Chronic Intrarenal Insulin Replacement Reverses Diabetes Mellitus–Induced Natriuresis and Diuresis

M. Marlina Manhiani, A. Daniel Duggan, Hunter Wilson, Michael W. Brands

Abstract—We showed recently that sustained natriuresis in type 1 diabetic dogs was attributed to the decrease in insulin rather than the hyperglycemia alone. The sodium-retaining action of insulin appeared to require hyperglycemia, and it completely reversed the diabetic natriuresis and diuresis. This study tested whether the sodium-retaining effect was attributed to direct intrarenal actions of insulin. Alloxan-treated dogs (D; n = 7) were maintained normoglycemic using 24-h/d IV insulin replacement. After control measurements, IV insulin was decreased to begin a 6-day diabetic period. Blood glucose increased from 84 ± 6 mg/dL to an average of 428 mg/dL on days 5 and 6, sodium excretion increased from 74 ± 8 to 98 ± 7 meq/d over the 6 days, and urine volume increased from 1645 ± 83 to 2198 ± 170 mL/d. Dir dogs (n = 7) were subjected to the same diabetic regimen, but, in addition, insulin was infused continuously into the renal artery at 0.3 mU/kg per minute during the 6-day period. This did not affect plasma insulin. Blood glucose increased from 94 ± 10 mg/dL to an average of 380 mg/dL on days 5 and 6, but sodium excretion averaged 76 ± 5 and 69 ± 8 meq/d during control and diabetes mellitus, respectively. The diuresis also was prevented. Glomerular filtration rate increased only in Dir dogs, and there was no change in mean arterial pressure in either group. This intrarenal insulin infusion had no effect on sodium or volume excretion in normal dogs. Intrarenal insulin replacement in diabetic dogs caused a sustained increase in tubular reabsorption that completely reversed diabetic natriuresis. Insulin plus glucose may work to prevent salt wasting in uncontrolled type 2 diabetes mellitus. (Hypertension. 2012;59[part 2]:421-430.)

Key Words: blood pressure ■ sodium excretion ■ insulin ■ diabetes mellitus ■ lithium ■ glomerular filtration rate

The chronic renal and cardiovascular actions of insulin are an enigma. Hyperinsulinemia correlates strongly with hypertension in metabolic syndrome and diabetes mellitus, but there is no consensus on whether there is a cause and effect relationship. On one hand, the possibility that hyperinsulinemia could cause hypertension is supported by acute insulin infusion studies that show stimulation of sodium reabsorption by insulin in animals and humans. Chronic sugar feeding studies in rats report hypertension linked to hyperinsulinemia and renal insulin action. In addition, chronic insulin infusion causes renal sodium retention and increases blood pressure in rats. Thus, insulin-mediated renal sodium retention is hypothesized to be at least one component that explains hypertension in hyperinsulinemic conditions, such as metabolic syndrome or type 2 diabetes mellitus.

On the other hand, there is no direct experimental evidence outside rat models for chronic sodium-retaining or hypertensive actions of insulin. In fact, numerous chronic insulin infusion studies in dogs have refuted the insulin hypotheses by failing to show hypertension or direct sodium retention. Chronic insulin infusion into the renal artery of normal dogs also failed to cause sustained sodium retention or hypertension. When considered along with the absence of hypertension in patients with insulinoma, it is understandable that other mechanisms, such as oxidative stress and aldosterone, have been proposed to explain the link among insulin resistance, renal sodium handling, and hypertension in metabolic syndrome and diabetes mellitus.

Therefore, hypotheses that insulin can cause sustained renal sodium retention and hypertension lack chronic experimental support outside of rat models. However, the failure of hyperinsulinemia to cause sustained sodium retention or hypertension in dogs and insulinoma patients does not necessarily warrant ignoring the supportive data and abandoning a potential sodium-retaining effect of insulin. This is because there have been no chronic experiments testing the renal actions of insulin under conditions that actually represent the metabolic syndrome or diabetic milieu. In other words, the euglycemic hyperinsulinemic approach used to isolate the effect of insulin in most insulin infusion experiments, including previous dog studies, does not represent the insulin-glucose relationship either in type 1 diabetes mellitus (low insulin-high glucose) or metabolic syndrome/type 2 diabetes mellitus (high insulin-high glucose).

We addressed that recently by testing whether the natriuresis caused by induction of type 1 diabetes mellitus was attributable, at least in part, to the loss of insulin and its sodium-retaining action rather than being attributed solely to the

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osmotic diuretic effect of hyperglycemia. We reported that the sustained natriuresis in type 1 diabetic dogs (400 mg/dL for 6 days) was obliterated if the decrease in circulating insulin was prevented. That was the first evidence in a nonrodent model for a sustained sodium-retaining action of insulin. The results also suggested that insulin only stimulates renal tubular sodium reabsorption in the presence of hyperglycemia. However, all of the manipulations of insulin in that study were systemic, and there is evidence that central actions of insulin can increase sympathetic nervous system activity. Therefore, in the present study we infused insulin 24 h/d for 6 days into the renal artery of diabetic dogs to test the hypothesis that insulin, only in the presence of hyperglycemia, acts directly on the kidneys to stimulate sodium reabsorption chronically.

Methods

Studies were conducted in conditioned male mongrel dogs weighing ~25 kg, and all of the experimental protocols were approved by the institutional animal care and use committee at Georgia Health Sciences University. Dogs were instrumented as follows during a single, sterile surgical procedure under isoflurane anesthesia: via a left flank incision, a flow probe (3PSB; Transonic, Ithaca, NY) was affixed around the left renal artery, and a Tygon catheter was inserted in the left renal artery using the method of Herd and Barger. The right kidney was removed via a right flank incision. A Data Sciences (DSI, St Paul, MN) TA11PA-D70 blood pressure unit was implanted in the right femoral artery, and standard fluid-filled Tygon catheters were implanted in the right femoral vein and in the left femoral artery and vein. Drinking water was available ad libitum.

Tygon catheters were implanted in the right femoral vein and in the left femoral artery and vein. The catheters and probe cable were tunneled subcutaneously to the scapular region and exteriorized, and the dogs were fitted with a polypropylene jacket equipped with a pocket to protect and hold the catheters and flow probe cable.

After 1 week of recovery, dogs were placed in individual metabolic cages and connected to the infusion pumps and flowmeter. Briefly, dogs are fitted with a curved, padded piece of plexiglas under their jacket. It serves as a platform to connect the catheters and flow probe cable to the infusion lines and flowmeter cable. It also is the base for the flexible stainless steel conduit that connects (ie, tethers) the dog to the customized electric/hydraulic swivel mounted at the top center of the cage. The infusion lines and flowmeter cable run through this conduit. The swivel connects to the infusion pumps and flowmeter. This system allows continuous intravenous and intrarenal infusion and electric connectivity, and the dogs have completely unrestricted, 360° freedom of movement in the cages 24 h/d. Approximately 2 weeks were allowed for the dogs to acclimate to the metabolic cages and to be trained to lie quietly for blood sampling.

Salt and Water Balance and Glucose Control

Immediately after placement in the cages, 24-h/d infusion of 0.9% saline (~475 mL/d) was begun in all dogs. Combined with feeding a low-sodium diet (Hills H/D; three 13-oz cans per dog per day), this enabled maintenance of constant sodium intake throughout the study. In addition, all dogs received ~975 mL of sterile water vehicle per day IV and 48 mL of heparinized (1%) sterile water vehicle per day through the renal artery catheter throughout the study. Drinking water was available ad libitum.
During this 2-week acclimation period, dogs were divided randomly into 2 groups: type 1 diabetes mellitus (D; n = 7) and type 1 diabetes mellitus with chronic intrarenal insulin infusion (Dir; n = 7). Alloxan (50 mg/kg) was given to all of the dogs to decrease endogenous insulin. This allowed us to control plasma insulin chronically throughout the experiment. To avoid potential renal complications from concentrating alloxan, the IV saline infusion was increased to 1000 mL/d for 2 days preceding alloxan administration, and each dog was given mannitol (50 mg/kg) was given to all of the dogs to decrease endogenous insulin. This allowed us to control plasma insulin chronically throughout the experiment.

**Experimental Protocol**

After the acclimation period, control period measurements were resumed in both groups, and normal glucose levels were reached in all of the dogs in both groups. On the same days, glomerular filtration rate (GFR) was determined from the total plasma clearance of I\(^{125}\) labeled iohlatable (Glofil; QOL Medical, Kirkland, WA) over a 3-hour period from 8:00 AM to 11:00 AM, while the dogs rested quietly in their cages. Blood pressure and renal blood flow (RBF) measurement began at 2:00 PM every day and continued through to 8:00 AM the next morning. The blood pressure and flow signals were sampled for 10 seconds each minute at 100 Hz using the A.R.T. software from DSI.

Urine sodium, potassium, and lithium concentrations were determined by atomic absorption, plasma electrolytes were measured by ion-sensitive electrodes (MEDICA Easy Electrolytes, Bedford, MA), and plasma renin activity (PRA) was measured by radioimmunoassay (Diasorin, Stillwater, MN). Daily electrolyte and glucose was measured with an Accu-Check meter (Roche, Indianapolis, IN), urine glucose was measured using a glucose assay kit (Sigma-Al.

**Blood Sampling and Analytic Procedures**

Fasting blood samples (21 hours postprandial) were drawn during the control period, on diabetes mellitus days 2 and 5, and during the recovery period in all of the dogs in both groups. On the same days, glucose, potassium, and lithium concentrations were determined by atomic absorption, plasma electrolytes were measured by ion-sensitive electrodes (MEDICA Easy Electrolytes, Bedford, MA), and plasma renin activity (PRA) was measured by radioimmunoassay (Diasorin, Stillwater, MN). Daily electrolyte and glucose was measured with an Accu-Check meter (Roche, Indianapolis, IN), urine glucose was measured using a glucose assay kit (Sigma-Al.

**Table 1. Plasma Composition and Extracellular Fluid Volume in D and Dir Dogs**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Control</th>
<th>Day 2</th>
<th>Day 5</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(_{\text{Insulin}})</td>
<td>D</td>
<td>4.3±1.1</td>
<td>2.1±0.5*</td>
<td>2.7±0.6</td>
<td>5.0±1.0</td>
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<tr>
<td></td>
<td>Dir</td>
<td>4.1±0.5</td>
<td>2.8±0.2</td>
<td>2.6±0.3*</td>
<td>4.1±0.7</td>
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<tr>
<td>PRA</td>
<td>D</td>
<td>0.33±0.13</td>
<td>0.12±0.06</td>
<td>0.20±0.05</td>
<td>0.83±0.33</td>
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<td></td>
<td>Dir</td>
<td>0.39±0.10</td>
<td>0.91±0.30*</td>
<td>0.68±0.20*</td>
<td>0.38±0.10*</td>
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<tr>
<td>P(_{\text{Na}})</td>
<td>D</td>
<td>144.0±1.1</td>
<td>137.3±0.5*</td>
<td>135.2±0.6*</td>
<td>144.0±1.0</td>
</tr>
<tr>
<td></td>
<td>Dir</td>
<td>144.6±0.4</td>
<td>140.1±2.0*</td>
<td>139.1±1.0†</td>
<td>143.7±0.4</td>
</tr>
<tr>
<td>P(_{\text{K}})</td>
<td>D</td>
<td>4.6±0.1</td>
<td>5.5±0.4</td>
<td>4.9±0.1</td>
<td>4.5±0.1</td>
</tr>
<tr>
<td></td>
<td>Dir</td>
<td>4.7±0.4</td>
<td>5.1±1.8</td>
<td>4.7±0.8</td>
<td>4.5±0.1</td>
</tr>
<tr>
<td>P(_{\text{Osm}})</td>
<td>D</td>
<td>311±2</td>
<td>323±4*</td>
<td>325±6*</td>
<td>307±4</td>
</tr>
<tr>
<td></td>
<td>Dir</td>
<td>310±1</td>
<td>313±3†</td>
<td>315±2†</td>
<td>311±4</td>
</tr>
<tr>
<td>Hct</td>
<td>D</td>
<td>42±2</td>
<td>38±2*</td>
<td>36±2*</td>
<td>39±2</td>
</tr>
<tr>
<td></td>
<td>Dir</td>
<td>43±1</td>
<td>41±1</td>
<td>42±1</td>
<td>40±1</td>
</tr>
<tr>
<td>P(_{\text{Protein}})</td>
<td>D</td>
<td>7.4±0.3</td>
<td>8.0±0.2</td>
<td>7.9±0.3</td>
<td>7.4±0.3</td>
</tr>
<tr>
<td></td>
<td>Dir</td>
<td>7.2±0.2</td>
<td>7.2±0.2</td>
<td>7.2±0.2</td>
<td>6.9±0.1</td>
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<tr>
<td>ECFV</td>
<td>D</td>
<td>7839±233</td>
<td>7738±194</td>
<td>7107±286*</td>
<td>7537±349*</td>
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<tr>
<td></td>
<td>Dir</td>
<td>7333±300</td>
<td>7140±115</td>
<td>7223±278</td>
<td>6466±53*</td>
</tr>
</tbody>
</table>

P\(_{\text{Insulin}}\) indicates plasma insulin (µU/mL); PRA, plasma renin activity (ng angiotensin I/mL/h); P\(_{\text{Na}}\), plasma sodium (mEq/L); P\(_{\text{K}}\), plasma potassium (mEq/L); P\(_{\text{Osm}}\), plasma osmolality (milliosmol/kg); Hct, hematocrit (%); P\(_{\text{Protein}}\), plasma protein (g/dL); ECFV, extracellular fluid volume (mL); D, diabetic dogs; Dir, diabetic dogs with chronic intrarenal insulin infusion. Data are mean±SEM. D group n = 7 and Dir group n = 7.

*P<0.05 vs control.
†P<0.05 vs D group.
Results

Glucose and Insulin
Figure 1 shows blood glucose in D dogs (Figure 1, top) and Dir dogs (Figure 1, bottom). During the control period, plasma insulin averaged 4.3 ± 1.1 and 4.1 ± 0.5 μU/mL (Table 1), and blood glucose averaged 84 ± 6 and 94 ± 10 mg/dL (Figure 1) in the D and Dir dogs, respectively. During diabetes mellitus, the increase in blood glucose was significant and not different between groups, increasing similarly on day 1, tending to be lower in the Dir dogs on days 2 to 4, and averaging ∼400 mg/dL in both groups on days 5 and 6 (Figure 1). Systemic plasma insulin concentration decreased significantly in both groups to ∼2.5 μU/mL with no difference between groups (Table 1). All of the values returned toward control levels when Insulin Rx was resumed for the recovery period.

Urine Sodium and Volume Excretion
Sodium intake averaged 88 ± 1 and 85 ± 2 meq/d for the D and Dir dogs, respectively. Urine sodium excretion (UNaV) increased in both groups on day 1 of diabetes mellitus (Figure 2). This is similar to the day 1 natriuresis we measured previously in type 1 D dogs and in diabetic dogs with circulating insulin maintained at control levels. The natriuresis was sustained over the 6-day period in the D group, with some waning near the end, likely because of the decreasing blood pressure and withdrawal of pressure natriuresis. In the Dir dogs, however, UNaV decreased rapidly to levels not different from control (Figure 2) despite sustained hyperglycemia (Figure 1, bottom). Cumulative sodium balance averaged (∼)187 ± 82 and (∼)25 ± 16 meq in the D and Dir dogs, respectively, after 6 days of diabetes mellitus. The different recovery period UNaV response pattern between the 2 groups also was interesting. Although UNaV decreased rapidly in the D dogs at the start of the recovery period, there was an opposite response in the Dir dogs. These patterns are consistent with a sodium-retaining response during diabetes mellitus in the Dir dogs and sodium-losing response in the D dogs. The increase in fractional lithium reabsorption (Figure 3) during the diabetic period also supports a sodium-retaining response in the Dir dogs. Moreover, the significant attenuation of urinary glucose excretion in the Dir dogs, considered together with lithium reabsorption, is consistent with stimulation of proximal sodium-glucose cotransport.

Urine volume (Figure 4) tracked with UNaV, and the elimination of diabetic diuresis by intrarenal insulin is consistent with the elimination of diabetic natriuresis in the Dir dogs. Extracellular fluid volume also decreased significantly only in the D dogs (Table 1), and there was a significantly greater decrease in cumulative water balance in D versus Dir dogs (Table 2). The greater increase in urinary glucose
excretion (Table 2) in the D versus Dir dogs is consistent with their greater urine sodium and water losses.

Hemodynamics
Mean arterial pressure (MAP) did not change significantly in either group, but there were trends toward decreased MAP in the D dogs versus increased MAP in the Dir dogs (Figure 5). RBF was measured continuously, 19 h/d with a Transonic flow probe, and there was no change in RBF in either group (remaining essentially “flat” in both groups) throughout all of the periods. GFR also did not change in the D group, but it increased significantly in the Dir group during diabetes mellitus (Table 2). PRA also increased only in the Dir group (Table 1).

Intrarenal Insulin in Normal Dogs
Our intrarenal insulin dose of 0.3 mU/kg per minute is the same dose shown previously not to cause sustained sodium retention or hypertension during chronic intrarenal infusion in normal dogs.21 We confirmed that in a separate group of 4 dogs surgically instrumented, housed, and maintained the same as the diabetic dogs. The only difference was that they were not given alloxan and, thus, were normoglycemic. Figure 6 shows that 6 days of intrarenal insulin infusion had no significant, sustained effect on UNaV. There was no change in MAP, and Table 3 shows that there were no changes in plasma insulin or blood glucose, consistent with previous results at this dose.21

Intrarenal Insulin Plus Glucose in Normal Dogs
Our previous25 and current data show that insulin stimulates sodium reabsorption only in the presence of hyperglycemia. To determine whether adding glucose to the intrarenal insulin infusion could trigger a sodium-retaining action, we infused insulin (0.3 mU/kg per minute) plus glucose (17 mg/min or 24 g/d) intrarenally in 7 dogs surgically instrumented, housed, and maintained the same as the diabetic dogs. This dose of glucose was calculated to raise intrarenal blood glucose by ≈14 mg/dL, similar to the modest increase early in metabolic syndrome. Infusing glucose alone at this dose in 6 dogs had no effect on UNaV or any measured variable, similar to the effect of insulin alone. However, the combined infusion significantly decreased UNaV over the 6-day period, followed by rebound natriuresis during the recovery period (Figure 7). There was no change in MAP, and Table 3 shows that there were no changes in plasma insulin or blood glucose, but PRA increased significantly (Table 3).

Discussion
The primary finding from this study is that intrarenal insulin replacement in type 1 D dogs completely reversed the natriuresis and diuresis caused by the onset of hyperglycemia. The antinatriuretic action was sustained for the 6-day diabetic period. This replicates our previous finding25 that 6 days of hyperglycemia in the 400-mg/dL range did not cause sus-
tained natriuresis or diuresis if plasma insulin was not allowed to decrease from normal levels. However, all of the experimental manipulation of insulin and glucose in that study was intravenous, which raised the possibility of contributions from systemic mechanisms, such as the sympathetic nervous system.26,27 This study isolated the sustained sodium-retaining effect of insulin to the kidney.

Our previous study25 had a complicated experimental design. Our premise was that the euglycemic, hyperinsulinemic approach used to isolate the effect of insulin in most insulin infusion experiments, including previous dog studies,18–20 does not represent the insulin-glucose relationship either in type 1 diabetes mellitus (low insulin-high glucose) or metabolic syndrome/type 2 diabetes mellitus (high insulin-high glucose). Therefore, euglycemic hyperinsulinemia could not test the effect of changes in the endogenous insulin system. We addressed this problem with a chronic study in dogs that tested the renal effects of the decrease in insulin that causes type 1 diabetes mellitus.25 We used chronic, 24-h/d IV insulin and glucose infusion in alloxan-treated dogs to create a 6-day condition of normal plasma insulin and 400 mg/dL of hyperglycemia. The hyperglycemia was not different from the type 1 diabetic control dogs; the only difference was that plasma insulin did not decrease from baseline levels. The primary finding was that the sustained natriuresis and diuresis measured in the type 1 diabetic dogs was reversed completely in the dogs in which insulin was not allowed to decrease.

Three important mechanistic questions arose. First, how did insulin cause antinatriuresis if the dogs were not hyperinsulinemic? Second, is the antinatriuretic effect of insulin attributed to direct renal actions? Third, what are the renal mechanisms for the sodium-retaining effect? The first question is important because the intravenous insulin infusion in our previous study25 did not induce hyperinsulinemia but simply maintained baseline insulin action in alloxan-treated dogs. Thus, the sodium-retaining effect occurred only when the dogs became overtly diabetic: hyperglycemia in the presence of baseline insulin triggered a sodium-retaining action that was powerful enough to reverse completely the diabetic natriuresis and diuresis. This suggested that insulin plus glucose, rather than isolated hyperinsulinemia, has a sustained sodium-retaining action.

The present study confirmed that, because intrarenal insulin infusion had a sodium-retaining effect only in hyperglycemic dogs. Hall et al21 used a very similar experimental model to show that chronic intrarenal insulin infusion (0.3 mU/kg per minute) did not cause sustained sodium retention or hypertension in normal dogs. We confirmed that using the same intrarenal insulin dose in normal dogs (Figure 6). However, when that intrarenal insulin dose was administered in dogs with type 1 diabetes mellitus, the diabetes-induced natriuresis and diuresis were reversed. We further explored this relationship by testing whether the addition of glucose to the intrarenal insulin infusion in normal dogs would cause sustained sodium retention. Figure 7 shows that the combined intrarenal infusion of insulin and

![Figure 4](http://hyper.ahajournals.org/)
was no spillover into the systemic circulation either in normal kidney. The plasma insulin and glucose data show that there increases sodium excretion, these results addressed our second question regarding how insulin and glucose might act on the kidneys to cause antinatriuresis.

Renal salt wasting in uncontrolled diabetes mellitus, rather than stimulating sodium reabsorption. First, it is important to speculate that the 0.3 mU/kg per minute intrarenal dose that Based on those data and our own data in normal dogs, we expect that the mechanism indeed was through tubular sodium reabsorption, because intrarenal insulin infusion in the diabetic dogs prevented the natriuresis, diuresis, and increased sodium clearance in the face of increased GFR and decreased renal vascular resistance. The proximal tubule in particular is implicated, because the increases in GFR, fractional lithium reabsorption, and PRA are consistent with the hypothesis that glucose stimulates increased proximal tubule sodium transport and causes withdrawal of the tubuloglomerular feedback signal at the macula densa.29,30 The marked attenuation of urinary glucose excretion in the Dir dogs, consistent with our previous study,25 provides further support by suggesting increased activity of the proximal tubule sodium glucose cotransporter. A puzzling aspect of our results, however, is that this proximal sodium reabsorption mechanism has been proposed as an explanation for the increase in GFR in normal type 1 diabetes mellitus.29 Indeed, we reported increased GFR, RBF, and PRA in chronic intrarenal insulin infusion. Data are mean ± SEM. D group, n=7; Dir group, n=7. n=7 per group for UGlucoseV.

Table 2. Renal Clearance and Excretion Data in D and Dir Dogs

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Control</th>
<th>Day 2</th>
<th>Day 5</th>
<th>Recovery</th>
</tr>
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<tbody>
<tr>
<td>GFR</td>
<td>D</td>
<td>54.2 ± 2</td>
<td>53.2 ± 2</td>
<td>56 ± 1</td>
<td>58.2 ± 2</td>
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<tr>
<td></td>
<td>Dir</td>
<td>53.2 ± 2</td>
<td>60 ± 1†</td>
<td>63.3 ± 1†</td>
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</tr>
<tr>
<td>U_oSmV</td>
<td>D</td>
<td>631.1 ± 114</td>
<td>1452 ± 249*</td>
<td>1440 ± 251*</td>
<td>929 ± 279</td>
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<tr>
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<td>Dir</td>
<td>671.1 ± 40</td>
<td>1133 ± 162†</td>
<td>988 ± 167†</td>
<td>803 ± 103</td>
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<tr>
<td>U_KaV</td>
<td>D</td>
<td>47.6 ± 4</td>
<td>64 ± 8*</td>
<td>47 ± 5</td>
<td>45 ± 7</td>
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<tr>
<td></td>
<td>Dir</td>
<td>45.4 ± 4</td>
<td>61 ± 4*</td>
<td>37 ± 8†</td>
<td>48 ± 4</td>
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<tr>
<td>U_GlucoseV</td>
<td>D</td>
<td>ND</td>
<td>233.5 ± 3*</td>
<td>208.5 ± 7*</td>
<td>ND</td>
</tr>
<tr>
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<td>Dir</td>
<td>ND</td>
<td>15 ± 3*</td>
<td>11 ± 3†</td>
<td>ND</td>
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<tr>
<td>C_Osm</td>
<td>D</td>
<td>1.64 ± 0.10</td>
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<td></td>
<td>Dir</td>
<td>1.50 ± 0.09</td>
<td>2.51 ± 0.36*</td>
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<td>1.78 ± 0.31</td>
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<tr>
<td>C_HDO</td>
<td>D</td>
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<td>-2.00 ± 0.22*</td>
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<td>-475 ± 186†</td>
<td>-468 ± 197†</td>
<td>-616 ± 555†</td>
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</table>

GFR indicates glomerular filtration rate (mL/min); U_oSmV, osmolar excretion (milliosmol/d); U_oSmV, potassium excretion (mEq/d); U_GlucoseV, urine glucose excretion (g/d); C_oSm, osmolar clearance (mL/min); C_HDO, free water clearance (mL/min); C_Li, lithium clearance (mL/min); C_Na, sodium clearance (mL/min); Bal_H2O, cumulative water balance; D, diabetic dogs; Dir, diabetic dogs with chronic intrarenal insulin infusion. Data are mean ± SEM. D group, n=7; Dir group, n=7. n=7 per group for UGlucoseV. *P<0.05 vs D group. †P<0.05 vs D group.

glucose caused mild but significant antinatriuresis, although neither one affected sodium excretion when infused individually. Further studies will be needed to determine whether a longer infusion period and/or higher doses of glucose and/or insulin would cause greater sodium retention and possibly hypertension in normal dogs. Our previous25 and present results suggest that the antinatriuretic effect functions as a preventer of renal salt wasting in uncontrolled diabetes mellitus, rather than causing increased sodium balance.

In addition to confirming that insulin plus glucose decreases sodium excretion, these results addressed our second mechanistic question by isolating the effect of insulin to the kidney. The plasma insulin and glucose data show that there was no spillover into the systemic circulation either in normal or diabetic dogs. This is similar to findings of Hall et al.,21 who also showed that spillover did occur in normal dogs at a 2-fold higher intrarenal insulin dose of 0.6 mU/kg per minute. Based on those data and our own data in normal dogs, we speculate that the 0.3 mU/kg per minute intrarenal dose that we used in the diabetic dogs, in which plasma insulin was decreased 50%, restored rather than increased renal plasma insulin levels. We cannot rule out whether renal plasma insulin increased slightly above normal, but the absence of spillover nonetheless indicates that insulin acted directly on the kidneys to cause antinatriuresis.

There are several intriguing possibilities to address our third question regarding how insulin and glucose might act on the kidneys to stimulate sodium reabsorption. First, it is important to establish that the mechanism indeed was through tubular sodium reabsorption, because intrarenal insulin infusion in the diabetic dogs prevented the natriuresis, diuresis, and increased sodium clearance in the face of increased GFR and decreased renal vascular resistance. The proximal tubule in particular is implicated, because the increases in GFR, fractional lithium reabsorption, and PRA are consistent with the hypothesis that glucose stimulates increased proximal tubule sodium transport and causes withdrawal of the tubuloglomerular feedback signal at the macula densa.29,30 The marked attenuation of urinary glucose excretion in the Dir dogs, consistent with our previous study,25 provides further support by suggesting increased activity of the proximal tubule sodium glucose cotransporter. A puzzling aspect of our results, however, is that this proximal sodium reabsorption mechanism has been proposed as an explanation for the increase in GFR in normal type 1 diabetes mellitus.29 Indeed, we reported increased GFR, RBF, and PRA in chronically instrumented type I diabetic rats and suggested that combined effects of increased proximal tubule sodium reabsorption and renal volume loss could play a role in those responses.31 We expected similar renal changes in the D dogs, perhaps being amplified by adding intrarenal insulin in the Dir dogs. However, there was no increase in lithium reabsorption, PRA, or GFR in the normal diabetic dogs in this study or our previous study.25 Although we cannot explain the apparent lack of increased fractional proximal sodium reabsorption in the D dogs, the data suggest strongly that it is increased in the Dir dogs. The mechanism is not known, but serum and glucocorticoid-
inducible kinase 1 has been shown to regulate proximal tubular glucose transport and to be stimulated by glucose and insulin. Therefore, an intriguing question for future study is whether insulin and glucose can drive proximal tubule sodium transport in type 2 diabetes mellitus through a mechanism involving serum and glucocorticoid-inducible kinase 1.

The stimulation of the renin-angiotensin system by intrarenal insulin infusion in the Dir dogs suggests angiotensin II is another possible explanation for the antinatriuresis. Indeed, the increase in GFR combined with no change in RBF is tempting to ascribe to increased angiotensin II, such that the increase in filtration fraction would stimulate proximal tubular

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**Figure 5.** Mean arterial pressure in diabetic dogs (D; n=7; A) and diabetic dogs with intrarenal insulin infusion (Dir; n=7; B). C indicates control day; D, diabetes mellitus day; IR, intrarenal insulin diabetes mellitus day; R, recovery; Insulin Rx, 24-h/d IV insulin replacement infusion. Data are mean±SEM. *P<0.05 vs control period.

**Figure 6.** Urinary sodium excretion in normal dogs with intrarenal insulin infusion (NI; n=4). C indicates control day; I, intrarenal insulin day; R, recovery. Data are mean±SEM.
Table 3.  Plasma Insulin, Glucose, and Renin Activity in NG, NI, and NGI Dogs

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Control</th>
<th>Day 2</th>
<th>Day 5</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{\text{insulin}}$</td>
<td>NG</td>
<td>3.2 ± 0.6</td>
<td>3.1 ± 0.6</td>
<td>3.2 ± 0.3</td>
<td>4.7 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>NI</td>
<td>2.4 ± 0.5</td>
<td>3.4 ± 0.6</td>
<td>3.3 ± 0.7</td>
<td>3.7 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>NGI</td>
<td>4.8 ± 1.4</td>
<td>4.3 ± 0.3</td>
<td>4.0 ± 0.5</td>
<td>5.0 ± 0.6</td>
</tr>
<tr>
<td>$B_{\text{glucose}}$</td>
<td>NG</td>
<td>94 ± 1</td>
<td>94 ± 2</td>
<td>102 ± 6</td>
<td>94 ± 4</td>
</tr>
<tr>
<td></td>
<td>NI</td>
<td>104 ± 6</td>
<td>92 ± 5</td>
<td>103 ± 7</td>
<td>91 ± 9</td>
</tr>
<tr>
<td></td>
<td>NGI</td>
<td>93 ± 5</td>
<td>99 ± 3</td>
<td>102 ± 2</td>
<td>97 ± 3</td>
</tr>
<tr>
<td>PRA</td>
<td>NG</td>
<td>0.4 ± 0.2</td>
<td>0.3 ± 0.2</td>
<td>0.4 ± 0.2</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>NI</td>
<td>0.2 ± 0.1</td>
<td>0.3 ± 0.2</td>
<td>1.0 ± 0.9</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>NGI</td>
<td>0.1 ± 0.1</td>
<td>1.2 ± 0.2*</td>
<td>2.5 ± 1.7*</td>
<td>0.4 ± 0.2</td>
</tr>
</tbody>
</table>

$P_{\text{insulin}}$ indicates plasma insulin (µU/ml); $B_{\text{glucose}}$, blood glucose (mg/dL); PRA, plasma renin activity (ng angiotensin I/mL/h); NG, normal dogs with chronic intrarenal infusion of glucose; NI, insulin; NGI, glucose + insulin. Data are mean ± SEM.

* P<0.05 vs control.

Insulin regulates ENaC, and, interestingly, glucose can activate ENaC. However, an interactive effect with insulin is not known. It also is interesting that insulin and aldosterone activate ENaC independently via serum and glucocorticoid-inducible kinase 1. This has been ascribed to interaction with insulin via ENaC. Thus, the potential role of ENaC in mediating the sustained, antinatriuretic effect of insulin we discovered in dogs is particularly exciting, because it may relate to resistant hypertension and metabolic syndrome.

**Perspectives**

It is tempting to hypothesize that the antinatriuretic effect of insulin and glucose could cause hypertension if it occurred in the context of impaired kidney function, thus providing a potential link to the hypertension of metabolic syndrome and type 2 diabetes mellitus. However, it is critical to note that the effect we measured in the type 1 diabetic dogs in this study and our previous study was to reverse diabetic natriuresis and diuresis. Thus, there was not an increase in cumulative sodium balance in either case. Rather, insulin acted to prevent, or reverse, the decrease in sodium and water balance. Because of this, we hypothesize that protecting against glucose-induced renal sodium loss is a normal, physiological function of insulin that has not been recognized previously. Therefore, maintenance of sodium balance during the progression of metabolic syndrome and uncontrolled type 2 diabetes mellitus, in which plasma insulin and glucose both are elevated chronically, may be because of a cooperative effect of insulin and glucose in the renal tubule to counteract...
and prevent progressive sodium loss from the sustained hyperglycaemia. This hypothesis, the tubular mechanism for the effect, and the potential that this mechanism could raise blood pressure in certain conditions remain to be tested.

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References

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