Abstract

Background. Insulin resistance and hyperinsulinaemia has been suggested as a pathogenetic mechanism in hypertension.

Methods. In this investigation the renal response to insulin was studied in normotensive subjects with a positive family history of hypertension in two generations (n = 14), in one weight-matched (n = 11) and one lean (n = 13) control group. During hyperinsulinaemia (euglycaemic hyperinsulinaemic clamp technique) we determined renal haemodynamics (clearances of $^{51}$Cr-EDTA and PAH) and urinary sodium excretion. Lithium clearance was used to estimate the segmental tubular reabsorption of sodium.

Results. In subjects with a positive family history of hypertension, hyperinsulinaemia did not influence renal plasma flow (RPF) or glomerular filtration rate (GFR) but urinary sodium excretion decreased by 50%. Estimated proximal tubular sodium reabsorption was unaffected by insulin while estimated distal fractional sodium reabsorption increased, $P<0.01$. At the end of the clamp a low-dose infusion of angiotensin II (0.1 ng/kg per min) was superimposed. GFR and RPF then decreased significantly concomitant with urinary excretion of sodium.

In control subjects hyperinsulinaemia caused an unchanged GFR in both groups, increased RPF in the lean control group and 15-25% reduction in sodium excretion. No alteration was seen in estimated proximal tubular sodium reabsorption, but estimated distal fractional sodium reabsorption increased ($P<0.05$) in the lean control group. Angiotensin II elicited a further increase in distal fractional tubular sodium reabsorption in both control groups ($P<0.05$).

Conclusions. In normotensive subjects with a positive family history of hypertension, in contrast to control subjects without such history, hyperinsulinaemia caused a marked decrease in urinary sodium excretion in presence of unchanged RPF and GFR indicating a renal tubular effect of insulin located at a distal site of the renal tubules. Angiotensin II caused further sodium retention, probably due to an effect on renal haemodynamics.

Key words: insulin resistance; hyperinsulinaemia; hypertension; family history; angiotensin II; tubular sodium reabsorption

Introduction

Hyperinsulinaemia and insulin resistance recently have been extensively reported in hypertensive patients, thus raising the hypothesis that insulin may be involved in the pathogenesis of essential hypertension [1,2]. Furthermore, insulin resistance has been correlated with the severity of hypertension and with the existence of an elevated erythrocyte sodium–lithium countertransport [3]. Insulin exhibits an antinatriuretic effect when administered acutely in vivo [4]. The hormone can act on the isolated toad bladder [5] as well as in intact dogs and humans to promote kidney tubule sodium reabsorption [6]. With respect to the mechanism by which insulin acts at the level of the kidney it is not clear whether sodium reabsorption is mainly located in the proximal tubule, the distal tubule, or both [7,8].

Acute and chronic infusion of insulin may give a sustained rise of blood pressure by causing sodium retention [6] but also by a stimulation of the sympathetic nervous system [9], alteration of cation transport [10] or through increased hypertrophy of smooth muscle cells in the absence of change in serum glucose [11]. The cellular effects of insulin, if present also in smooth muscle cells, could explain the increase in total body sodium and blood pressure in salt-sensitive hypertensive patients. It is true that insulin infusion induces hypertension in the rat, but not in the dog, and it is still not clear whether humans are more like dogs or rats [12].

One way to investigate pathophysiological mechanisms of importance for development of high blood pressure is to study prehypertensive individuals, i.e. normotensive relatives of patients with established hypertension, who run an increased risk of developing...
As a part of a primary preventive study a random third 
\( (n=9,996) \) of the male population aged 47–54 years in 
Gothenburg were screened for cardiovascular risk factors 
between 1970 and 1973 [16]. All subjects \( (n=686) \) with two 
consecutive blood-pressure recordings above 175 mmHg sys-
tolic or 115 mmHg diastolic or undergoing treatment for 
hypertension were followed at the Hypertension Unit, 
Sahlgrenska Hospital, Gothenburg, Sweden. After cases of 
secondary hypertension had been excluded it was established 
that 49 of the hypertensive men had parents (both) who in 
turn had been treated for hypertension or suffered a stroke 
before the age of 65. These 49 patients had 24 sons who 
lived near Gothenburg. Of those eligible 14 agreed to participate 
and they constituted the group with a positive family 
history of hypertension. In four cases their mothers were on 
antihypertensive medication.

Twenty-four non-hypertensive subjects with a negative 
family history of hypertension were recruited from fathers 
who at the same screening examination had a blood pressure 
below 130/90 mmHg and who had a negative family history of hypertension. All mothers were contacted on telephone to 
ensure that none was hypertensive according to recent med-
ical examination. Since the group with a positive family 
history of hypertension was moderately overweight the control 
group was divided into one lean control group and one 
control group matched for body mass index. Hence, the 
study groups of the present examination comprised 
three age-matched groups (mean age 41 ±2 years), one with 
a positive family history of hypertension \( (n=14, BMI 
27.6±0.8) \), one control group with a negative family history 
of hypertension with a normal body mass index \( (n=13, BMI 
22.9±0.4) \), and one control group with a body mass index 
that was matched with the group with a positive family 
history of hypertension \( (n=11, BMI 27.0±0.8) \). No dietary 
advice was given. All subjects were free of intercurrent illnesses.

Hyperinsulinaemic/euglycaemic clamp

A hyperinsulinaemic euglycaemic clamp was performed as 
described previously [17]. Insulin (Actrapid, Novo Nordisk, 
Copenhagen, Denmark) was dissolved in saline \( (0.5 \text{IU/ml}) \) 
and infused through an antecubital venous catheter by means 
of a digital infusion pump (IMED 922, San Diego, CA). 
Target plasma insulin levels of about 60 μU/l were achieved 
by a 10-min priming infusion followed by a constant infusion 
of 0.08 IU/kg per min during 150 min. A second catheter 
was placed in a contralateral hand vein. This arm was 
warmed by heating pads. Blood samples for determining 
plasma glucose concentrations at sight \( (Reflolux^\text{®}, 
Boehringer Mannheim, Germany) \) were drawn every 5 min 
and samples for later determination of blood glucose with 
the glucose oxidase method \( (Kabi, Stockholm) \) were drawn 
with 10-min intervals. Blood glucose levels were maintained 
at control levels by a variable infusion of 20% glucose. 
Concomitantly potassium was infused at a rate of 5 mmol/h. 
Plasma insulin levels were measured using a radioimmuno-
assay technique using Phadebas insulin kit \( (Pharmacia, 
Stockholm) \).

During the last 30 min of the clamp \( (i.e. after 120 \text{min}) \) an 
infusion of angiotensin II \( (Hypertension, Ciba-Geigy) \) at a 
rate of 0.1 ng/kg/min was superimposed.

Renal haemodynamics

Renal plasma flow and glomerular filtration rate were measured 
as the paraaminohippurate (PAH) and \( ^{51}\text{Cr} \) ethylened-
Insulin and sodium reabsorption

Iaminetetraacetic acid (Cr-EDTA) clearances respectively. The technique of continuous infusion and urine collection was used. The patients initially received a priming dose of Cr-EDTA (0.6 multiplied by body surface area equals megabecquerel) and PAH (0.04 multiplied by weight/ml 20% solution), followed by an intravenous infusion (both at a rate of 0.83 ml/min) to produce a plasma concentration of 500 counts/min per ml and 50–100 μmol/l respectively. The subjects were initially hydrated with tap water, 10 ml/kg body weight, to ensure diuresis. When urine flow was established the priming doses of Cr-EDTA and PAH were given. The equilibration period (45 min) started when the subject had emptied his bladder. The patients were supine throughout the study but were allowed to stand up to void for each urine collection. This procedure resulted in a complete bladder emptying according to ultrasound examination. Thereafter two 30-min baseline periods followed where the subject emptied his bladder at the end of each period. Between the periods they drank the same volume of urine passed in the preceding period. These two urine portions were pooled for the renal haemodynamic assessments. Plasma and urine were assayed for PAH and Cr-EDTA. Clearance was calculated as urine concentration multiplied with diuresis (ml/min).

When the two baseline periods had been completed the hyperinsulinaemic clamp started. When insulin is raised appropriately, fourfold higher than basal, hepatic glucose production is suppressed. The bladder was emptied after 60 min of hyperinsulinaemia but no clearance measurements were performed at that time. During the second hour of the clamp when steady-state had been reached, renal haemodynamics were assessed. During the last 30 min of the clamp (i.e. after 120 min) an infusion of angiotensin II (0.1 ng/kg/min) was superimposed. Bladder emptying was performed at the end of this period for the third renal haemodynamic assessment.

Renal tubular sodium reabsorption

At 2100 hours the day before each study day, 600 mg (16.2 mmol) of lithium (Li) carbonate was given orally. Blood samples were drawn from the left antecubital catheter at 1000 hours the next day and then every clearance period until 1300 for analysis of serum Na and Li. One-hour urine samples were collected from 1000 to 1300 and analysed for Na and Li. Mean serum values for each clearance period were used in calculations of renal clearance (in ml/min).

Based upon the assumption that Li is absorbed solely in the proximal tubules and to the same degree as Na and water, Li-clearance (C Li) equals the output of isotonic fluid from the proximal tubule [18]. Using the GFR, C Li, Na-clearance (C Na) and serum concentration of Na (SNa) the following values were calculated:

\[
PFR_{Na} = \frac{(1 - C_{Li}/GFR)}{\times 100%}
\]

\[
DFR_{Na} = \frac{(C_{Li} - C_{Na})/C_{Li}}{\times 100%}
\]

where PFR Na is proximal fractional reabsorption and DFR Na is distal fractional Na reabsorption.

The GFR, RPF, lithium clearance, and calculated tubular parameters were all standardized to a body surface area of 1.73 m².

Plasma and urinary concentrations of lithium were measured by atomic absorption and sodium and potassium concentrations by flame photometry.

Blood pressure

The blood pressure was measured by means of an automatically inflated and deflated rubber cuff, 12 x 35 cm. Korotkoff V was taken as diastolic pressure. Signals from a sound microphone placed over the brachial artery and cuff pressure were registered on a Mingograph 82 (Siemens-Elema, Stockholm). Heart rate was calculated from the blood pressure recordings.

Protocol

All subjects were asked to avoid heavy physical exercise on the day before the study. After an overnight fast they arrived at the laboratory at 0730. They were fitted with the two venous catheters and the experimental procedure started at around 0800. Throughout the investigation the patients remained in a comfortable semirecumbent position (except when voiding).

Statistics

Values are given as means ±SD. Statistical significances of differences within or between groups were calculated by two-way or one-way analyses of variance respectively, followed by post-hoc testing by Fischer to allow for multiple comparisons. P<0.05 was considered statistically significant.

Results

Blood pressure and plasma insulin

The group with a positive family history of hypertension had a significantly elevated systolic and diastolic blood pressure at baseline compared to the lean control group but not to the control group matched for BMI (Table 1). Hyperinsulinaemia caused a significant rise in systolic but not in diastolic blood pressure in the group with a positive family history of hypertension and the weight-matched control group. During the superimposed angiotensin II infusion a further rise in systolic and diastolic blood pressure was seen in the hypertension-prone subjects. In the weight-matched control group systolic blood pressure only rose, while no change occurred in the lean control group. No effect on heart rate was seen in any of the groups during hyperinsulinaemia and angiotensin II infusion. Plasma glucose and insulin concentrations at baseline, at steady state 90–120 min after start of clamp and during the angiotensin II infusion are shown in Table 2. There was no significant difference between the three groups at any time studied. Despite the potassium infusion during the clamp serum potassium fell (Table 2) but there was no difference between the groups in this respect.

Renal haemodynamics

During hyperinsulinaemia renal plasma flow (RPF) increased in the lean control group (P<0.05) while no
Table 1. Effect of euglycaemic hypennsulinaemia alone or in combination with an infusion of angiotensin II (0.1 ng/kg per min) on blood pressure and renal haemodynamics in subjects with a positive family history of hypertension (n = 14), and in subjects with a negative family history of hypertension with (n = 11) or without (n = 13) overweight

<table>
<thead>
<tr>
<th></th>
<th>Positive family history of hypertension</th>
<th>Negative family history of hypertension (overweight)</th>
<th>Negative family history of hypertension (normal weight)</th>
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<tbody>
<tr>
<td><strong>SBP (mmHg)</strong></td>
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<tr>
<td>Baseline</td>
<td>120 ± 11#</td>
<td>118 ± 7</td>
<td>109 ± 11</td>
</tr>
<tr>
<td>Hyperinsulinaemia</td>
<td>123 ± 11**</td>
<td>122 ± 10*</td>
<td>112 ± 11</td>
</tr>
<tr>
<td>Hyperins/Angll</td>
<td>133 ± 11***</td>
<td>126 ± 7*</td>
<td>115 ± 11</td>
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<tr>
<td><strong>DBP (mmHg)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>73 ± 11#</td>
<td>69 ± 7</td>
<td>66 ± 11</td>
</tr>
<tr>
<td>Hyperinsulinaemia</td>
<td>74 ± 11</td>
<td>67 ± 7</td>
<td>64 ± 14</td>
</tr>
<tr>
<td>Hyperins/Angll</td>
<td>79 ± 11*</td>
<td>70 ± 10</td>
<td>65 ± 11</td>
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<td><strong>HR (beats/min)</strong></td>
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<tr>
<td>Baseline</td>
<td>62 ± 7</td>
<td>60 ± 7</td>
<td>58 ± 7</td>
</tr>
<tr>
<td>Hyperinsulinaemia</td>
<td>62 ± 7</td>
<td>61 ± 2</td>
<td>60 ± 4</td>
</tr>
<tr>
<td>Hyperins/Angll</td>
<td>61 ± 7</td>
<td>60 ± 1</td>
<td>59 ± 7</td>
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<tr>
<td><strong>GFR (ml/min)</strong></td>
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<tr>
<td>Baseline</td>
<td>107 ± 17</td>
<td>106 ± 22</td>
<td>97 ± 11</td>
</tr>
<tr>
<td>Hyperinsulinaemia</td>
<td>105 ± 20</td>
<td>107 ± 22</td>
<td>102 ± 14</td>
</tr>
<tr>
<td>Hyperins/Angll</td>
<td>97 ± 13*</td>
<td>118 ± 18</td>
<td>115 ± 27*</td>
</tr>
<tr>
<td><strong>RPF (ml/min)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>607 ± 206</td>
<td>573 ± 137</td>
<td>605 ± 149</td>
</tr>
<tr>
<td>Hyperinsulinaemia</td>
<td>610 ± 194</td>
<td>669 ± 129*</td>
<td>689 ± 154</td>
</tr>
<tr>
<td>Hyperins/Angll</td>
<td>517 ± 122*</td>
<td>671 ± 117</td>
<td>671 ± 151</td>
</tr>
</tbody>
</table>

Values are means ±SD.

# indicates significant difference vs NFHN (between groups at baseline only).

*P < 0.05, **P < 0.01, ***P < 0.001 indicate significant difference vs baseline (in case of hypennsulinaemia) and vs hyperinsulinaemia (in case of hyperinsulinaemia/AngII).

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate.

Table 2. Plasma glucose and insulin concentrations and serum potassium during euglycaemic hyperinsulinaemia alone or in combination with an infusion of angiotensin II (0.1 ng/kg per min) in subjects with a positive family history of hypertension (n = 14) and in subjects with a negative family history of hypertension with (n = 11) or without (n = 13) overweight

<table>
<thead>
<tr>
<th></th>
<th>Positive family history of hypertension</th>
<th>Negative family history of hypertension (overweight)</th>
<th>Negative family history of hypertension (normal weight)</th>
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<tbody>
<tr>
<td><strong>Plasma glucose (mmol/l)</strong></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>4.7 ± 0.4</td>
<td>4.8 ± 0.2</td>
<td>4.7 ± 0.4</td>
</tr>
<tr>
<td>Hyperinsulinaemia</td>
<td>4.9 ± 0.7</td>
<td>4.9 ± 0.6</td>
<td>4.8 ± 0.7</td>
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<tr>
<td>Hyperins/Angll</td>
<td>4.8 ± 0.5</td>
<td>4.8 ± 0.6</td>
<td>5.0 ± 0.8</td>
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<tr>
<td><strong>Plasma insulin concentrations (mU/l)</strong></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>5.6 ± 3.2</td>
<td>7.4 ± 6.7</td>
<td>4.6 ± 2.6</td>
</tr>
<tr>
<td>Hyperinsulinaemia</td>
<td>65 ± 19</td>
<td>58 ± 13</td>
<td>57 ± 25</td>
</tr>
<tr>
<td>Hyperins/Angll</td>
<td>67 ± 22</td>
<td>58 ± 13</td>
<td>57 ± 25</td>
</tr>
<tr>
<td><strong>Serum potassium (mmol/l)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>4.3 ± 0.3</td>
<td>4.3 ± 0.3</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>Hyperinsulinaemia</td>
<td>3.8 ± 0.2**</td>
<td>3.8 ± 0.2**</td>
<td>3.8 ± 0.1***</td>
</tr>
<tr>
<td>Hyperins/Angll</td>
<td>3.8 ± 0.2**</td>
<td>3.8 ± 0.2**</td>
<td>3.8 ± 0.1***</td>
</tr>
</tbody>
</table>

Values are means ±SD.

**P < 0.01 indicates significant difference vs baseline.

change was seen in the group with a positive family history of hypertension. Glomerular filtration rate (GFR) was unaffected in all three groups.

Angiotensin II infusion caused a significant decrease in RPF and GFR (P < 0.05 for both) in the group with a positive family history of hypertension. RPF was unchanged in both control groups during angiotensin II infusion while GFR rose significantly in the lean control group.

Sodium excretion and renal tubular function

The 24-h urinary sodium excretion (mmol) on the day prior to examination was: positive family history of
Insulin and sodium reabsorption

hypertension 204 ±86, control group, normal weight, 175 ±83 and control group, weight-matched, 182 ±70 (NS).

Urinary sodium excretion values in the three groups are given in Table 3. There was a decrease in urinary sodium excretion during hyperinsulinaemia in the group with a positive family history of hypertension, the weight-matched control group and the lean control group by 50, 19 and 28% respectively. The response in the hypertension-prone subjects differed significantly from the two control groups (P < 0.05 for both). During angiotensin II infusion a further decrease in sodium excretion (P < 0.05) was demonstrated in subjects with a positive family history of hypertension but not in the control groups.

Estimated proximal fractional tubular sodium reabsorption was not altered in any of the groups during hyperinsulinaemia.

In subjects with a positive family history of hypertension a highly significant increase in estimated distal fractional tubular sodium reabsorption was found during hyperinsulinaemia. In sodium-replete human subjects, determination of lithium clearance and fractional excretion of lithium has been used to estimate proximal tubular sodium reabsorption [19]. In the present study estimation of distal tubular sodium reabsorption disclosed a clear increase in the hypertension-prone subgroup during hyperinsulinaemia. Also in the lean control group a significant increase in distal tubular sodium reabsorption concomitant with an increase in RPF was seen during hyperinsulinaemia.

Discussion

The results of the present study demonstrate that normotensive subjects with a strong positive family history of hypertension, during hyperinsulinaemia exhibit a marked reduction in urinary sodium excretion in presence of unaltered renal haemodynamics indicating a tubular action of the hormone. In sodium-replete human subjects, determination of lithium clearance and fractional excretion of lithium has been used to estimate proximal tubular sodium reabsorption [19]. In the present study estimation of distal tubular sodium reabsorption disclosed a clear increase in the hypertension-prone subgroup during hyperinsulinaemia. Also in the lean control group a significant increase in distal tubular sodium reabsorption concomitant with an increase in RPF was seen during hyperinsulinaemia.

Table 3. Effect of euglycaemic hyperinsulinaemia alone or in combination with an infusion of angiotensin II (0.1 ng/kg/per min) on urinary sodium excretion (U-Na), lithium clearance (Cl), urine flow (U-flow), proximal and distal, fractional sodium reabsorption (PFRNa, and DFRNa) in subjects with a positive family history of hypertension (n = 14) and in subjects with a negative family history of hypertension with (n = 11) or without (n = 13) overweight

<table>
<thead>
<tr>
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<th>Positive family history of hypertension</th>
<th>Negative family history of hypertension</th>
<th>Negative family history of hypertension</th>
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<tbody>
<tr>
<td></td>
<td>(overweight)</td>
<td>(normal weight)</td>
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<tr>
<td>U-Na (mmol/min)</td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>0.24 ±0.10</td>
<td>0.19 ±0.07</td>
<td>0.20 ±0.07</td>
</tr>
<tr>
<td>Hyperins/AngII</td>
<td>0.12 ±0.01**</td>
<td>0.16 ±0.01</td>
<td>0.15 ±0.06*</td>
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<td></td>
<td>0.08 ±0.03*</td>
<td>0.16 ±0.07</td>
<td>0.14 ±0.06*</td>
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<tr>
<td>U-flow (ml/min)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>13.6 ±3.2</td>
<td>13.3 ±3.0</td>
<td>12.9 ±1.1</td>
</tr>
<tr>
<td>Hyperinsulinaemia</td>
<td>12.2 ±3.1</td>
<td>10.5 ±4.2*</td>
<td>11.9 ±3.5</td>
</tr>
<tr>
<td></td>
<td>11.9 ±3.2</td>
<td>10.3 ±3.2</td>
<td>13.5 ±4.1</td>
</tr>
<tr>
<td>Clj (ml/min)</td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>32.4 ±14.6</td>
<td>27.5 ±5.2</td>
<td>30.9 ±6.1</td>
</tr>
<tr>
<td>Hyperinsulinaemia</td>
<td>33.0 ±11.3</td>
<td>28.8 ±5.2</td>
<td>32.5 ±6.7</td>
</tr>
<tr>
<td>Hyperins/AngII</td>
<td>28.3 ±7.4</td>
<td>32.7 ±6.4</td>
<td>35.6 ±10.5</td>
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<tr>
<td>PFRNa (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>70.2 ±10.0</td>
<td>73.6 ±4.1</td>
<td>67.9 ±6.9</td>
</tr>
<tr>
<td>Hyperinsulinaemia</td>
<td>68.9 ±5.9</td>
<td>72.3 ±6.2</td>
<td>67.0 ±11.9</td>
</tr>
<tr>
<td>Hyperins/AngII</td>
<td>70.2 ±6.6</td>
<td>72.0 ±4.7</td>
<td>67.8 ±13.3</td>
</tr>
<tr>
<td>DFRNa (%)</td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>95.2 ±2.3</td>
<td>95.8 ±0.9</td>
<td>95.6 ±1.4</td>
</tr>
<tr>
<td>Hyperinsulinaemia</td>
<td>97.8 ±1.0**</td>
<td>96.5 ±1.2</td>
<td>96.8 ±1.8*</td>
</tr>
<tr>
<td>Hyperins/AngII</td>
<td>98.2 ±0.6</td>
<td>97.1 ±1.1*</td>
<td>97.2 ±1.5*</td>
</tr>
</tbody>
</table>

Values are means ± SD.

* P < 0.05, ** P < 0.01 indicate significant difference vs baseline (in case of hyperinsulinaemia) and vs hyperinsulinaemia (in case of hyperinsulinaemia/Ang-II).
emia, while the insulin effect did not reach statistical significance in the weight-matched control group.

Lithium clearance, considered to be the best available method to assess proximal sodium handling in human subjects, however, has its limitations. Lithium reabsorption in the late distal and collecting tubules is negligible in sodium-replete human subjects, but lithium reabsorption in the loop of Henle has been estimated to 10% of total lithium reabsorption. An effect of hyperinsulinaemia on lithium reabsorption could not be excluded in the present study.

In addition, lithium carbonate may increase sodium clearance and renal plasma flow in normal males [20,21], but since similar plasma lithium levels were noted in our three groups it should not have influenced the differences noted.

Insulin increases volume reabsorption in the isolated perfused proximal tubule by stimulating Na+/H+ exchange in proximal tubule brush border membrane vesicles [22], but human studies have disagreed as to whether the antinatriuretic effect of insulin occurs at a distal or a proximal tubular site [7,8]. The absence of a change in lithium clearance during hyperinsulinaemia suggests that the antinatriuretic action of insulin occurred in a segment distal to the proximal tubule. As shown by Eadington et al. the natriuretic effect of insulin was not modified by inhibition of angiotensin converting enzyme, implying that insulin-dependent sodium retention is not mediated by angiotensin II generation [23].

Trevisan et al. demonstrated that subcutaneous insulin infusion resulting in circulating plasma insulin levels in normotensive control subjects comparable to those in IDDM patients, inhibited natriuresis and increased proximal tubule reabsorption at the level of the kidney and inhibited an adequate ANP stimulation caused by saline infusion [8]. The present data are at variance with those findings but in keeping with that of DeFronzo et al. [6], Friedberg et al. [24], Stenvinkel et al. [25], and Kageyama et al. [26] showing that insulin influences kidney sodium handling mainly at the level of the distal tubule. DeFronzo et al. used a micropuncture technique, while the others used the lithium clearance approach as in the present study.

In this context it could be of interest to mention that in a similar previous study disparate effects of insulin on the proximal sodium reabsorption in hypertensive and normotensive type 2 diabetics was demonstrated [27].

We could, in contrast to DeFronzo et al. [6], Friedberg et al. [24], and Stenvinkel et al. [25], not demonstrate any significant effect of hyperinsulinaemia on the proximal tubular sodium handling. The reason is not clear, but compared with at least two of the mentioned studies we used lower, more physiological plasma insulin levels.

Evidence that the antinatriuretic effect of insulin is secondary to hypokalaemia was reported by Friedberg et al. [24]. In that study a 16% decrease in plasma potassium concomitant with signs of sodium retention during insulin infusion was found in seven healthy control subjects. When potassium infusion to prevent the fall in plasma potassium was given insulin infusion had no sodium retaining effect. We also infused potassium during the hyperinsulinaemia but still serum potassium decreased 12% in the group with a positive family history of hypertension. A similar decrease was, however, noted in the two control groups speaking against a more pronounced hypokalaemia in that group as the underlying mechanism of sodium retention during hyperinsulinaemia.

A vasodilatory response to insulin has been demonstrated in the kidneys [25,28] as well as other vascular beds [29]. Whether this is a local vascular action of insulin or an indirect neurohumoral response is not known.

The blunted renal vasodilatory effect of insulin infusion in the group with a positive family history of hypertension found in the present study could be of interest in relation to several recent reports on a blunted vasodilatory effect of insulin in states characterized by insulin resistance [30–32]. It is true that the hypertension-prone subjects in the present study have a decreased insulin sensitivity compared with the lean control group, but it did not differ from that found in the weight-matched control group [15].

Acute or sustained elevations of plasma insulin levels may thus give rise to a blunted sodium excretion as previously reported in the subjects with a positive family history of hypertension [3]. This effect by insulin is a direct function of the concentration achieved and it may be a direct tubular effect of the hormone. The plasma insulin concentration after 120 min in our study was 10% higher (not significantly) in the subjects with a positive family history of hypertension, as compared with the two control groups which was similar, and this may have contributed to the differences noted. High concentrations of insulin also causes an activation of the adrenergic system, which is as a rule signalled by an increase in circulating noradrenaline concentrations and by small increases in heart rate and blood pressure. An activation of the adrenergic system will by itself enhance renal tubular sodium reabsorption by means of an effect on the innervated renal tubules. We could of course not exclude that at least a part of the increase in tubular sodium reabsorption was mediated through stimulation of renal sympathetic nerve activity. There was, however, no detectable increase in heart rate but a slight increase in blood pressure in the group with a positive family history of hypertension and the weight-matched control group during hyperinsulinaemia in the present study.

The superimposed low-dose angiotensin II infusion dose caused an increased diastolic blood pressure concomitant with a diminished RPF, GFR and urinary sodium excretion in the group with a positive family history of hypertension. The decreased GFR causing a diminished filtered load of sodium was presumably responsible for the further decrease in urinary sodium excretion seen during angiotensin II infusion, since no alteration in estimated tubular sodium reabsorption was noted in the hypertension-prone subgroup under
these circumstances. The influence by angiotensin II on GFR in the group with a positive family history of hypertension and in the lean control group may explain the tendency to changes in CLi (see Table 3).

This enhanced renal vascular response to the very low angiotensin II dose in the group with a positive family history of hypertension is remarkable, and it confirms previous observations of an enhanced angiotensin II sensitivity in hypertensive disease [14,33]. It also emphasizes the possible role of an increased sensitivity/action of the renin–angiotensin system as a pathophysiological mechanism in hypertension.

Eiskjær et al. gave a higher dose of angiotensin II (1.5 ng/kg/min) to normotensive healthy subjects and found a marked fall in renal plasma flow and GFR [34]. Using lithium clearance a rise in both estimated proximal and distal tubular sodium reabsorption was demonstrated.

In conclusion, we have demonstrated that in normotensive subjects with a strong positive family history of hypertension hyperinsulinaemia caused a marked decrease in urinary sodium excretion in presence of an unchanged RPF and GFR, indicating a direct renal tubular effect of insulin. Estimations of proximal and distal tubular sodium reabsorption by means of lithium clearance measurements suggested an effect of insulin at a distal site of the renal tubules. The subjects with a positive family history of hypertension also showed an enhanced sensitivity to a superimposed angiotensin infusion in the systemic circulation and in the renal vascular bed as compared with subjects without such history. During angiotensin II a further sodium retention was seen in the hereditarily predisposed subjects, supposedly due to a renal haemodynamic effect.

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