ± 9	75 ± 8	82 ± 8 ^d	77 ± 12 ^d	79 ± 7 ^c
Heart rate (/min)	Control	90 ± 9	69 ± 7	85 ± 8	83 ± 10	69 ± 9	80 ± 9
	DN-	83 ± 8	71 ± 10	80 ± 8	83 ± 9	70 ± 7	79 ± 9
	DN+	85 ± 7	69 ± 11	81 ± 7	83 ± 8	68 ± 11	80 ± 8

Results are in means ± SD, $n = 15$ per group.

^a $P < 0.05$ vs DN+.

^b $P < 0.01$ vs DN+.

^c $P < 0.05$ high vs low salt intake.

^d $P < 0.01$ high vs low salt intake.

As shown in Figure 2, consistently higher MAP values were found during daytime and night-time in offspring of patients with DN.

Table 3 shows systolic and diastolic BP and heart rate during the low and high salt periods in the three groups studied. In offspring of DN+ patients, a significant increase of systolic and diastolic BP was observed on high salt intake. No significant increment of BP on high salt intake was found in offspring of DN- patients and control subjects. Nevertheless night-time systolic BP on low salt intake in the group of offspring of DN+ patients was significantly higher

than in controls. On high salt intake, systolic BP (daytime, night-time and 24 h) was significantly higher in offspring of DN+ patients when compared with offspring of DN- patients and control subjects.

On high salt intake, diastolic BP (daytime and night-time) was significantly higher in the offspring of DN+ patients when compared with controls.

Using established and generally accepted criteria [12] three out of 15 offspring of DN- diabetic patients and 10 out of 15 DN+ patients were salt sensitive ($P < 0.05$). The respective proportion for the control group was four out of 15. The heart rate was

Table 4. Hormonal measurements in low and high salt periods

		Low salt	High salt
PRA (ng/ml/h)	Controls	4.9 ± 2.4 ^a	0.6 ± 0.7
	DN–	5.5 ± 2.9 ^a	0.8 ± 1.2
	DN+	4.9 ± 1.9 ^a	0.8 ± 0.7
Aldosterone (pg/ml)	Controls	157 ± 77.7 ^b	22 ± 4
	DN–	151 ± 66 ^b	26 ± 9
	DN+	134 ± 50 ^b	23 ± 13
ANP (pmol/l)	Controls	19 ± 10	22 ± 11
	DN–	17 ± 9	20 ± 11
	DN+	21 ± 10	21 ± 10

Results are means ± SD, *n* = 15 per group.

Offspring of patients without (DN–) or with (DN+) nephropathy.

^a*P* < 0.05 high salt vs low salt.

^b*P* < 0.01 high salt vs low salt.

PRA = plasma renin activity; ANP = atrial natriuretic peptide.

comparable in all study groups. It was not different between the periods of low and high salt intake.

Hormonal measurements (Table 4)

PRA, plasma aldosterone and ANP did not differ significantly between the study groups during low and high salt intake, respectively.

Urinary steroid metabolites (Figure 3)

The urinary (THF + 5αTHF)/THE ratio at the end of the low salt period was 0.97 ± 0.31 in controls, 1.24 ± 0.36 in offspring of DN+ patients (*P* < 0.05 vs controls) and 1.04 ± 0.36 in offspring of DN– patients. The individual values are displayed in Figure 3. There was a significant difference of the urinary (THF + 5αTHF)/THE ratio between salt-sensitive individuals (*n* = 15) and salt-resistant individuals (*n* = 30), i.e. 1.23 ± 0.36 vs 0.99 ± 0.33 (*P* < 0.02).

Discussion

The present study shows that BP is more salt sensitive in offspring of type 2 diabetic patients with DN as compared with offspring of type 2 diabetic patients without DN despite a similar duration of diabetes. This finding is compatible with the notion that salt sensitivity is an intermediate phenotype related to the pathogenesis of DN.

There is no universally accepted definition of salt sensitivity. We adopted a modified and generally accepted version of the procedure proposed by Sharma *et al.* [13]. We are conscious of the fact that to categorize individuals as salt sensitive or salt insensitive is somewhat arbitrary. However, the difference of BP responses to changes in dietary sodium was still significantly different between the groups when the BP was treated as a continuous variable.

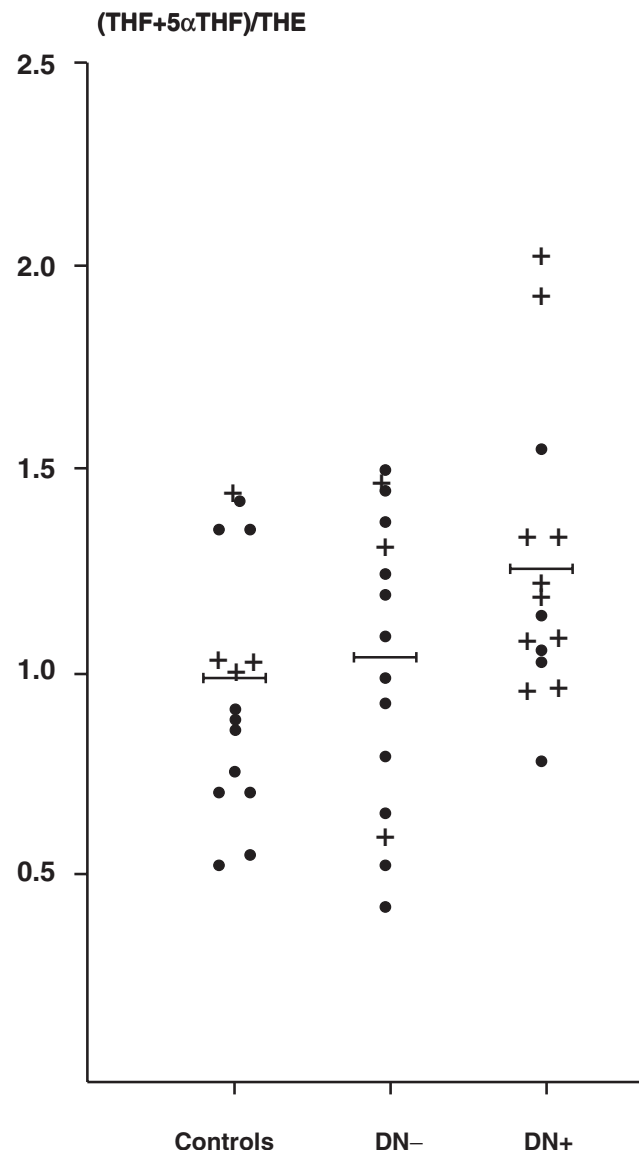


Fig. 3. Ratio of cortisol to cortisone metabolites in the urine (THF + 5αTHF/THE ratio) at the end of the low salt diet. Salt-sensitive individuals (+), salt-resistant individuals (filled circles). Controls = offspring of non diabetic patients; DN– = offspring of diabetic patients without diabetic nephropathy; DN+ = offspring of diabetic patients with nephropathy. A significant difference between salt-sensitive and salt-resistant subjects was found (1.23 ± 0.36 vs 0.99 ± 0.33, *P* < 0.02) when all subjects were considered as two groups.

The study has certain limitations. We took great care to make certain that the subjects were in sodium equilibrium after 5 days. Equilibrium was clearly documented by daily measurements of urinary sodium as in a past study [15]. The subjects were remarkably compliant and this was reflected by the measured urinary sodium excretion rates. Based on fasting glycaemia, the study subjects were all non-diabetic, even if the recent downward revision of fasting glycaemia criteria is taken into consideration.

The study thus includes individuals at high genetic risk of diabetes mellitus, but without demonstrable pre-diabetes or diabetes. Some hint of a latent pre-diabetic state may be the fact that the body mass index was higher and kidney size tended to be greater (in DN+), even though normal glucose and HbA1c levels indicated normal glucose tolerance.

We took care to exclude obvious confounding factors such as hormonal contraception in females. All study subjects had no medication whatsoever that could have confounded BP measurements.

The observation of higher BP in offspring of type 2 diabetic patients with DN confirms our previous finding [7]. Higher BP was associated with a higher left ventricular mass index (LVMI), but we cannot exclude confounding by insulin concentrations. Several possibilities to explain the increased salt sensitivity of BP were considered. The offspring of diabetic patients tended to have higher insulin concentrations and thus might possibly have had a more marked metabolic syndrome. This possibility would also be in line with the observation of a significantly higher body mass index. Renal ultrasonography showed a tendency for kidneys to be larger (Table 1).

It is possible that higher pre-diabetic BP predisposes to future DN. This hypothesis is supported by the observation of Nelson *et al.* that pre-diabetic BP determines the risk of DN after onset of type 2 diabetes, at least in Pima Indians [16]. It would also be consistent with the observation that BP values and frequency of hypertension are higher in families where there is at least one propositus with diabetic [17] or non-diabetic glomerular disease. In this context, one could also refer to the hypothesis of Brenner and Chertow [18] that individuals predisposed to hypertension and renal disease have lower numbers of nephrons. This hypothesis has to some extent been confirmed, at least as far as hypertension is concerned [19]. We acknowledge, however, that we cannot distinguish whether salt sensitivity of BP is causally linked to hypothetical future nephropathy, whether it is caused by a latent renal abnormality predisposing to nephropathy, e.g. nephron underdosing, or whether it is an epiphenomenon caused by some confounding factor.

Salt sensitivity of BP is noted in a high proportion of type 1 diabetic patients even in the absence of nephropathy, but salt sensitivity is also a well known characteristic of established DN [15]. Tuck *et al.* found salt-sensitive BP in hypertensive, but not non-hypertensive patients with type 2 diabetes [20]. Several factors have been implied for the tendency of diabetes to cause Na retention, i.e. increased renal tubular Na-K-ATPase or Na⁺/proton exchange, increased action of aldosterone and increased proximal tubular sodium reabsorption via the Na-glucose co-transporter. The latter is presumably excluded in the present study because of the consistent absence of glycosuria.

Confirming previous results in our laboratory [14], we found a significantly higher urinary (THF + 5 α THF)/THE ratio in salt-sensitive than

in salt-resistant individuals, indicating a decreased 11 β HSD2 activity. Although we are fully aware of the limited biostatistical power because of the relatively small sample size, it is remarkable that a tendency for decreased 11 β HSD2 activity in salt-sensitive individuals was noted even in this relatively small sample. Increased salt sensitivity as a potential intermediate phenotype may be of interest to the geneticist. Salt sensitivity has recently been related to adducin [21], the epithelial sodium channel of the distal tubule and cortical collecting ducts [22], and to 11 β HSD2 enzyme [11,14]. A recent communication found changes in 11 β HSD2 expression and activity in experimental diabetes [23], but acquired changes in enzyme activity are not an issue in the present study.

We conclude that the present study documents greater salt sensitivity of BP in offspring of diabetic parents with DN. Whether salt sensitivity is an intermediate phenotype predisposing to future DN requires further investigation.

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Conflict of interest statement. None declared.

[See related Editorial by Weir, p. 2022]

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