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Effect of insulin on renal sodium handling in hyperinsulinaemic Type 2 (non-insulin-dependent) diabetic patients with peripheral insulin resistance

P. Skøtt[1, 2], A. Vaag[1], N. E. Bruun[2], O. Hother-Nielsen[1], M.-A. Gall[1], H. Beck-Nielsen[1] and H.-H. Parving[1]

[1] Hvidøre Hospital, Klampenborg, and [2] Department of Clinical Physiology and Nuclear Medicine, Glostrup University Hospital, Copenhagen, Denmark

Summary. The sodium retaining effect of insulin was studied in ten Type 2 (non-insulin-dependent) diabetic patients (mean age 56 (43–73) years, mean body mass index 29.5 (24.2–33.7) kg/m^2) and eight age-matched control subjects (mean age 57 (43–68) years, mean body mass index 23.4 (20.8–26.6) kg/m^2). The renal clearances of ^{99m}Tc-DTPA, lithium, sodium and potassium were measured over a basal period of 90 min. Then insulin was infused at a rate of 40 mU · min^{-1} · m^{-2}. After an equilibration period of 90 min, the clearance measurements were repeated during a new 90 min period. Blood glucose was clamped at the basal level (diabetic patients: 9.9 ± 3.5, control subjects: 5.3 ± 0.5 mmol/l) by a variable glucose infusion. Basal plasma insulin concentration was elevated in the diabetic patients (0.12 ± 0.05 vs 0.05 ± 0.02 pmol/ml, $p < 0.01$). Insulin infusion resulted in comparable absolute increments in plasma insulin concentrations in the diabetic group and in the control group (0.44 ± 0.13 vs 0.36 ± 0.07 pmol/ml, NS). The metabolic clearance rate of glucose during the last 30 min of insulin infusion was lower in the diabetic patients (155 ± 62 vs 320 ± 69 ml · min^{-1} · m^{-2}, $p < 0.01$), reflecting peripheral insulin resistance. The decline in sodium clearance during insulin infusion was similar in diabetic subjects (1.8 ± 1.1 vs 0.7 ± 0.4 ml · min^{-1} · 1.73 m^{-2}, $p < 0.01$) and in control subjects (1.7 ± 0.3 vs 0.8 ± 0.3 ml · min^{-1} · 1.73 m^{-2}, $p < 0.01$). The glomerular filtration rate and lithium clearance was unchanged, consequently calculated distal tubular fractional sodium reabsorption increased (diabetic patients: 92.9 ± 4.1 vs 97.1 ± 1.5, $p < 0.01$, control subjects: 93.1 ± 1.1 vs 96.5 ± 0.6%, $p < 0.01$). Estimated extracellular fluid volume was 10% higher in the diabetic subjects (16.3 ± 2.1 vs 14.8 ± 2.0 l · 1.73 m^{-2}, NS). In conclusion, the sodium retaining effect of insulin is preserved in Type 2 diabetic patients with peripheral insulin resistance. Insulin may contribute to sodium and fluid retention and thus to the increased frequency of hypertension in hyperinsulinaemic Type 2 diabetic patients.

Key words: Glomerular filtration rate, lithium clearance, sodium excretion, potassium excretion, isoglycaemic clamp, insulin infusion.

Several conditions are characterized by peripheral insulin resistance (i.e. relative resistance to the glucose lowering effect of insulin), and concomitantly by elevated circulating plasma insulin levels. Prominent are the syndromes of Type 2 (non-insulin-dependent) diabetes mellitus, obesity, and essential hypertension [1–3].

Recently we found, that elevation of plasma insulin concentration within the physiological range has a marked antinatriuretic action located distally to the proximal renal tubules in young healthy subjects [4]. It has been proposed, that enhanced renal tubular sodium reabsorption due to hyperinsulinaemia may in part explain the sodium and fluid retention and the increased frequency of hypertension in obesity and Type 2 diabetes [1, 2, 5]. In contrast, it has been argued that the associated peripheral insulin resistance will counteract this renal effect [6].

The aim of the present study was to evaluate the renal effects of physiological increments in plasma insulin concentration in a state characterized by marked peripheral insulin resistance. Therefore, we studied a representative sample of Type 2 diabetic patients.

Subjects and methods

Subjects

We studied ten consecutive patients (two female, eight male) with Type 2 diabetes not receiving insulin treatment (Table 1) mean age 56 years (range 43–73 years). Four were treated with diet alone and six received oral hypoglycaemic agents, which were withheld on the study day and the day before. Four patients had established arterial hypertension, but antihypertensive treatment had been withheld for

Table 1. Clinical data in ten patients with Type 2 (non-insulin-dependent) diabetes mellitus and eight healthy control subjects

	Type 2 diabetic patients	Healthy subjects
Number (female/male)	10 (2/8)	8 (2/6)
Age (years)	56 (43–73)	57 (43–68)
Body mass index (kg/m^2)	29.5 (24.2–33.7)[a]	23.4 (20.8–26.6)
Blood pressure (mm Hg)	134 ± 15/81 ± 12[a]	116 ± 18/72 ± 12
Haemoglobin A_{1c}	7.9 ± 2.2[b]	5.6 ± 0.5
Retinopathy (none/background/proliferative)	6/4/0	–

Mean ± SD or mean (range) indicated. [a] $p < 0.05$; [b] $p < 0.01$

at least four weeks before the study. Previous antihypertensive therapy was thiazide diuretic (two patients, one of these also received prazosin), captopril (one patient) and metoprolol (one patient). All patients had urinary albumin excretion rates < 300 mg/24 h. The control group comprised eight age-matched healthy subjects (two female, six male) mean age 57 years (range 43–68 years). They were all normotensive and none was taking medication. In all control subjects an oral glucose tolerance test was normal, and none of them had a family history of diabetes. In both groups changes in the diet taken prior to the investigation were avoided. All subjects agreed to participate in the study after receiving oral and written information. The procedure was performed according to the principles of the Helsinki Declaration and the study was approved by the regional ethical committee.

Methods

The subjects received 600 mg (16.2 mmol) of lithium carbonate (DAK Laboratory, Copenhagen, Denmark) orally the evening before the clearance study. The subjects fasted from midnight until the end of the study. During the clearance studies all the subjects were in the supine position, except when voiding urine. The subjects drank 200 ml of tap water per h beginning one h before the start of the first clearance period.

During the investigation, each subject kept one hand in a heated plexiglas box to enable arterialized venous blood to be obtained. Blood glucose concentration was monitored in arterialized blood with a continuous glucose analyser (Biostator CGIIS, Life Science Instruments, Miles Laboratories, Elkhart, Ind., USA) connected to a forearm vein. Arterialized blood samples were drawn from a plastic catheter placed in another forearm vein on the same arm and after each blood sampling, the catheter was flushed with 5 ml NaCl 154 mmol/l in water. Insulin (Insulin Actrapid, Novo, Bagsværd, Denmark) dissolved in NaCl 154 mmol/l in water was infused through an antecubital vein in the contralateral arm. The insulin infusion was given at a constant rate of 11.6 ml/h with insulin added to provide an infusion rate of 40 mU · m^{-2} · min^{-1}. Through the same vein a variable infusion of 20% glucose was administered during insulin infusion. For determination of glucose turnover rates an adjusted primed-continuous infusion of 3-^{3}H-glucose was given. 3-^{3}H-glucose (New England Nuclear, Boston, Mass., USA) was dissolved in NaCl 154 mmol/l in water and infused at a rate of 25 ml/h. During the study about 29 mmol Na was removed by blood sampling and about 36 mmol Na was given by infusions and flushing of catheters.

The renal clearances of ^{99m}Tc-DTPA, lithium, potassium and sodium were measured over a basal period of 90 min beginning 30 min after i.v. injection of approximately 100 MBq ^{99m}Tc-DTPA (diethylenetriaminepentaacetic acid (TCK-6, CIS bioindustries, Gif Sur Yvette, Cedex, France). At the end of the basal period, an insulin infusion was started. Blood glucose concentration was clamped at the mean fasting value obtained in the basal period with a variable glucose infusion via the Biostator CGIIS. After an equilibration period of 90 min the renal clearance measurements were repeated over a 90 min clearance period. The steady-state glucose infusion rate during the last 30 min of this period was calculated. Residual urine was determined by measuring radioactivity from ^{99m}Tc-DTPA in the pubic region before and after each voiding. After correction for background radioactivity determined over the chest, residual urine was calculated [7]. The clearance values obtained were corrected if calculated residual urine exceeded 20 ml.

Blood pressure was measured in duplicate by a semiautomatic device (Takeda UA-751, Japan) about 10 min after the injection of ^{99m}Tc-DTPA.

Blood samples for measurements of various substances were timed as follows. Plasma glucose: approximately every 30 min throughout the investigation and every 10 min for the last 30 min of each of the clearance periods. For immediate adjustment of the readings of the continuous glucose analyser in the Biostator CGIIS, plasma glucose concentration was measured with an automated glucose oxidase method (Glucose Analyser 2, Beckman Instruments, Fullerton Calif., USA) and the continuous glucose analyser adjusted accordingly. Plasma insulin and C-peptide: every 30 min during each of the clearance periods. 3-^{3}H-glucose: every 30 min throughout the study and every 10 min for the last 30 min of each of the clearance periods. ^{99m}Tc-DTPA: every 30 min. Lithium, sodium and potassium: at the beginning and end of each clearance period.

Calculations. The renal clearances of ^{99m}Tc-DTPA, lithium (C_{Li}), potassium and sodium were calculated as the ratio between the urinary excretion rate and the mean plasma concentration during the 90 min clearance periods. For ^{99m}Tc-DTPA and lithium, the plasma concentration was calculated as the mean interpolated plasma concentration [8]. As a measure of extracellular fluid volume, the apparent volume of distribution of ^{99m}Tc-DTPA was calculated for the last clearance period, which started at least 210 min after the injection of ^{99m}Tc-DTPA where the final slope of the time-activity curve for ^{99m}Tc-DTPA has been reached [9]. During steady-state, the excreted amount of a tracer ($U \cdot V$), is equal to the amount of tracer present in the subject at the beginning of a clearance period ($t = 1$) minus the amount of tracer present in the subject at the end of the clearance period ($t = 2$). Assuming that the plasma concentration at any time, P_t reflects the true mean concentration in the volume of distribution for the tracer (V_D), the relationship can be written

$$(1)\ U \cdot V = P_1 \cdot V_D - P_2 \cdot V_D,$$

which is rearranged:

$$(2)\ V_D = U \cdot V/(P_1 - P_2).$$

The absolute proximal tubular reabsorption rate of water was calculated as (glomerular filtration rate (GFR) – C_{Li}), and the absolute proximal reabsorption rate of sodium as plasma sodium concentration multiplied by the absolute proximal reabsorption of water. The fractional reabsorption of sodium and water in the proximal tubules was determined as $1 - (C_{Li}/GFR)$. The absolute distal reabsorption rate of water was calculated as C_{Li} – urine flow and the fractional distal water reabsorption was calculated as 1 – urine flow/C_{Li}. The absolute distal reabsorption rate of sodium was calculated as plasma sodium concentration multiplied by the difference between C_{Li} and sodium clearance, and the fractional distal sodium reabsorption was determined as 1 – sodium clearance/C_{Li}.

In the diabetic patients osmolar clearance induced by glucosuria was calculated from the urinary glucose excretion rate and estimated plasma osmolarity (two times the sum of plasma sodium and plasma potassium concentrations plus plasma glucose concentration) as previously described [10].

As measure of insulin sensitivity, the metabolic clearance rate of glucose during the last 30 min of each clearance period was calculated as the rate of glucose infusion per unit surface divided by the ambient plasma glucose concentration. Since this approach has been criticized, the insulin sensitivity index was calculated according to Bergman et al. [11] as the difference between the rate of disappearance of glucose (R_d) during the last 30 min of insulin infusion and during the basal period divided by the product of the ambient plasma glucose concentration during this period and the increment in plasma insulin concentration produced by insulin infusion. For this purpose, plasma glucose concentration was measured every 10 min dur-

Table 2. Effects of insulin infusion on plasma insulin concentration, plasma glucose concentration and metabolic clearance rate of glucose in ten Type 2 (non-insulin-dependent) diabetic patients and eight healthy control subjects

	Type 2 diabetic patients		Healthy subjects	
	Basal	Insulin	Basal	Insulin
Plasma insulin (pmol/ml)	0.12 ± 0.05[b]	0.56 ± 0.15[a]	0.05 ± 0.02	0.41 ± 0.07
CV (insulin) (%)	12.2 ± 4.7	6.3 ± 3.5	21.7 ± 10.0	8.3 ± 4.9
Plasma glucose (mmol/l)	9.9 ± 3.5[a]	10.0 ± 4.0[a]	5.3 ± 0.5	5.2 ± 0.4
CV (glucose) (%)	1.0 ± 0.5	3.2 ± 1.8	1.4 ± 1.3	5.1 ± 1.9
Metabolic clearance rate of glucose ($ml \cdot min^{-1} \cdot m^{-2}$)		155 ± 62[b]		320 ± 69
Insulin sensitivity index ($l \cdot min^{-1} \cdot m^{-2} \cdot pmol^{-1} \cdot ml$)		0.26 ± 0.21[c]		0.68 ± 0.20

CV: Coefficient of variation. Mean ± SD indicated
[a] $p < 0.05$ as compared to the corresponding clearance period in the control subjects; [b] $p < 0.01$ as compared to the corresponding clearance period in the control subjects; [c] $p < 0.005$ as compared to the corresponding value in the control subjects

Table 3. Effects of insulin infusion on kidney function and serum electrolytes in ten Type 2 (non-insulin-dependent) diabetic patients and eight healthy control subjects

	Type 2 diabetic patients		Healthy subjects	
	Basal	Insulin	Basal	Insulin
Glomerular filtration rate ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$)	104 ± 24	105 ± 27	91 ± 11	91 ± 8
Lithium clearance ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$)	26 ± 3	25 ± 4	24 ± 4	24 ± 7
Renal sodium clearance ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$)	1.8 ± 1.1	0.7 ± 0.4[b]	1.7 ± 0.3	0.8 ± 0.3[b]
Plasma sodium concentration ($mmol \cdot l^{-1}$)	137 ± 2	137 ± 2	137 ± 2	138 ± 2[a]
Fractional excretion of sodium (%)	1.8 ± 1.0	0.7 ± 0.4[b]	1.8 ± 0.5	0.9 ± 0.3[b]
Urine flow rate ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$)	3.0 ± 1.3	3.1 ± 0.9	3.2 ± 2.0	4.5 ± 1.6
Renal potassium clearance ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$)	16 ± 9	7 ± 2[b]	15 ± 3	6 ± 2[b]
Plasma potassium concentration ($mmol \cdot l^{-1}$)	4.1 ± 0.1	3.6 ± 0.2[b]	3.9 ± 0.2	3.5 ± 0.2[b]

Mean ± SD indicated.
[a] $p < 0.05$ basal period vs insulin infusion period; [b] $p < 0.01$ basal period vs insulin infusion period

ing the steady-state periods. Thus, the insulin sensitivity index indicates the change in the metabolic clearance rate of glucose per unit change in plasma insulin concentration. During insulin infusion, the glucose infusion rate necessary to maintain a constant blood glucose concentration during the last 30 min of the clearance period was used as R_d assuming suppression of hepatic glucose production, for the basal period R_d was calculated as previously described [12].

Analyses. Urine and serum concentrations of lithium were determined by atomic absorption spectrophotometry (Perkin Elmer 1100 B, Norwalk, Conn., USA) as described by Amdisen [13]. Urine and plasma concentrations of sodium and potassium were determined by routine flame emission spectrophotometry. Plasma and urinary glucose concentrations were measured by the hexokinase method [14].

Plasma insulin concentration and plasma C-peptide concentrations were measured by radioimmunoassay [15, 16].

Statistical analysis

For comparisons within groups the Wilcoxon test for paired comparisons with Pratts correction was used [17]. For comparisons between groups, the Mann-Whitney test was used. A p-value < 0.05 was considered significant.

Results

Results are shown in Tables 1, 2, 3, and 4 and in Figure 1. As compared to the age-matched control group, the patients with Type 2 diabetes had significantly higher body mass index ($p < 0.05$), blood pressure ($p < 0.05$), plasma glucose concentration ($p < 0.05$) and fasting plasma insulin concentration ($p < 0.01$) (Table 1 and Table 2). Fasting plasma C-peptide concentrations were significantly elevated in the diabetic subjects (0.94 ± 0.40 vs 0.49 ± 0.19 pmol/ml, $p < 0.01$). Plasma insulin and glucose concentrations during each of the clearance periods are shown in Table 2. During insulin infusion, the plasma insulin concentration in the patients was significantly higher than in the control subjects, but the mean absolute increment in plasma insulin concentrations produced by insulin infusion was about the same in the diabetic subjects and the control subjects (0.44 ± 0.13 vs 0.36 ± 0.07 pmol/ml, NS). Both the metabolic clearance rate of glucose during the last 30 min of insulin infusion and the calculated insulin sensitivity index were significantly lower in the diabetic patients ($p < 0.01$ and $p < 0.005$, respectively) (Table 2).

Sodium clearance declined during insulin infusion in both diabetic and control subjects, while the GFR and the output of water from the straight part of the proximal tubules into the thin descending limb of Henle's loop as estimated by lithium clearance remained unchanged (Table 3). Consequently, the proximal reabsorption rate of sodium and water was unchanged. Fractional distal sodium reabsorption increased significantly, while distal water reabsorption was unchanged. Calculated absolute distal reabsorption rate of sodium did not change signifi-

Table 4. Effects of insulin infusion on calculated segmental renal tubular reabsorption in ten Type 2 (non-insulin-dependent) diabetic patients and eight healthy control subjects

	Type 2 diabetic patients		Healthy subjects	
	Basal	Insulin	Basal	Insulin
Absolute proximal reabsorption rate of water ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$)	79 ±22	81 ±25	67 ±11	67 ±10
Absolute proximal reabsorption rate of sodium ($mmol \cdot min^{-1} \cdot 1.73\ m^{-2}$)	10.8 ± 3.0	11.1 ± 3.4	9.2 ± 1.5	9.2 ± 1.4
Fractional proximal reabsorption rate of sodium and water (%)	75 ± 4	76 ± 5	73 ± 5	73 ± 7
Absolute distal tubular reabsorption rate of sodium ($mmol \cdot min^{-1} \cdot 1.73\ m^{-2}$)	3.3 ± 0.4	3.3 ± 0.6	3.1 ± 0.6	3.2 ± 0.9
Absolute distal tubular reabsorption rate of water ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$)	23 ± 3	21 ± 4	21 ± 5	20 ± 7
Fractional distal tubular reabsorption rate of sodium (%)	92.9 ± 4.1	97.1 ± 1.5[a]	93.1 ± 1.1	96.5 ± 0.6[a]
Fractional distal tubular reabsorption rate of water (%)	88 ± 5	87 ± 4	87 ± 8	79 ±11

Mean ± SD indicated.
[a] $p < 0.01$ basal period vs insulin infusion period

cantly (Table 4). Potassium clearance and plasma potassium concentrations declined with increasing plasma insulin concentrations. Plasma sodium concentration did not change significantly in the diabetic subjects, in the healthy subjects a minor but significant increase was observed during insulin infusion. No significant difference in the pattern of response of any of the parameters mentioned was detected between the diabetic subjects and the control subjects. In the control group insulin infusion reduced sodium clearance to 51 ± 13% of the baseline value, while in the diabetic group, it declined to 40 ± 10% of the baseline value. The median reduction in sodium clearance during insulin infusion was 12% higher in the diabetic group (95% confidence interval for this difference was −3% to 22%). The concentration-response curve for the effect of insulin on renal sodium clearance was nearly identical in Type 2 diabetic patients and in control subjects (Fig. 1).

Glucose-induced osmolar clearance was minimal in the diabetic patients both in the basal clearance period (median value 0.01 ml/min, range 0–0.6) and during insulin infusion (median value 0.01 ml/min, range 0–0.8) corresponding to 0.0% (range 0.0–1.9) and 0.0% (range 0.0–3.3) of lithium clearance during the basal clearance period and during insulin infusion, respectively.

When compared to the control group, baseline GFR was significantly higher in the diabetic patients without correction for body surface area (120 ± 27 vs 97 ± 17 $ml \cdot min^{-1}$, $p < 0.05$), but after correction for surface this difference was no longer significant ($p = 0.17$), although mean GFR was still 14 $ml \cdot min^{-1} \cdot 1.73\ m^{-2}$ higher in the diabetic patients. Similarly, estimated extracellular fluid volume was significantly elevated in the diabetic patients without correction for body surface area (18.9 ± 2.9 vs 15.6 ± 2.1 l, $p < 0.05$) but not after correction for body surface (16.3 ± 2.1 vs 14.8 ± 2.0 $l \cdot 1.73\ m^{-2}$, $p = 0.20$). No differences in calculated segmental tubular sodium handling emerged, specifically both absolute and fractional proximal tubular sodium reabsorption rates were not significantly elevated in the diabetic patients.

Residual urine exceeding 20 ml was observed in 10 of 32 voidings in the healthy control group, and in 18 of 40 voidings in the diabetic patients. There was no predictable pattern in the amount of residual urine observed and in several subjects only one or two voidings were associated with significant residual urine. To exemplify the impact of residual urine on renal excretion measurements, the coefficient of variation for GFR measured in the basal period and during insulin infusion was 5.9% in the diabetic patients when using correction for residual urine and 23.3% without.

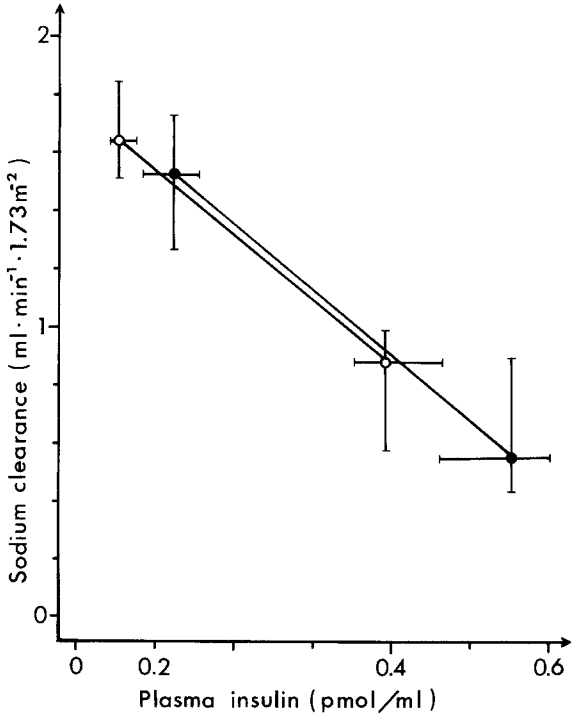

Fig. 1. The effect of insulin infusion on sodium clearance and plasma insulin concentration during isoglycaemia in ten patients with Type 2 (non-insulin-dependent) diabetes mellitus (filled circles) and eight healthy subjects (open circles). Median and interquartile ranges indicated

Discussion

In our study the sodium retaining effect of insulin is preserved in Type 2 diabetic patients with peripheral insulin resistance and fasting hyperinsulinaemia. The association between obesity, hypertension and Type 2 diabetes is well known [1, 2]. Recent longitudinal studies demonstrated, that blood pressure and body weight increases several years before the development of Type 2 diabetes [18], and that hyperinsulinaemia is still seen 5 years after the diagnosis of diabetes [19] as previously suggested [20]. In our patients plasma immunoreactive insulin and plasma C-peptide values were elevated. True plasma insulin was not measured, but despite elevated levels of proinsulin, true plasma insulin is also elevated in Type 2 diabetic subjects [21]. Thus, hyperinsulinaemia remains a candidate for explaining the sodium and water retention and the increased frequency of hypertension observed in Type 2 diabetes [22, 23].

The 95% confidence interval for the reduction in sodium clearance induced by insulin only leaves the possibility for a 3% greater response in the healthy subjects. Therefore, the risk that our study did not detect a resistance to the sodium retaining effect of insulin similar to the marked peripheral insulin resistance in the diabetic subjects is negligible. Originally, Rocchini et al. found that the sodium retaining effect of insulin was normal in young, obese non-diabetic subjects with peripheral insulin resistance [24]. Using the same dose of insulin, they observed a reduction in renal sodium excretion very similar to that observed in the present study.

The clinical characteristics of our study population is representative of patients with Type 2 diabetes attending our clinic [25]. Since the basic question was, whether peripheral insulin resistance was associated with renal insulin resistance, we included patients with more than one cause for insulin resistance (i.e. obesity, hypertension). Although antihypertensive treatment was stopped at least four weeks prior to study, a slight residual effect of antihypertensive treatment on insulin sensitivity is possible. However, the changes in insulin sensitivity induced by antihypertensive treatment are less than the 62% difference in insulin sensitivity index observed in the present study [26, 27].

The evaluation of dose response curves is conceptually difficult. Since baseline insulin levels differed despite similar sodium excretion rates, the absolute plasma insulin concentrations cannot be used directly in the interpretation of results. Thus, the changes in plasma insulin levels must be related to the baseline values. In our previous study using two different rates of insulin infusion, there was a linear relation between the mean absolute increment in plasma insulin and the mean reduction in sodium clearance [4]. Therefore, the absolute increment in plasma insulin levels was evaluated in relation to the change in sodium excretion. If the fractional change in circulating insulin levels is related to the change in renal sodium clearance, the conclusion would be that the sodium retaining effect of insulin is enhanced about two-fold in Type 2 diabetes.

Our subjects were studied at their ambient plasma glucose concentration. This is the pathophysiologically relevant situation and avoids a confounding influence of semiacute changes in plasma glucose on renal function and metabolic parameters. In particular euglycaemia obtained by insulin infusion prior to the study would have led to a reduction in renal sodium excretion and simultaneously an aggravation of hyperinsulinaemia as can be observed in previous studies [28]. Recently the importance of performing metabolic studies at the patient's own ambient glucose concentration was stressed by Bergman et al. [11].

Lithium clearance is a measure of endproximal tubular fluid delivery during osmotic diuresis [29], and during osmotic diuresis induced by glucosuria there is a dissociation of distal tubular delivery of water and of sodium [30]. However, glucosuria did not appreciably affect the present results.

The present study localized the renal tubular site of action of insulin distally to the proximal renal tubules in Type 2 diabetic patients as did several previous studies in healthy subjects [4, 31, 32]. In contrast, a study in five normal subjects studied on two occasions with and without overnight infusion of insulin at a low dose reported that insulin increased 1-(C_{Li}/GFR) slightly from 70.08 to 70.92% ($p < 0.05$), while fractional distal reabsorption rate of sodium increased non-significantly from 28.20 to 28.92% (NS) (the unusual numerical value of fractional distal reabsorption rate is calculated as (C_{Li}-sodium clearance)/GFR instead of (C_{Li}-sodium clearance)/C_{Li}) [28]. Estimated from the mean values reported, (C_{Li}-sodium clearance)/C_{Li} increased from about 93.6 to 94.7% during insulin infusion. The minor change in 1-(C_{Li}/GFR) in a study with five subjects does not allow any firm conclusions.

A plausible cellular mechanism for insulin-induced renal sodium retention is stimulation of Na-K-ATPase, which is stimulated by insulin [4, 33]. Animal experiments found no functional relationship between the effect of insulin on the Na^+-K^+ pump and on cellular glucose metabolism [34]. In man, some authors have reported that cellular sodium-pump activity is insulin-resistant in obesity [35], while others have demonstrated a dissociation between the effects of insulin on forearm glucose uptake and sodium-potassium exchange [36]. In contrast to a study in young obese subjects [37], we observed a similar lowering effect of insulin on the plasma concentration of potassium in diabetic and non-diabetic subjects. There is no explanation for this discrepancy.

Recently, the sodium retaining effect of insulin was ascribed to the decline in plasma potassium, since it could be reversed by a simultaneous infusion of potassium [32]. However, there is ample *in vitro* evidence for direct effects of insulin on cellular electrolyte transport [33, 38]. Experiments in isolated kidneys showed insulin-induced sodium retention despite an unchanged plasma potassium level [39], and the effects of chronic insulin infusion to reduce sodium excretion did not bear any relation to changes in plasma potassium levels [40]. Moreover, potassium loading *per se* is natriuretic in normal man. Although the effect has been related to concurrent changes in plasma potassium and aldosterone concentrations, the mechanism is poorly understood [41].

In Type 1 (insulin-dependent) diabetic patients absolute and fractional proximal reabsorption rate of fluid and sodium is enhanced as compared to healthy subjects in a comparable sodium balance, while lithium clearance is normal [42–44]. In the present study in Type 2 diabetic patients, we found no significant difference in any index of segmental tubular function, although both absolute and fractional proximal reabsorption rate of sodium and water was slightly elevated in the diabetic patients. In a previous study, Mbanya et al. [45] reported that both fractional lithium clearance and fractional excretion of sodium in the distal tubule was lower in hypertensive Type 2 diabetic patients, but not significantly different from control subjects in normotensive diabetic patients. Although our study was not designed to detect such differences, there was no tendency to a similar difference. Further studies are necessary to clarify this issue. In non-diabetic patients with essential hypertension, most studies concluded that renal tubular function is normal [46].

A relationship between hyperinsulinaemia and high blood pressure has been established in several studies [1, 2, 47]. Hypertension in diabetes is accompanied by sodium and volume retention [22, 23, 48, 49]. The preserved sodium retaining effect of insulin suggests that insulin induced sodium retention may contribute to the pathogenesis of hypertension in Type 2 diabetes. However, long-term insulin infusion in dogs did not elevate blood pressure [40], and the frequency of hypertension is normal in patients with Type 1 diabetes without renal complications [50]. In a clinical study of the effect of short-term strict metabolic control in long-term Type 1 diabetic patients, intensified insulin treatment resulted in an increase in extracellular fluid volume without any detectable change in GFR or blood pressure [51]. Further studies on the role of insulin-induced sodium retention – if any – in the pathogenesis of hypertension are warranted.

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Dr. P. Skøtt
Hvidøre Hospital
Emiliekildevej 1
DK-2930 Klampenborg
Denmark