DIETARY PROTEIN AND CIRCULATING amino acids have im-
portant effects on renal function. Both human and animal studies have shown that progression of diabetic kidney disease can be slowed by low-protein diets (10, 42, 62, 63). However, this lifestyle strategy, like many others, is difficult for patients to accomplish or maintain. Furthermore, the present popularity of high-protein diets for glycemic control and weight loss is cause for concern in people with diabetes (11, 52). In animal models and our previous studies of humans, those with diabetes had an augmented glomerular hyperfiltration response to a high-protein diet or amino acid infusion (57, 58, 62). In rats, glomerular hyperfiltration produced by diabetes and/or a high-protein diet is accompanied by glomerular hypertension, a major mecha-

nism of progressive renal injury (5, 22, 62). The search for specific hormonal or metabolic mediators of glomer-
ular hyperfiltration has been elusive. However, the consistent response to dietary protein or amino acids provides an opportunity to evaluate candidate mediators and potential treatment targets that could conceivably allow a more liberal intake of protein while providing renoprotection, as observed with low-protein diets.

In response to a protein meal or amino acid infusion, increased plasma glucagon levels have been reported to correlate with glomerular hyperfiltration (12, 16, 61). Infusion of glucagon can produce a rise in glomer-
ular filtration rate (GFR) and renal plasma flow (RPF), although supraphysiological doses have usually been administered (40, 41). In addition, a previous study suggested that people with diabetes may have an enhanced glomerular hyperfiltration response to gluca-
gon infusion (40). Vasodilatory prostaglandins may be required for the renal hemodynamic responses to glu-
cagon or amino acids, but this finding has been vari-
able (12, 21, 33, 46, 59). The main objectives of this study were to evaluate whether glucagon mediates the augmented glomerular hyperfiltration response to amino acids in diabetes and whether the responses to amino acids and glucagon depend on prostaglan-
dins. Patients with type 1 diabetes mellitus (n = 12) and normal control subjects (n = 12) were studied in a series of six experiments, each on different occasions. Baseline GFR and filtration fraction were higher in diabetes. In response to amino acid infusion, GFR increased more and filtration fraction was greater among those with diabetes. Their augmented GFR response to amino acids was not inhibited by octreotide or indomethacin. Participants with diabetes also had enhanced GFR and renal plasma flow responses to glucagon infusion, both of which were inhibited by indomethacin. Glomerular hyperfiltration responses induced by amino acids or glucagon occur by di-
vergent pathways in diabetes; only the response to glucagon is prostaglandin dependent.

prostaglandins; glomerular hyperfiltration; octreotide; indom-
ethacin

METHODS

Study Participants

Individuals with type 1 diabetes mellitus (n = 12) and normal control subjects (n = 12) were invited to participate in the study. The control group was matched to the diabetic group for age, weight, and gender. The group with diabetes received conventional insulin therapy and had hemoglobin A1C values ≥8% for the 3 mo before entering the study. None of the study subjects received medication, except for insulin, other than thyroid replacement in one participant with dia-
abetes. All of the participants had normal blood pressure, normal renal function, and urinary albumin excretion <40 µg/min. At the screening evaluation, vital signs and a 24-h urine collection for creatinine clearance and excretion of albumin and urea were obtained. In the subjects with dia-
abetes, hemoglobin A1C was measured at screening and on the occasions of the third and sixth studies.

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The study was reviewed and approved by the Institutional Review Board-Spokane. All subjects gave written informed consent to participate.

Experimental Procedures

A series of six experiments was performed, each on different days and after an overnight fast. Measurements were obtained during a 2-h baseline period, followed by a 3-h experimental period. Conventional insulin and p-aminohippurate (PAH) clearance techniques were used to measure GFR and estimate RPF, respectively. Priming doses of insulin (4 mg/kg) and PAH (40 mg/kg) were given as intravenous boluses, followed by continuous infusion at 20 and 10 mg/min, respectively. Insulin and PAH were diluted in one-half normal saline and infused at 5 ml/min. An oral water load (7 ml/kg) was given with the priming doses of insulin and PAH. Each voided urine specimen was quantitatively replaced to keep the urine flow rate ≥7 ml/min. After a 1-h equilibration period, timed urine specimens were collected every 30 min. Blood was drawn at the midpoint of urine collections for measurement of insulin, PAH, and glucose and every 60 min for insulin and glucagon. Blood pressure was measured every 30 min.

The following six experiments were performed (Table 1): amino acid infusion (study AA); AA with octreotide to inhibit glucagon secretion (study AA-O); AA after pretreatment with indomethacin to inhibit prostaglandin production (study AA-I); time control study of octreotide infusion with basal hormone replacement (study O); glucagon infusion to achieve physiological elevation of levels, along with octreotide to inhibit endogenous secretion (study O-G); and glucagon and octreotide infusion after pretreatment with indomethacin (study O-G-I). The amino acid solution used for infusion was 10% Travasol (Baxter, Deerfield, IL).

In the participants with diabetes, only regular insulin was started in the diabetic patients on arrival at the Clinical Research Unit. The insulin dose was titrated to achieve plasma glucose values of 5–6 mmol/l during the baseline period. In this study, the intent was to maintain a constant insulin level. Therefore, insulin was continued at the baseline rate during the experimental periods. The dose was 0.19 ± 0.01, with a range of 0.10–0.20 μU·kg⁻¹·min⁻¹. In control subjects, insulin was infused at 0.10·μU·kg⁻¹·min⁻¹ only during experimental periods employing octreotide infusion (studies AA-O, O, O-G, and O-G-I).

Analytic Methods

Plasma glucose was determined by the glucose oxidase technique (Beckman II glucose autoanalyzer, Beckman Instruments, Brea, CA). Serum insulin and plasma glucagon were measured by radioimmunoassays using commercially available kits (Diagnostic Products, Los Angeles, CA). Insulin and PAH in plasma and urine were analyzed by standard manual colorimetric assays. Because plasma glucose values generally remained within the range below the renal tubular threshold for glycosuria, urine was not alkalinized for the PAH measurement. Hemoglobin A1C was assessed by latex agglutination inhibition (DCA 2000, Ames, Elkhart, IN). Albuminuria was measured by radioimmunoassay (Diagnostic Products). Urinary urea was measured by the glutamate dehydrogenase method, and sodium was determined by an ion-specific electrode (Synchron CX5 Delta Chemistry Analyzer, Beckman Instruments). For study AA, urinary nitrate/nitrite were measured by the cadmium reduction method of Vodovotz (60).

Data Analysis

Calculations. Dietary protein intake was estimated from 24-h urinary excretion of nitrogen according to the following formula: protein intake (g/day) = (urinary urea nitrogen + non-urea nitrogen) × 6.25. Nonurea nitrogen was calculated to be 0.031g·kg⁻¹·day⁻¹, assuming nitrogen balance. Mean arterial blood pressure (MAP) was determined as MAP (mmHg) = diastolic blood pressure + 0.33 (systolic blood pressure – diastolic blood pressure).

Table 1. Test substances and doses administered during experimental periods

<table>
<thead>
<tr>
<th>Test Substances (Dose)</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Travasol-10% amino acid solution (0.043 ml·kg⁻¹·min⁻¹ iv)</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>Octreotide (0.8 μg/min iv)</td>
<td>+</td>
</tr>
<tr>
<td>Hormone replacement</td>
<td></td>
</tr>
<tr>
<td>Growth hormone (0.01 μg·kg⁻¹·min⁻¹ iv)</td>
<td>+</td>
</tr>
<tr>
<td>Insulin</td>
<td>+</td>
</tr>
<tr>
<td>Control (0.10 μU·kg⁻¹·min⁻¹ iv)</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>Diabetes mellitus (0.19 μU·kg⁻¹·min⁻¹ iv)</td>
<td>+</td>
</tr>
<tr>
<td>Glucagon (1 ng·kg⁻¹·min⁻¹ iv)</td>
<td>+</td>
</tr>
<tr>
<td>Glucagon</td>
<td></td>
</tr>
<tr>
<td>0 to +60 min (1 ng·kg⁻¹·min⁻¹ iv)</td>
<td>+</td>
</tr>
<tr>
<td>+60 to +120 min (3 ng·kg⁻¹·min⁻¹ iv)</td>
<td>+</td>
</tr>
<tr>
<td>+120 to +180 min (5 ng·kg⁻¹·min⁻¹ iv)</td>
<td>+</td>
</tr>
<tr>
<td>Indomethacin (150 mg po 2 h before experimental period)</td>
<td>+</td>
</tr>
</tbody>
</table>

AA, amino acid infusion; AA-O, infusion of amino acids and octreotide with basal hormone replacement; AA-I, AA after pretreatment with indomethacin; O, infusion of octreotide with basal hormone replacement; O-G, infusion of octreotide with glucagon escalation and basal doses of insulin and growth hormone; O-G-I, O-G after pretreatment with indomethacin. Composition of Travasol-10% amino acid solution (L-isomers; g/l) was the following: 11.5 arginine, 7.3 leucine, 6.0 isoleucine, 5.8 lysine, 5.8 valine, 5.6 phenylalanine, 4.8 histidine, 4.2 threonine, 4.0 methionine, 1.8 tryptophan, 20.7 alanine, 10.3 glycine, 6.8 proline, 5.0 serine, and 0.4 tyrosine. In diabetic subjects, insulin was titrated to achieve plasma glucose values of 5–6 mmol/l in the baseline period. The insulin infusion during the experimental period was fixed at the mean dose during the baseline period, 0.19 ± 0.01 (range 0.10–0.20) μU·kg⁻¹·min⁻¹.
Urine albumin, m experimental hours. as the mean of measurements made at the second and third urinary sodium, and urinary nitrate/nitrite were calculated Experimental values for MAP, glucose, insulin, glucagon, inulin and PAH clearance determined changes from baseline. as the mean of measurements performed during the baseline clearance. Baseline values of all parameters were calculated fraction (FF) was calculated as the ratio of inulin to PAH estimated RPF due to incomplete renal extraction. Filtration Hemoglobin A1c, % 9.4 Duration of diabetes, yr 8.0 Protein intake, g 6 Systolic blood pressure, mmHg 116 Weight, kg 70 Age, yr 29 Baseline measurements of renal hemodynamics and blood pressure of these hemodynamic parameters and included variables Linear regression was employed to develop predictive models Relationships between candidate predictors and GFR and RPF responses to amino acids and glucagon were assessed by Statistical tests. Renal hemodynamics and blood pressure were evaluated by two-way ANOVA, with contrasts between study groups and experiments and between study groups and changes from baseline. Relationships between candidate predictors and GFR and RPF responses to amino acids and glucagon were assessed by Pearson’s product-moment correlations. Stepwise multiple linear regression was employed to develop predictive models of these hemodynamic parameters and included variables with P < 0.10 in univariate analysis. Statistical tests were performed with SPSS, version 10 (SPSS, Chicago, IL).

RESULTS

Characteristics of Study Participants

The group with diabetes and the control group were similar in gender distribution, age, weight, and blood pressure (Table 2). Dietary protein intake and urinary albumin excretion tended to be higher in those with diabetes, but the differences were not statistically significant. In participants with diabetes, baseline hemoglobin A1C was 9.4 ± 1.3% at screening and remained stable throughout the study, 9.1 ± 1.6 and 9.4 ± 1.6% at the third and sixth studies, respectively.

Renal Hemodynamics and Blood Pressure

Throughout the series of experiments, baseline GFR, RPF, and MAP were not significantly different between control subjects and patients with diabetes (Table 3). However, baseline FF values were consistently higher in the group with diabetes (Table 3).

In the three studies including amino acid infusion (AA, AA-O, and AA-I), GFR and RPF increased above baseline in both groups. However, for those with diabetes, the increase in GFR was significantly greater than in controls during amino acid infusion (Fig. 1A, study AA, 26 ± 7 compared with 18 ± 8 ml·min⁻¹·1.73 m²⁻¹ (P = 0.021). The increase in RPF was the same for both groups, 127 ± 92 and 127 ± 72 ml·min⁻¹·1.73 m²⁻¹ (Fig. 1B, study AA). FF remained higher in those with diabetes vs. controls, 0.23 ± 0.04 and 0.19 ± 0.03 (P = 0.012), without a change from baseline (Fig. 1C, study AA). No significant change in MAP was observed (Fig. 1D, study AA). When octreotide was administered with amino acids, the GFR response was partly blocked in normal subjects but not in those with diabetes (Fig. 1A, study AA-O). Increments in RPF were reduced in both groups (Fig. 1B, study AA-O). FF was higher in the diabetic group than controls, 0.25 ± 0.03 and 0.21 ± 0.03 (P = 0.020), and the change from baseline was significantly greater than during amino acid infusion alone (Fig. 1C, study AA-O). MAP increased with-

Table 2. Characteristics of study participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control (n = 12)</th>
<th>Diabetes (n = 12)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (women/men)</td>
<td>6/6</td>
<td>6/6</td>
<td>0.605</td>
</tr>
<tr>
<td>Age, yr</td>
<td>29 ± 7</td>
<td>(21–42)</td>
<td>(18–44)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>70 ± 14</td>
<td>74 ± 10</td>
<td>0.449</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>116 ± 10</td>
<td>115 ± 12</td>
<td>0.737</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>73 ± 6</td>
<td>75 ± 8</td>
<td>0.536</td>
</tr>
<tr>
<td>Protein intake, g·kg⁻¹·day⁻¹</td>
<td>1.06 ± 0.13</td>
<td>1.14 ± 0.17</td>
<td>0.236</td>
</tr>
<tr>
<td>Urine albumin, µg/min</td>
<td>3.6 ± 2.6</td>
<td>9.0 ± 9.5</td>
<td>0.080</td>
</tr>
<tr>
<td>Duration of diabetes, yr</td>
<td>8.0 ± 3.5</td>
<td>(2–13)</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin A1c, %</td>
<td>9.4 ± 1.3</td>
<td>(8.1–12.4)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD and (range), except for gender (no. of women vs. no. of men).

For each 30-min collection period, clearances of inulin and PAH were calculated by standard formulas and corrected to body surface area of 1.73 m². PAH clearance represents estimated RPF due to incomplete renal extraction. Filtration fraction (FF) was calculated as the ratio of inulin to PAH clearance. Baseline values of all parameters were calculated as the mean of measurements performed during the baseline period. During the experimental period, maximal values for inulin and PAH clearance determined changes from baseline. Experimental values for MAP, glucose, insulin, glucagon, urinary sodium, and urinary nitrate/nitrite were calculated as the mean of measurements made at the second and third experimental hours.

Statistical tests. Data are expressed as means ± SD. Characteristics of study participants in the diabetic and control groups were compared by unpaired t-test. Renal hemodynamics and blood pressure were evaluated by two-way ANOVA, with contrasts between study groups and experiments and between study groups and changes from baseline. Relationships between candidate predictors and GFR and RPF responses to amino acids and glucagon were assessed by Pearson’s product-moment correlations. Stepwise multiple linear regression was employed to develop predictive models of these hemodynamic parameters and included variables with P < 0.10 in univariate analysis. Statistical tests were performed with SPSS, version 10 (SPSS, Chicago, IL).

Table 3. Baseline measurements of renal hemodynamics and blood pressure

<table>
<thead>
<tr>
<th>Study</th>
<th>AA</th>
<th>AA-O</th>
<th>AA-I</th>
<th>O</th>
<th>O-G</th>
<th>O-G-I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFR</td>
<td>117 ± 8</td>
<td>117 ± 8</td>
<td>114 ± 14</td>
<td>114 ± 16</td>
<td>117 ± 11</td>
<td>114 ± 10</td>
</tr>
<tr>
<td>RPF</td>
<td>578 ± 73</td>
<td>568 ± 79</td>
<td>548 ± 90</td>
<td>536 ± 63</td>
<td>564 ± 67</td>
<td>524 ± 67</td>
</tr>
<tr>
<td>FF</td>
<td>0.21 ± 0.03</td>
<td>0.21 ± 0.02</td>
<td>0.21 ± 0.03</td>
<td>0.21 ± 0.03</td>
<td>0.21 ± 0.03</td>
<td>0.22 ± 0.03</td>
</tr>
<tr>
<td>MAP</td>
<td>87 ± 9</td>
<td>84 ± 8</td>
<td>87 ± 10</td>
<td>86 ± 9</td>
<td>83 ± 9</td>
<td>85 ± 9</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFR</td>
<td>124 ± 16</td>
<td>120 ± 17</td>
<td>126 ± 17</td>
<td>124 ± 10</td>
<td>120 ± 14</td>
<td>123 ± 15</td>
</tr>
<tr>
<td>RPF</td>
<td>538 ± 86</td>
<td>516 ± 83</td>
<td>514 ± 90</td>
<td>558 ± 69</td>
<td>527 ± 97</td>
<td>491 ± 82</td>
</tr>
<tr>
<td>FF</td>
<td>0.23 ± 0.03*</td>
<td>0.24 ± 0.04*</td>
<td>0.25 ± 0.04*</td>
<td>0.23 ± 0.03*</td>
<td>0.23 ± 0.03*</td>
<td>0.26 ± 0.05*</td>
</tr>
<tr>
<td>MAP</td>
<td>89 ± 9</td>
<td>86 ± 7</td>
<td>89 ± 9</td>
<td>90 ± 8</td>
<td>87 ± 8</td>
<td>90 ± 9</td>
</tr>
</tbody>
</table>

Values are means ± SD. GFR, glomerular filtration rate; RPF, renal plasma flow; FF, filtration fraction; MAP, mean arterial pressure. *P = 0.040 (ANOVA) for difference in FF between subjects with diabetes and controls.

AJP-Renal Physiol • VOL 282 • JANUARY 2002 • www.ajprenal.org
out an interaction by diabetes status (Fig. 1D, study AA-O). Indomethacin did not significantly change GFR or RPF responses to amino acids in either group (Fig. 1, A and B, study AA-I). However, FF declined and MAP increased during the experimental period without interactions by diabetes status (Fig. 2C, study AA-I).

During the octreotide infusion with basal hormone replacement, GFR and FF increased slightly in both groups (Fig. 2, A and C, study O). Changes from baseline were not significant for RPF or MAP (Fig. 2, B and D, study O). In subjects with diabetes, infusion of glucagon with a physiological dose escalation increased GFR and RPF responses, which were 21 ± 12 (P = 0.001) and 77 ± 91 ml·min⁻¹·1.73 m²⁻¹ (P = 0.036), respectively (Fig. 2, A and B, study O-G). There was a trend for FF to increase in both groups (P = 0.060) (Fig. 2C, study O-G). A small increase in MAP without an interaction by diabetes status was observed (Fig. 2D, study O-G). In the group with diabetes, the effect of the glucagon infusion to increase GFR and RPF responses was inhibited by indomethacin (Fig. 2, A and B, study O-G-I), and FF increased due to a relatively larger decline in RPF response (Fig. 2C, study O-G-I). MAP did not change (Fig. 2D, study O-G-I).

Glucose and Hormones

Baseline glucose values ranged from 4.7 ± 0.3 to 6.2 ± 0.7 mmol/l in controls and from 5.4 ± 1.4 to 6.0 ± 1.0 mmol/l in diabetes. In the control group, baseline insulin levels were 37 ± 14 to 50 ± 14 pmol/l. For study AA, the baseline insulin level was 49 ± 14 pmol/l and increased to 92 ± 10 pmol/l during the experimental period in the control group. For the group with diabetes, baseline and experimental insulin levels were 128 ± 48 to 152 ± 69 and 113 ± 56 to 141 ± 62 pmol/l, respectively. Because insulin was not increased during experimental periods, glycemia could not be held constant. Experimental glucose values ranged from 6.2 ± 2.4 to 10.5 ± 3.5 mmol/l in the diabetic group. Glycemia also increased during experimental periods when octreotide was given to normal subjects. Plasma glucose values ranged from 6.6 ± 1.0 to 10.1 ± 1.4 mmol/l in these studies (AA-O, O, O-G, and O-G-I).

Baseline plasma glucagon levels were similar in participants with diabetes, 79 ± 29 to 87 ± 34 ng/l, and controls, 81 ± 38 to 88 ± 46 ng/l. In both groups, glucagon increased to the same level during amino acid infusion (study AA), 97 ± 39 and 97 ± 45 ng/l for
Fig. 2. Changes (means ± SD) in GFR (a), RPF (b), FF (c), and MAP (d) during experimental procedures utilizing glucagon and octreotide infusion. GFR increased above baseline in these studies (P < 0.05). RPF increased only among subjects with diabetes in the study using octreotide infusion with glucagon escalation and basal doses of insulin and growth hormone (O-G; P = 0.008). For FF, change from baseline was significant without an interaction by diabetes status in studies with octreotide with basal hormone replacement (O; P = 0.036) and O-G after pretreatment with indomethacin (O-G-I; P = 0.010). For MAP, change from baseline was significant (P = 0.045) for study O-G without an interaction by diabetes status. *P < 0.05, diabetes vs. control. †P < 0.05, O-G vs. O. ‡P < 0.05, O-G-I vs. O-G.
not exclude the possibility that glucagon could mediate glomerular hyperfiltration under other conditions.

In contrast to those with diabetes, the response to amino acids was partly dependent on glucagon in normal individuals. However, physiological elevation of circulating glucagon produced an enhanced GFR and RPF response only in the diabetic group. Their response was inhibited by indomethacin, indicating that prostaglandins mediate glucagon-induced renal hemodynamic changes in diabetes. As in our nondiabetic human subjects, animal studies have shown that physiological increases in circulating glucagon do not alter renal function in the absence of diabetes (44). Supraphysiological doses of glucagon must be given in the peripheral circulation to achieve portal vein concentrations that mimic pancreatic secretion after a meat meal or amino acid infusion (13, 43). Glucagon acts on the liver, whereby the second messenger cAMP is released into the circulation (1, 43). Both glucagon and cAMP are required to raise GFR and RPF (1). On the basis of our data, we cannot determine in the case of diabetes whether the increased renal responsiveness to glucagon is mediated via the kidney, liver, or elsewhere.

Indomethacin did not reduce the GFR response to amino acids in either group, but FF declined due to disproportionately larger RPF responses. Because this study did not include measurements of prostaglandins or their metabolites, we can only speculate about relevant mechanisms. Diabetic rats were recently reported to have increased cyclooxygenase-2 in the macula densa and glomeruli, and a specific inhibitor reduced FF and the thromboxane B2 metabolite while maintaining RPF (31). If thromboxane were also preferentially reduced by indomethacin during amino acid stimulation, this could explain the observed decrease in FF. Although glomerular hemodynamics cannot be directly measured in humans, the data are consistent with a reduction in glomerular pressure. However, changes in FF must be interpreted with caution because they do not necessarily indicate selective alterations in glomerular vascular resistance and may be influenced by changes in glomerular permeability and surface area (7).

Other candidate predictors of renal hemodynamic responses were also evaluated. Plasma glucose did not predict GFR or RPF responses to amino acids, although levels were normal or only modestly elevated at the time of study. By contrast, the GFR response to glucagon correlated negatively with baseline glucose in the diabetic group, implying that even mild hyperglycemia may impair acute responsiveness to glucagon. These data support other findings from the study, which indicate that renal hemodynamic changes induced by amino acids or glucagon occur through divergent pathways in diabetes.

Among those with diabetes, the plasma insulin level correlated negatively with GFR and RPF responses to amino acids. Such a relationship was not observed in controls. However, insulin levels were three- to fourfold higher in diabetic patients. A recent study of people with a family history of hypertension also found higher insulin levels than in controls and an inverse correlation between insulin and the GFR response to a mixed amino acid infusion (39). Higher insulin levels indicate the development of insulin resistance, which may lead to impaired endothelial-dependent vasodilation (51). To the contrary, in young healthy subjects, insulin was shown to at least partly mediate peripheral vasodilation induced by L-arginine infusion (18). Although insulin levels approximately doubled during infusion of mixed amino acids in our control subjects, insulin did not predict their GFR or RPF responses. However, the mixed amino acid infusion was designed to be physiological and did not increase insulin as much as after the pharmacological L-arginine infusion (18). It is possible that greater hyperinsulinemia may be required to produce vasodilation in normal individuals. Furthermore, control of the renal circulation may not directly follow peripheral vascular responses. In young healthy men, infusion of L-arginine has been
reported to produce a decrease in GFR and FF, with stable RPF (20, 29). A mathematical model indicated that this response may have been due to selective dilation of the efferent arteriole (29).

An independent, negative correlation was observed between level of blood pressure and the GFR response to amino acids (study AA) and GFR and RPF responses to glucagon (study O-G) in diabetes. Nevertheless, blood pressure increased during the experimental phase of some studies (AA-O, AA-I, and O-G), suggesting the possibility of systemic effects on renal hemodynamics. However, in these experiments, blood pressure increased in both groups and there were no interactions with diabetes status that would explain their disparate renal hemodynamic responses. Among individuals with a family history of hypertension, who had higher systolic blood pressure (even though normal by conventional criteria), a blunted GFR response to amino acids was also observed (39). In another study, people with a family history of severe hypertension had a diminished urine flow response to L-arginine infusion (20). Overall, the data demonstrate that a tendency to higher blood pressure reduces renal responsiveness, even though it may be within the conventional normal range.

Nitric oxide, the mediator of endothelium-dependent vasodilation, has been implicated in renal hemodynamic changes induced by diabetes or a high protein diet (3, 28, 30, 32, 35, 55, 56). In the present study, nitrate/nitrite excretion did not correlate with GFR or RPF responses to amino acids. Nitrate/nitrite excretion also did not differ between normal and diabetic subjects, or before and after amino acid infusion (data not shown). However, a role for nitric oxide cannot be excluded in the renal hemodynamic responses we have observed. Other indicators of nitric oxide production, such as cGMP or citrulline, could detect changes not apparent by measuring nitrate/nitrite. Excretion of nitrate/nitrite or cGMP may also be affected by alterations in proximal tubule sodium reabsorption (53). In addition, inhibition of nitric oxide synthesis could detect relevant differences. For example, in experimental diabetes, urinary nitrate/nitrite excretion was not increased, but enhanced reductions of GFR and RPF were observed after inhibition of neuronal nitric oxide synthase, indicating increased activity of this isoform (30, 32). Furthermore, when rats were rendered euglycemic, they had normalization of their enhanced response (30). Therefore, the aggregate data suggest a contribution of nitric oxide to the aberrant renal hemodynamics in diabetes.

A reciprocal relationship between nitric oxide and angiotensin II is believed to determine the renal hemodynamic response to amino acids in normal and disease states (14). Studies of both humans and animals with diabetes have yielded conflicting data. Renal hemodynamic responses to amino acid infusion or a protein meal vary across the spectrum from absent to augmented (4, 6, 9, 26, 38, 47, 57, 58). However, in diabetic humans or animals with impaired responses, treatment with either an angiotensin-converting enzyme inhibitor or an angiotensin receptor blocker restores responsiveness (9, 49, 50). These observations indicate that the response is not fixed and implicate intrarenal angiotensin II in blunting the response to amino acids. Although angiotensin II promotes proximal tubular sodium reabsorption, at higher levels it may do the opposite and activate the vasoconstrictor effect of tubuloglomerular feedback (14, 15). Diabetes has been characterized by increased renal renin-angiotensin system activity (2, 36). In the human studies where responses to amino acid infusion or a protein meal were impaired, patients often had a long duration of diabetes, had renal disease, or were older (4, 6, 38, 47). These are situations in which this system could be further enhanced. Experimental diabetes has also been associated with an increased renal vasoconstrictor response to angiotensin II (27). If tubular effects were also enhanced, this could contribute to impaired proximal solute reabsorption and activation of tubuloglomerular feedback in established diabetes. We have also shown that mesangial cells cultured in high concentrations of a mixed amino acid solution increase expression of the angiotensin II type 1 receptor (8). Expression of aminopeptidase A, a metallopeptase that degrades angiotensin II, is suppressed by amino acid treatment (8). Both effects are potential mechanisms by which responsiveness to angiotensin II could be increased.

The acute renal hemodynamic response to amino acids or a protein meal depends on baseline GFR. If subjects are studied while hyperfiltering, their response is generally impaired (26, 47). Measurements performed without fasting or control of dietary protein intake are likely to be affected. This issue is a particular concern, because diabetes is characterized by hyperphagia, and patients may try high-protein diets to control blood glucose or lose weight (11, 17, 52). In our previous studies, and in the present one, we have shown that people with either type 1 or type 2 diabetes and suboptimal glycemic control typically have a normal GFR when they are studied while fasting and dietary protein intake is normal (57, 58). However, they have an augmented glomerular hyperfiltration response to amino acids under these conditions. Therefore, our study design has separated effects of glycemia from protein intake. Chronic hyperglycemia exerts a permissive role to augment the glomerular hyperfiltration response to amino acids. In our view, this response indicates a greater sensitivity to amino acids for producing renal hemodynamic disturbances in diabetes. This view is supported by both animal and human data and may explain the greater benefit of a low-protein diet in diabetic nephropathy than in nondiabetic renal disease (19, 34, 42, 62). In addition, strict glycemic control can normalize the increased renal sensitivity to amino acids (57, 58). However, the success of such therapy is limited by difficulty with long-term compliance.

Our study did not identify a specific mediator of the response to amino acids or of glomerular hyperfiltration in diabetes. However, the enhanced sensitivity to
both amino acids and glucagon, which produce hemodynamic changes by divergent pathways, suggests that there is a redundancy of mechanisms that raise GFR. Alterations in common response pathways could explain a generalized increased sensitivity to vasoactive mediators. Increased activity of the protein kinase C-diacylglycerol-extracellular signal-regulated pathway, which can be activated by a number of mediators, is associated with glomerular hyperfiltration and increased FF in experimental diabetes (24). ATP-sensitive potassium channels have also been reported to be upregulated in kidneys of diabetic rats (23). These channels regulate voltage-gated calcium flux and vascular tone, and afferent arteriolar dilation was increased with a series of agonists in this model (23). In another report, a primary increase in renal tubular growth was proposed to enhance proximal solute reabsorption and decrease distal delivery, an effect that could potentiate GFR and RPF through suppression of tubuloglomerular feedback in early diabetes (54). These common response pathways are important areas for further study because they provide new opportunities to interrupt hemodynamic mechanisms of injury and perhaps other mechanisms as well.

The data from this study also raise concern about the potential therapeutic use of octreotide because GFR and FF increased consistently in the studies where it was used. The experimental data regarding diabetic nephropathy are varied. In diabetic rats, glomerular hyperfiltration may worsen or fail to improve with octreotide treatment (37, 45). Octreotide administration has also been associated with higher FF, greater proteinuria, and worsened histological lesions in diabetic rats (37). To the contrary, octreotide has been shown to decrease renal growth, and when combined with captopril, to reduce urinary albumin excretion as much as insulin treatment in established diabetes (17). In humans with diabetes, a small short-term (3-wk) study of octreotide treatment reported a decrease in glomerular hyperfiltration, but a longer term (9-mo) study showed no sustained benefit (25, 48).

In conclusion, people with diabetes had augmented glomerular hyperfiltration responses to both amino acids and glucagon. Only their glucagon response was prostaglandin dependent. The glucagon-prostaglandin pathway was not responsible for the augmented GFR response to amino acids in diabetes. However, our data do not exclude a role for glucagon and prostaglandins in producing glomerular hyperfiltration under other conditions, such as more severe hyperglycemia. In contrast to those with diabetes, the glomerular hyperfiltration response to amino acids in normal individuals was partly dependent on glucagon. Our data support an important role for amino acids in producing the characteristic renal hemodynamic disturbances of diabetes. Experimental models have demonstrated several underlying abnormalities that could potentiate responsiveness to various mediators (23, 24, 30–32, 54). These are key targets for further investigation and new therapies. In the meantime, close attention to established treatments, including avoidance of excess protein intake and protein restriction for those with diabetic nephropathy, is prudent.

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