Renal and Metabolic Effects of Insulin Lispro in Type 2 Diabetic Subjects With Overt Nephropathy

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OBJECTIVE — To assess whether the insulin analog lispro may antagonize the renal effects of IGF-1, a mediator of glomerular hyperfiltration involved in the progression of diabetic and nondiabetic chronic nephropathies.

RESEARCH DESIGN AND METHODS — In a randomized crossover study, we compared the renal and metabolic responses to regular or lispro insulin (0.1 units/kg body wt) administered after a euglycemic clamp and 5 and 30 min before a standard meal to 11 type 2 diabetic patients with macroalbuminuria.

RESULTS — Two- and four-hour postprandial changes (vs. preprandial euglycemia) in glomerular filtration rate (GFR) followed a significantly different trend (5.8 ± 5.0 vs. −6.3 ± 4.7, P < 0.05; and 11.0 ± 6.8 vs. 0.7 ± 5.1%, P < 0.05) after regular insulin and lispro injection, respectively. After lispro, postprandial GFR changes were negatively correlated (r = −0.48, P = 0.0001) with plasma insulin concentration. After regular insulin, renal plasma flow increased in parallel with a decrease in renal vascular resistances. Both changes were fully prevented by lispro. Postprandial blood glucose maximum concentration (278 ± 16 vs. 240 ± 16 mg/dl, P < 0.01) and area under the curve (79,381 ± 19,237 vs. 72,810 ± 16,211 mg/dl per min, P < 0.05) were significantly lower after insulin lispro than after regular insulin injection, respectively, despite comparable postprandial insulin profiles. Changes in total and gluconeogenic amino acids followed a similar trend. Changes in blood glucose and plasma amino acids did not correlate with concomitant changes in GFR.

CONCLUSIONS — In overt nephropathy of type 2 diabetes, lispro prevents glomerular hyperfiltration and offsets the renal effects of meal or meal-associated hyperglycemia by mechanisms possibly related to IGF-1 antagonism.


Landmark experimental studies in the 1970s (1) led to formulate the hypothesis that chronic nephropathies progress through a common mechanism initiated by focal nephron loss and sustained—even after the initial disease activity has abated—by adaptive responses of surviving units, such as glomerular hyperfiltration and hypertrophy. This initially helps the kidney to accommodate the load of nitrogenous wastes that accompanies protein metabolism but ultimately proves detrimental to the kidney. Over time, glomerulosclerosis and tubular atrophy reduce nephron mass and fuel a self-perpetuating cycle of nephron destruction that culminates in uremia (2). This process is amplified and accelerated in diabetes, where preglomerular vasodilation sustains glomerular hyperfiltration and hyperfiltration even before a substantial loss of nephrons occurs (3).

Several mediators, including growth hormone (4), glucagon (5), gluconeogenic amino acids (6), and ketone bodies (7), may sustain glomerular hyperfiltration associated with kidney failure. Some studies, however, suggest a central role for IGF-1 that, in addition to directly modulating renal hemodynamics (8), may also mediate the effects of some of the above factors, such as growth hormone (9). The hypothesis that IGF-1 increases renal hemodynamics is consistent with the finding that IGF-1 serum levels are elevated in individuals with chronic renal failure (10,11) or diabetes (12) and that these levels are positively correlated with the the glomerular filtration rate (GFR) (13). Moreover, IGF-1 infusion increases both GFR and renal plasma flow (RPF) in diabetic and nondiabetic animals and humans with or without nephropathy (14–18); the increase in GFR is sustained by an increase in both RPF and glomerular barrier hydraulic permeability, and that in RPF is sustained by a decrease in renal vascular resistances (RVR) (15). Experimental evidence is available to confirm that these effects are exerted through specific receptors—the expression of which increases in hyperfiltering animals (20)—on mesangial cell surface (19) and are fully blunted by IGF-1 antagonists (21,22). In particular, increased expression of IGF-1 and of the related factor IGF-2 or of their binding proteins and receptors has been involved in the pathogenesis of glomerular hyperfiltration and hypertrophy in experimental and human diabetes (20,22–25).

In this regard, it has been recently found that a recombinant insulin analog (insulin lispro), because of the transposition of proline and lysine at position 28...
and 29 in the COOH-terminal of the b α-chain, has an enhanced homology with the corresponding regions of IGF-1 (26,27). This results in a twofold increased IGF-1 receptor affinity relative to human insulin and in an increased competitive binding to IGF-1 receptors (26). Thus, lispro, in addition to acting as an hypoglycemic agent (28), might also act as a specific IGF-1 antagonist and therefore offset its renal effects, in particular in the setting of overt diabetic nephropathy, since both diabetes and reduced nephron mass converge to sustain glomerular hyperfiltration (29).

To test this hypothesis, we took advantage of the fact that lispro is now standard therapy for type 1 and insulin-treated type 2 diabetes with or without the presence of nephropathy (28). Because of its peculiar pharmacokinetic properties, lispro is routinely administered to diabetic patients immediately before meals (30,31). This is finalized to minimize postprandial hyperglycemia without inducing late hypoglycemia (32). Thus, we evaluated whether and to what extent the renal effects could be in the potential confounding effect of the acute increase in renal hemodynamics expected after the meal (33) and meal-induced hyperglycemia (34), the renal response to lispro was compared with that of an identical dose of human insulin administered to the same patients under the same experimental conditions.

RESEARCH DESIGN AND METHODS

This was a short-term study aimed to evaluate the acute renal response to a standard meal in 11 type 2 diabetic subjects (World Health Organization classification) with macroalbuminuria after a single subcutaneous injection of regular insulin or insulin lispro (0.1 unit/kg body wt.) on two separate occasions 2 weeks apart. We secondarily evaluated to what extent the renal effects could be influenced by the different bioavailability and the metabolic effects of the two drugs.

Patients

Patients on continued treatment with oral antidiabetic agents and/or insulin for at least 1 year, who had a serum creatinine <2.0 mg/dl and an albuminuria persistently ≥200 μg/min in two of the three urinary collections for at least 6 months, were asked to submit within 2 weeks three consecutive timed overnight urine collections for the centralized measurement of urinary albumin excretion rate (UAER). Those with UAER ≥200 μg/min in two of the three urinary samples entered the study. Patients with any evidence of nondiabetic renal disease; urinary tract infection; stroke; acute myocardial infarction or unstable angina in the last 6 months; severe liver or hematological disease; collagen vascular disease; cancer; treatment with cimetidine, steroid, or nonsteroidal anti-inflammatory agents over the last 2 months; or any condition that in the investigator’s judgment might prevent study completion or affect data interpretation were not included. Pregnancy or childbearing potential were also exclusion criteria. All patients provided written informed consent according to the declaration of Helsinki (1975 and 1983).

Study design

At the initial screening visit, data concerning clinical history, antidiabetic and antihypertensive therapy, and concomitant medications were recorded. Blood pressure was measured by standard procedures. An ultrasound evaluation was performed to evaluate kidney size and perfusion after bladder voiding. Patients with any evidence of renovascular disease, urinary tract obstruction, or incomplete bladder voiding were not selected for study participation. Blood and urine were sampled from eligible patients for measurement of fasting and postprandial blood glucose, fructosamine, and HbA1c; complete blood count; routine laboratory tests, including serum creatinine, total, LDL and HDL cholesterol, triglycerides, plasma electrolytes, venous pH, and creatinine clearance and also for the evaluation of urine sediment. Baseline evaluations were repeated every month for 3 months, and patients with changes in HbA1c and BMI <5% vs. baseline with no change in antidiabetic/antihypertensive therapy and diet were admitted at the Outpatient Division of the Clinical Research Center for Rare Diseases “Aldo e Cele Dacco” Villa Camozzi for the clearance studies.

Clearance studies

Patients were fasting and without antidiabetic therapy from the evening before. The morning of the clearance study, regular insulin was infused through an antecubital vein and infusion rate was adjusted according to blood glucose concentration in order to maintain stable blood glucose levels (range 80–120 mg/dl) throughout the clearance study (34). After 2-h blood glucose stabilization within the target range (80–120 mg/dl), intravenous regular insulin infusion was stopped and the clearance studies were started (Fig. 1). As previously described, GFR, RPF, and glomerular permselectivity were evaluated by measuring inulin, paraminohippuric acid (PAH), and albumin fractional clearances under a steady state of diuresis induced by oral water loading (34). A priming dose containing 25% inulin and 20% PAH was infused followed by continuous administration of insulin and PAH to maintain constant plasma concentrations of ~15 and 1.5 mg/dl, respectively. After an equilibration period of ~45 min, two urine collections of 1 h each were done under conditions of sustained euglycemia for basal evaluations. After completion of baseline clearance studies, patients randomly received a subcutaneous insulin injection of a standard dose of regular insulin or lispro (0.1 unit/kg body wt) into the abdominal wall of the periumbilical region (35). The site of injection was recorded in order to ensure that the two insulins were injected exactly in the same position. Then, 30 and 5 min after regular or lispro insulin administration, respectively, patients ate a standard meal made of pasta, turkey, bread, fresh tomatoes, an apple, and olive oil, containing 692 kcal (54.2% carbohydrates, 17.4% proteins, and 28.4% lipids) within 20 min (30). The broad aim was to achieve postprandial blood glucose concentrations >250 mg/dl after the subcutaneous injection of a standard dose (0.1 unit/kg body wt) of regular insulin (29). Patients voided at the beginning of the meal, and six consecutive 1-h urine collections were made for postprandial evaluations. Blood was sampled at the beginning and end of each postprandial urine collection. Urine and plasma samples were collected for the measurement of insulin, PAH, and albumin. Basal (euglycemic) GFR and RPF were defined as the mean of insulin and PAH clearances during the two 1-h pre-
Prandial clearance periods. Similarly, postprandial GFR and RPF were evaluated by averaging inulin and PAH clearances measured every two consecutive 1-h clearance periods (from 0 to 120 min, from 120 to 240 min, and from 240 to 360 min after meal, respectively). Fractional clearances of albumin were computed as \((U/P)_m/(U/P)_{IN}\), where \((U/P)_m\) and \((U/P)_{IN}\) are the urine to plasma concentration ratios of albumin and inulin, respectively. Again, basal and postprandial albumin fractional clearances were calculated by the mean of two consecutive 1-h clearances before and after the meal, respectively. Throughout the functional studies, the blood glucose was measured every 30 min. In a subgroup of four patients at the same time points, additional samples were taken for the measurement of total (taurin, aspartic acid, hydroxyproline, threonin, serine, asparagin, glutamic acid, glutamin, proline, glycine, alanin, citrulin, valine, cystin, methionin, isoleucine, leucine, tyrosin, phenylalanine, homocystein, isine, histidine, and arginine) and gluconeogenic (34) (serine, proline, glycine, cystin, methionin, and arginine) plasma amino acid concentration. Immediately before the meal, every 30 min up to 120 min, and every 60 min up to study end-plasma samples were collected and stored for the measurement of insulin and C-peptide plasma concentrations. Recumbent systolic and diastolic blood pressure were measured every hour, and mean arterial blood pressure (MAP) was calculated at each measurement by adding one-third of the pulse pressure to the diastolic blood pressure.

At completion of the clearance studies, patients were discharged and asked to continue their previous antidiabetic and antihypertensive therapy. No change was introduced in diet or pharmacological treatments. Two weeks later they attended the clinical research center for the second clearance study. Exactly the same procedure described for the first clearance study was adopted for the second one, with the exception of insulin treatment. Patients who previously received insulin lispro were given regular insulin. Again, regular and lispro insulin (0.1 unit/kg body wt) were given 30 or 5 min before the standard meal. At completion of the second clearance study, the patients were discharged.

**Measurements**

Plasma-free insulin concentrations were determined by radioimmunoassay after polyethylene glycol precipitation (36) and C-peptide concentration was measured by radioimmunoassay. Albumin concentration was measured on fresh plasma and urine samples by immunoturbidimetry. Amino acid plasma concentration was measured by a high-performance liquid chromatography fluorimetric method (37). Inulin and PAH concentrations in plasma and urine samples were determined using methods previously described (34). Other laboratory measurements were done by routine laboratory techniques. Blood glucose and plasma amino acid areas under the curve and mean GFR, RPF, filtration fraction (FF), RVR, and albumin fractional clearances were calculated using standard formulas. GFR and RPF were normalized for body surface area.

**Sample size**

The primary efficacy variable of the study was the percent change in postprandial GFR achieved by lispro versus regular insulin. Based on previous studies of type 2 diabetic subjects with a similar degree of renal dysfunction studied under sustained euglycemia (38), our patients were predicted to have a mean ± SD basal GFR under sustained euglycemia of 60 ± 10
Table 1—MAP and renal functional parameters measured during the euglycemic studies before regular or lispro insulin injection

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Regular</th>
<th>Lispro</th>
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<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>105.9 ± 3.0</td>
<td>101.7 ± 3.8</td>
</tr>
<tr>
<td>GFR (ml·min⁻¹·1.73 m²⁻¹)</td>
<td>57.4 ± 7.6</td>
<td>58.4 ± 7.2</td>
</tr>
<tr>
<td>RPF (ml·min⁻¹·1.73 m²⁻¹)</td>
<td>247.9 ± 37.6</td>
<td>283.6 ± 43.5</td>
</tr>
<tr>
<td>FF</td>
<td>0.247 ± 0.021</td>
<td>0.229 ± 0.025</td>
</tr>
<tr>
<td>RVR (mmHg·ml⁻¹·min⁻¹)</td>
<td>0.319 ± 0.051</td>
<td>0.284 ± 0.059</td>
</tr>
<tr>
<td>Albumin clearance</td>
<td>115.7 ± 29.9</td>
<td>112.9 ± 25.3</td>
</tr>
<tr>
<td>(×10⁻⁶)</td>
<td>6.75 ± 1.75</td>
<td>6.56 ± 1.70</td>
</tr>
</tbody>
</table>

Data are means ± SEM.

RESULTS — Eleven patients (seven men), aged 59.3 years (range 42–72), completed the study. Their diabetes had been diagnosed for 16 years (7–28). At study entry, the BMI was 31.4 kg/m² (26.1–41.9), HbA₁c was 8.4% (6.8–10.4), serum creatinine was 1.20 mg/dl, and albumin fractional clearance was 746 mg/min (352–535). Main clinical and laboratory parameters measured during the euglycemic studies before regular or lispro injection are shown in Table 1.

Clearance studies
MAP, GFR, RPF, FF, RVR, albumin clearance, and albumin fractional clearance measured during the euglycemic studies before regular or lispro injection were comparable (Table 1). During the postprandial period, MAP slightly increased to 106 ± 4 mmHg after lispro and marginally decreased to 105 ± 3 mmHg after regular insulin injection (P > 0.05 vs. preprandial values). Postprandial changes in renal hemodynamic parameters were shown in Fig. 2. Two- and four-hour postprandial GFR changes (vs. preprandial euglycemia) followed a significantly different trend (5.8 ± 5.0 vs. −6.3 ± 4.7, P < 0.05; and 11.0 ± 6.8 vs. 0.7 ± 5.1, P < 0.05) after regular insulin and lispro injection, respectively. After regular insulin injection, GFR and RPF progressively increased in parallel with a concomitant increase in RVR. FF did not change throughout the entire postprandial period (Fig. 2).

Metabolic studies
After the euglycemic state was achieved during the clamp, no patient required additional insulin infusion to maintain stable blood glucose levels throughout the euglycemic studies. Blood glucose concentrations achieved during the euglycemic studies were constantly within the target levels (Fig. 3). Basal free plasma insulin and total and gluteonecogenic plasma amino acid concentrations measured before regular or lispro injection were comparable as well (Table 2). The area under the postprandial blood glucose profile (area under the curve [AUC]) was significantly lower after lispro than after regular insulin injection (Fig. 3, Table 2). Average postprandial blood glucose levels also tended to be lower after insulin lispro than after regular insulin (Fig. 3, Table 2). Differences in blood glucose concentrations achieved the statistical significance from 60 to 180 min after the meal (Fig. 3). Time to maximum blood glucose concentrations was

ml·min⁻¹·1.73 m²⁻¹. A standard meal of 692 kcal (54.2% carbohydrate, 17.4% protein, and 28.4% lipid) given after a subcutaneous injection of 0.1 unit · kg⁻¹·body wt⁻¹ of regular insulin was predicted to result in a mean ± SD GFR increase versus basal of 20%. This effect was predicted to be fully blunted by the administration of an identical dose of insulin lispro. It was estimated that in order to give the study a 90% power to detect as statistically significant (P < 0.05) the difference in postprandial GFR changes versus baseline, 10 patients had to complete the study.

Statistical analyses
Continuous data were evaluated by means of paired t test. Correlation analyses were carried out using Pearson’s r correlation coefficient. Multivariate analysis was carried out using multiple linear regression models. All data were analyzed with SAS software (Version 8.0). Data are mean ± SEM or percent frequency, unless otherwise stated.
comparable in the two experimental settings (Table 2).

The postprandial total and gluconeogenic amino acid AUCs and average plasma concentration were significantly lower after lispro than after regular insulin injection (Fig. 4, Table 2). Basal insulin levels were comparable before administration of regular insulin or insulin lispro (Table 2). Postprandial insulin AUCs and average plasma insulin levels tended to be lower after insulin lispro than after regular insulin (Fig. 5, Table 2), but differences in plasma insulin concentrations did not achieve the statistical significance at any time point after the meal. Time to maximum plasma insulin concentration was significantly shorter after lispro than after regular insulin injection (Table 2).

On both study days, plasma C-peptide concentrations were comparable during the euglycemic and postprandial period (Fig. 5).

**Correlation and multivariate analyses**

After insulin lispro injection, postprandial GFR changes were significantly and negatively correlated with plasma-free insulin concentration (Fig. 6). No correlation was found between postprandial GFR and plasma-free insulin concentration after regular insulin injection. Changes in GFR did not correlate with absolute blood glucose levels (or with percent blood glucose changes versus basal) after either lispro or regular insulin injection. Differences in post-lispro versus post–regular insulin GFR changes also did not correlate with differences in corresponding blood glucose levels (or with changes in blood glucose levels versus basal). At linear regression analyses, differences in GFR changes during the first hour after insulin injection were significantly and negatively correlated with plasma-free insulin concentration.

**Table 2** — Preprandial (basal) mean concentration and postprandial mean, AUC, maximum (Cmax) concentration, and time to Cmax (Tmax) of blood glucose, plasma insulin, and total and gluconeogenic plasma amino acids during the metabolic and clearance studies with regular or lispro insulin

<table>
<thead>
<tr>
<th></th>
<th>Basal period</th>
<th>Mean of the whole period</th>
<th>Postprandial period</th>
<th>Median Tmax (IQ25–75, range)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(mU/ml)</td>
<td>(mU/ml)</td>
<td>(mU·ml⁻¹·min)</td>
<td>(mU/ml)</td>
</tr>
<tr>
<td>Plasmin insulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lispro</td>
<td>29 ± 6</td>
<td>43 ± 4</td>
<td>14,837 ± 2,383</td>
<td>60 (60–105)</td>
</tr>
<tr>
<td>regular</td>
<td>28 ± 6</td>
<td>49 ± 3</td>
<td>17,328 ± 2,031</td>
<td>150 (120–150)</td>
</tr>
<tr>
<td>P</td>
<td>0.916</td>
<td>0.1750</td>
<td>0.1371</td>
<td>0.002</td>
</tr>
<tr>
<td>Blood glucose</td>
<td></td>
<td></td>
<td>(mg/dl per min)</td>
<td></td>
</tr>
<tr>
<td>Lispro</td>
<td>95 ± 6</td>
<td>207 ± 14</td>
<td>72,810 ± 4,888</td>
<td>180 (150–240)</td>
</tr>
<tr>
<td>regular</td>
<td>100 ± 6</td>
<td>224 ± 17</td>
<td>79,381 ± 5,800</td>
<td>180 (150–225)</td>
</tr>
<tr>
<td>P</td>
<td>0.2103</td>
<td>0.0657</td>
<td>0.0473</td>
<td>0.3244</td>
</tr>
<tr>
<td>Total plasma amino acids</td>
<td></td>
<td></td>
<td>(mmol/l per min)</td>
<td></td>
</tr>
<tr>
<td>Lispro</td>
<td>2,490 ± 142</td>
<td>2,750 ± 134</td>
<td>985,084 ± 42,785</td>
<td>120 (60–195)</td>
</tr>
<tr>
<td>regular</td>
<td>2,433 ± 193</td>
<td>2,884 ± 127</td>
<td>1,032,578 ± 42,394</td>
<td>120 (120–165)</td>
</tr>
<tr>
<td>P</td>
<td>0.7933</td>
<td>0.0376</td>
<td>0.0504</td>
<td>0.6042</td>
</tr>
<tr>
<td>Gluconeogenic plasma amino acids</td>
<td></td>
<td></td>
<td>(mmol/l per min)</td>
<td></td>
</tr>
<tr>
<td>Lispro</td>
<td>532 ± 36</td>
<td>619 ± 41</td>
<td>221,295 ± 13,897</td>
<td>75 (60–113)</td>
</tr>
<tr>
<td>regular</td>
<td>533 ± 45</td>
<td>655 ± 39</td>
<td>233,415 ± 13,064</td>
<td>120 (113–120)</td>
</tr>
<tr>
<td>P</td>
<td>0.9712</td>
<td>0.0061</td>
<td>0.0107</td>
<td>0.7027</td>
</tr>
</tbody>
</table>

Data are means ± SEM.
and second clearance period were still statistically significant ($P < 0.05$), even after correction for concomitant differences in blood glucose concentration.

**CONCLUSIONS** — In this study, we have found that in type 2 diabetic subjects with overt nephropathy, a standard meal preceded by regular insulin injection induced a progressive increase in glomerular filtration and kidney perfusion that was fully blunted when the meal was preceded by the injection of an identical dose of insulin lispro. Actually, the administration of lispro was associated with a transient postprandial decrease in glomerular filtration and renal perfusion. Arterial blood pressure and renal vascular resistance decreased after regular and increased after lispro insulin injection. Albumin fractional clearance increased after regular insulin and, to a lesser extent, after insulin lispro.

Of note, after lispro injection, maximum postprandial GFR reductions occurred at maximum free plasma insulin concentrations, and postprandial changes in GFR and plasma insulin were negatively correlated, suggesting that lispro has a direct effect of limiting hyperfiltration in human diabetes. This effect is probably achieved through inhibition of arteriolar vasodilatation, as suggested by the prevention of meal-induced RVR decrease. These findings provide support to our working hypothesis that lispro may inhibit IGF-1–dependent renal vasodilation (26,27), possibly through antagonism for the IGF-1 receptors located on mesangial cell surface. This effect is specific for lispro, since previous studies found that human insulin may have opposite effects on renal hemodynamics, thereby resulting in a trend to increase GFR and RPF (39) and to synergize the vasodilator effects of blood glucose (40). Regardless of the involved mechanisms, in the present study, modulating renal hemodynamics most likely limited the increase in albuminuria sustained by postprandial hyperperfusion and hyperfiltration.

It is unlikely that these results were due to factors other than differences in the type of insulin used. A randomized design and 2-week washout period were used between the study protocols to prevent a carry-over effect. There were no differences in the main basal (premeal) clinical features, including arterial pressure, blood glucose, serum HbA1c and creatinine, and albuminuria in the two studies, nor were there changes in diet or antidiabetic and antihypertensive therapy, and no new concomitant therapy was introduced throughout the entire observation period. The two studies were carried out under strictly controlled experimental conditions to achieve euglycemia before the clearance studies. Moreover, in all patients, the intravenous infusion of regular insulin was withdrawn before the start of the euglycemic studies to ensure that there was no possibility of a carry-over effect of intravenously infused insulin that might affect the metabolic and renal response to the meal.

In theory, the different effect on renal hemodynamics could, at least in part, de-
Lispro in type 2 diabetes

However, because of its prompter absorption, postprandial lispro profile was virtually superimposable to that of regular insulin despite the different timing of subcutaneous injection (i.e., 5 and 30 min before the meal for lispro and regular insulin, respectively). As expected, equivalent doses of insulin lispro prevented the acute increase in blood glucose concentration induced by a standard meal more effectively than regular insulin and did not induce late hypoglycemia. However, findings that after lispro and regular insulin injection the opposite changes in GFR did not correlate with blood glucose levels and were not predicted by the differences in blood glucose profiles ruled out the possibility that improved postprandial metabolic control account for the renal hemodynamic response to lispro administration.

An original finding of the present study, with potential clinical implications, was that lispro, in addition to its capability to ameliorate postprandial hyperglycemia, also limited the postprandial increase in plasma amino acids and, in particular, the gluconeogenic ones that may directly modulate renal hemodynamics (4). Again, however, correlation and multivariate analyses failed to demonstrate any relationship between plasma amino acid levels and postprandial changes in GFR. A confounding effect of endogenous insulin production was also excluded by our data, since postprandial C-peptide levels were comparable after lispro or regular insulin administration. Finally, the finding that postprandial plasma profiles of lispro and human insulin were virtually identical renders it unlikely that the two insulins affected renal hemodynamics in different ways, through various effects on glucagon (5), growth hormone (4), or ketone body (7) production.

In conclusion, in type 2 diabetic subjects with nephropathy, a meal preceded by a dose of regular insulin allowed a moderate blood glucose increase and induced an acute kidney hyperperfusion and hyperfiltration that was paralleled by an increase in albumin excretion rate. Equivalent doses of lispro fully blunted postprandial hyperfiltration and limited the increase in blood glucose and plasma amino acid concentration and excess urinary albumin excretion. The mechanism(s) by which insulin lispro modulates renal hemodynamics is not known. Several lines of evidence consistently indicate a possible role for lispro-IGF-1 receptor binding, which prevents glomerular hyperfiltration and offsets the renal effects of meal or meal-associated hyperglycemia.

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Appendix

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References
18. Miller SB, Hansen VA, Hammerman MR:  
16. Guler HP, Eckardt KU, Zapf J, Bauer C,  
11. Miller SB, Rotwein P, Bortz JD, Bechtel PJ,  
13. Verrotti A, Cieri F, Petitti MT, Morgese G,  
6. Christiansen JS, Gammelgaard J, Frand-  
8. Hirschberg R, Kopple JD: Increase in re-  
9. Hammerman MR: The growth hormone-  
10. Enberg G, Hall K: Immunoreactive IGF-II in  
11. Miller SB, Rogers SA, Hammerman MR: Renal expression of IGF-I in hypersoma-  
18. Miller SB, Hansen VA, Hammerman MR: Effects of growth hormone and IGF-I on renal function in rats with normal and re- 
23. Flyvbjerg A, Landau D, Domene H, Her- 
24. Flyvbjerg A: Role of growth hormone, in- 
30. Pampaloni S, Torlone E, Lalli C, Del Sin- 
33. Woods LL: Mechanisms of renal hemody- 
34. Remuzzi A, Viberti G, Ruggenenti P, Bat- 
35. Heinemann L, Heise T, Wall L, Traut- 
36. Nakagawa SH, Nakayama T, Sasaki Y, Yochimok K, Shinozaki S, Akoi S, Mo- 
37. Olsen DC: Determination of aminocids in physiological fluids using liquid chromato- 
39. Baron AD: Hemodynamic actions of insu- 
40. Luksch A, Polak K, Matulla B, Dollinger S, Kapiotis S, Woltz M, Schmetterer L: Glu- 
41. Miller SB, Hansen VA, Parving HH: Kidney function and size in type I diabetic pa- 
43. Christiansen JS, Gammelgaard J, Parv.