Altering Dietary Protein Type and Quantity Reduces Urinary Albumin Excretion without Affecting Plasma Glucose Concentrations in BKS.cg-m+/Lepr<sup>db</sup>+/Lepr<sup>db</sup> (db/db) Mice<sup>1,2</sup>

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ABSTRACT  Protein restriction is used conventionally in the prevention and treatment of diabetic nephropathy. Recently, the use of soy protein instead of animal protein has been postulated as a new preventive and treatment option. The aim of this study was to determine the qualitative and quantitative effects of dietary protein on biomarkers of diabetic nephropathy in a Type 2 diabetes mellitus mouse model (BKS.cg-m+/Lepr<sup>db</sup>+/Lepr<sup>db</sup> mice). Diabetic (+Lepr<sup>db</sup>+/Lepr<sup>db</sup>) and control (m+/m+) mice (n = 24/group) consumed one of four different diets ad libitum [20% casein, 20% soy protein, 12% casein or 12% soy protein (energy-based percentages)] from 35 ± 4 d of age until termination (184–217 d of age). Blood and urine were collected throughout the study to measure biomarkers of diabetes and diabetic nephropathy. Kidney tissue was collected at the end of the study for weight.

In diabetic mice, a 20% casein diet increased urinary albumin excretion to macroalbuminuric levels, whereas a 20% soy protein diet led to no major changes in urinary albumin excretion. Low protein diets (12%), independently of protein type, decreased urinary albumin excretion to low microalbuminuric levels. There were no significant differences in plasma glucose concentrations. These findings show lower urinary albumin excretion when a soy protein diet or a low casein diet is fed, suggesting a delay in the progression of diabetic nephropathy.

KEY WORDS:  • soy protein • low protein diet • diabetic mice • urinary albumin excretion

More than six decades ago, Chanutin et al. (1) and Farr et al. (2) showed that in rats with induced chronic renal failure (CRF), a high protein diet led to increases in proteinuria, renal histological damage and mortality, whereas dietary protein restriction protected the kidney from further damage. Since then, protein restriction has been advocated by some as a preventive measure for kidney disease (3), but refuted by others (4). The lack of a consensus may be due to the fact that dietary protein restriction does not have as strong an effect on kidney disease prevention as other therapeutic measures, such as tight blood pressure control. Nevertheless, a beneficial effect is consistently seen with protein restriction if the patients are shown to follow the prescribed diets (3,5–8). This effect seems to be even stronger in patients with diabetic nephropathy (7,8). However, the disadvantage of protein restriction is that low protein diets are typically associated with poor compliance and the risk of malnutrition (3). Although most studies did not show a significant deleterious effect on nutritional status (3), close nutritional and compliance monitoring is essential for a successful outcome. This is particularly important for diabetic patients, who may require other dietary modifications as well (9).

Over the past two decades, the possibility of maintaining the quantity of protein intake while changing its type has been investigated. In particular, the work of Williams and Walls in the late 1980s (10–12) sparked considerable interest in studying the effects of soy protein. The authors showed that in the remnant kidney rat model, soy protein diets at 12 or 24% led to higher survival, lower urinary protein excretion, less renal hypertrophy and less histological damage than casein diets with equivalent quantities of protein. Although considerable attention has focused on the effects of soy protein in the remnant kidney rat model and in animal models of polycystic kidney disease (PKD) (13), studies in animal models of diabetic nephropathy are presently lacking. Thus, the objective of this study was to investigate the effects of protein type and quantity on diabetic nephropathy in male BKS.cg-m+/Lepr<sup>db</sup>+/Lepr<sup>db</sup> diabetic mice, which are commonly used as a model of type 2 diabetes mellitus (14) and diabetic nephropathy (15–20). We hypothesized that soy protein consumption, compared with casein, would result in lower urinary albumin excretion throughout the study, which would suggest protec-


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<sup>4</sup>Abbreviations used: 12C, 12% casein; 12S, 12% soy protein; 20C, 20% casein; 20S, 20% soy protein; BW, body weight; CON, control mice; CRF, chronic renal failure; dA, arrival day; DB, diabetic mice; dF, final day; FI, food intake; GFR, glomerular filtration rate; ISP, isolated soy protein; PGLUC, plasma glucose; PKD, polycystic kidney disease; SALB, serum albumin; SU, serum urea; TC, serum total cholesterol; UAE, urinary albumin excretion; UALB, urinary albumin; UCREAT, urinary creatinine; ΔUAE, change in urinary albumin excretion.

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tion from diabetic nephropathy. We expected the effect of protein type to be more pronounced at the higher protein intake.

**Materials and Methods**

**Animals.** Male BKS.Cg-m mice (n = 48; formerly designated as C57BLKS/+ Lepr<sup>dm</sup> ), comprising 24 diabetic (+ Lepr<sup>dm</sup> ) and 24 control (+/+ Lepr<sup>dm</sup> ), were obtained from Jackson Laboratory (Bar Harbor, ME) at ~4 wk of age (27 ± 3 d). On arrival, each diabetic mouse (DB) was matched to a control mouse (CON) on the basis of body weight. Mice were housed individually in sterile conditions in a humidity- and temperature-controlled (21–22°F) facility with a 12-h light:dark cycle for the duration of the study. Principles of laboratory animal care were followed and the study was approved by the University of Illinois Laboratory Animal Care Advisory Committee.

**Experimental design.** Mice were fed an AIN-330 (21) pelleted diet for acclimation from the arrival day (time point designated as dA) until age 35 ± 4 d. During this period, mice were maintained in metabolic cages to collect urine for glucose measurement. At age 35 ± 4 d (designated as d 0), mice were randomly assigned to one of four dietary treatments, casein or soy protein at 20 or 12% energy, and followed for 180 d or until terminal stage, whichever occurred first (185–215 ± 4 d of age). The duration of the study was decided on the basis of the average lifespan of the DB mice (~8 mo). The final time point was designated as the final day (dF) and was determined for each DB mouse if two of the following three findings were observed: body weight (BW) loss of >30%, persistent urinary ketone bodies or straightened hind-limb pose indicative of neuropathy. Each DB mouse and its paired control were killed at the same time. During the intervention period, the mice were maintained in plastic shoebox cages and placed in metabolic cages every 30 d (d 30, 60, 90, 120, 150 and dF) of intervention for 24-h urine collections. At d 0, 30, 60, 120 and dF of intervention, 50 µL of blood was collected from the saphenous vein for blood glucose measurements. BW was measured twice a week up to age 166 ± 4 d and daily thereafter.

**Diets.** During the dietary intervention period, mice were fed one of four semipurified, pelleted and isonitrogenous diets (Table 1). The diets were modifications of the AIN-93G (21) containing different types and amounts of protein. They were designated as 20C (20% total energy) or a moderately reduced protein intake (12% total energy). These amounts were chosen to be easily achievable in rodent growth (21).

**Diet analysis.** Treatment diets were analyzed in duplicate for dry and organic matter (22), crude protein and amino acids. Crude protein was determined using the Kjeldahl procedure (22,23). Amino acid composition was determined by ion-exchange chromatography, and sample preparation was performed according to Spitz (24). Total and bioavailable phosphorus was calculated for the four diets. The ISP was supplemented with ~300 mg bioavailable phosphorus (calcium phosphate)/100 g ISP, making ~50% of the total phosphorus bioavailable. All of the phosphorus in casein was bioavailable.

**Blood analyses.** At d 0, 30, 60, 120 and dF of intervention, 50 µL of blood was collected from the saphenous vein, and blood glucose was measured in duplicate using a glucose meter (ONE TOUCH Basic, LifeScan, Milpitas, CA). All blood collections were performed in the afternoon from fed rats, except for dF, which was performed in the afternoon after a 7-h period of food deprivation. Blood glucose concentrations were converted to plasma glucose (PGLUC) concentrations according to Wettigasser et al. (25). Because the ONE TOUCH Basic glucose meter measured concentrations only up to 33.3 mmol/L, any blood with glucose concentrations above the maximum was diluted 1:2 with saline and reanalyzed in duplicate. The dilution method was verified in a preliminary study and the dilution was found not to have any considerable effect on the glucose measurements (CV <5%).

At the final time point (dF), mice were food deprived for 7 h, weighed and anesthetized by intraperitoneal injection of pentobarbital (40–90 mg/kg body) followed by cardiac puncture for blood collection. Blood was collected into evacuated tubes without preservatives, allowed to clot for ~60 min and centrifuged at 550 × g for 20 min at 4°C to obtain serum. Aliquots of serum samples were placed into storage tubes and stored at ~70°C until analysis. Concentrations of serum urea (SU), total cholesterol (TC) and albumin (SALB) were analyzed enzymatically in a Hitachi 911 system (Hitachi, Indianapolis, IN) at the Pathology Laboratory, Department of Veterinary Medicine, University of Illinois at Urbana-Champaign. Interassay CV were <5% for SU, <5% for TC and <3.5% for SALB.

**Urine analyses.** Urine collections (24-h) were performed daily from dA to d 0 and at d 30, 60, 90, 120, 150 and dF of intervention. Urine was collected in polypropylene vials containing 2 mL of mineral oil to reduce evaporation. Samples were refrigerated promptly after collection. Urine was centrifuged at 413 × g for 5 min at 4°C to remove the mineral oil or any solid debris, and stored at ~70°C until analysis. Urine from dA to d 0 was used to measure urinary glucose concentrations. Urine collected at d 0, 30, 60, 90, 120, 150 and dF was analyzed for creatinine (UCREAT) and albumin (UALB) concentrations, and the presence of ketone bodies. UCREAT was analyzed in a preliminary study and the dilution was found not to have any considerable effect on the glucose measurements (CV <5%).

**Table 1. Diet Composition**

<table>
<thead>
<tr>
<th>Diets</th>
<th>12C</th>
<th>20C</th>
<th>12S</th>
<th>20S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (% energy)</td>
<td>12</td>
<td>20</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>Casein</td>
<td>125</td>
<td>210</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Soy protein&lt;sup&gt;3&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>130</td>
<td>219</td>
</tr>
<tr>
<td>Carbohydrates (% energy)</td>
<td>71</td>
<td>63</td>
<td>71</td>
<td>63</td>
</tr>
<tr>
<td>Cornstarch</td>
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<td>143</td>
<td>145</td>
<td>143</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Sucrose from MX &amp; VX</td>
<td>17</td>
<td>17</td>
<td>34</td>
<td>34</td>
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<tr>
<td>Soybean oil</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Sulfur amino acids supplements, g/kg</td>
<td>0.89</td>
<td>0.89</td>
<td>0.89</td>
<td>0.89</td>
</tr>
</tbody>
</table>

<sup>1</sup> Composition as provided by Dyets, Inc. (Bethlehem, PA) Composition in g per kg diet unless otherwise indicated.

<sup>2</sup> 12C, 12% casein; 20C, 20% casein; 12S, 12% soy protein; 20S, 20% soy protein.

<sup>3</sup> Isolated soy protein aglycone composition as provided by Protein Technologies International; genistein = 1.31 mg/g protein; daidzein = 1.00 mg/g protein; glycitein = 0.24 mg/g protein; total aglycone = 2.55 mg/g protein.
lyzed enzymatically in a Hitachi 911 system as described above. The interassay CV was <1.6%. Urinary albumin concentration was determined using an ELISA (Albuwell M, Exocell, Philadelphia, PA), with a detection limit of 0.04 mg/L (calculated as 2 SD above blank) (15). The interassay CV was <7%. Urinary albumin excretion (UAE, in mg albumin/mg creatinine) was determined as the ratio between UAE and UCREAT. The change in UAE from d 0 (UAUE) was also calculated for each time point. The presence of urinary ketone bodies was detected with urine test strips (Chemstrip uGK, Roche Diagnostics, Indianapolis IN).

**Tissues analyses.** After cardiac puncture, the mice were killed by cervical dislocation, and kidney tissue was rapidly excised and weighed.

**Statistical analysis.** Data are presented as means ± SEM. For outcomes measured at multiple time points of the study (BW, FI, PGLUC, and ΔUAUE), they were first analyzed by repeated-measures ANOVA (split-plot approach) with time as a within-subject factor. Between-subject factors included protein type (casein or soy) and quantity (20 or 12%) as main factors and diabetes (DB or CON) as a covariate. Subjects were effects-coded. For ΔUAUE, the UAE concentrations at d 0 (referred to as baseline hereafter) were used as an additional covariate. If significant interactions between time and the main factors were detected, a separate analysis was performed for each time point. Otherwise, only dA (for BW) and d 0 were analyzed. At each time point, multiple linear regression for multifactorial experiments (26) was used to analyze the effects of the between-subject factors above, which were protein type, protein quantity, diabetes and baseline UAE (for ΔUAUE only). Effects coding was used to code for the main factors and the covariate diabetes (26). The covariate “baseline UAE concentrations” was coded as the difference between baseline UAE for each mouse and the mean baseline for all mice (26). Two- and three-way interactions among the main factors and the covariates were analyzed for all outcome variables. If the interactions were not significant, they were removed as a block and the statistical analysis was rerun with the reduced model. Significance for individual main factors was examined only if the multiple R² for the model was significant (P < 0.05). All statistical analyses were conducted with an a level of 0.05, using SAS (version 8.01; SAS Institute, Cary, NC). One-tailed P-values were used to evaluate treatment effects with directional hypotheses (ΔUAUE and kidney weight), whereas two-tailed P-values were used for nondirectional hypotheses and all interactions.

**RESULTS**

**Diets.** Crude protein on a dry-matter basis was 20.3 g/100 g (21.7%), 19.0 g/100 g (20.3%), 12.2 g/100 g (13.0%) and 12.0 g/100 g (12.8%) for 20C, 20S, 12C and 12S, respectively. All diets had the same amount of total phosphorus, whereas the amount of bioavailable phosphorus was different due to the phytic acid content of soy protein (Table 2).

**Body weight.** The effect of diabetes and diet on BW varied with time (time × diabetes, P < 0.0001; time × protein type, P = 0.0215; Fig 1). DB mice were ~40% heavier than CON mice (P < 0.0001) upon arrival (dA). Most DB mice continued to be heavier than CON mice at later time points, but the difference in BW became smaller toward the end of the study, due to considerable weight loss of the DB mice.

For DB mice, differences in BW among the dietary groups began to emerge at d 30 (protein type, P = 0.0470). These differences resulted from the lower BW of the 12S group. The lower BW of this group became more evident by d 60 (protein type, P = 0.0001; protein quantity, P = 0.0221; protein type × protein quantity, P = 0.0216).

**TABLE 2**

<table>
<thead>
<tr>
<th>Dietary total and bioavailable phosphorus</th>
<th>Diets¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20C</td>
</tr>
<tr>
<td>g/kg</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3.125</td>
</tr>
<tr>
<td>Bioavailable</td>
<td>3.125</td>
</tr>
</tbody>
</table>

¹ 20C, 20% casein; 20S, 20% soy protein; 12C, 12% casein; 12S, 12% soy protein. Bioavailable phosphorus in soy protein calculated based on usual 70% bioavailable phosphorus in isolated soy protein + 0.3 g/100 g added bioavailable phosphorus.

**FIGURE 1** Body weight of BKS.cg-m + Lepr°/°/Lepr°/° (DB) and m+/ +/+ (CON) mice consuming casein (C) or soy protein (S) at 12 or 20% of energy. Data are means ± pooled SEM, n = 5 or 6, df, final day. Time × diabetes × protein type (P = 0.044), time × protein type (P = 0.0001), time × diabetes × protein type (P = 0.0001), protein type (P = 0.0001), protein quantity (P = 0.021) (by repeated Measures ANOVA); d0: diabetes × protein type × protein quantity (P = 0.0247), diabetes × protein type × protein quantity (P = 0.0027), diabetes × protein type (P = 0.0014), protein type (P = 0.0111), protein type (P < 0.0001), protein type (P = 0.0042), d00: diabetes × protein type × protein quantity (P = 0.0216), diabetes × protein type × protein quantity (P = 0.0055), diabetes × protein type (P = 0.0006), protein quantity (P = 0.0100), d120: diabetes × protein type × protein quantity (P = 0.0090), diabetes × protein type (P = 0.0072), diabetes × protein type (P = 0.0014), protein quantity (P = 0.0221), d150: diabetes × protein type (P = 0.0114), diabetes × protein type (P = 0.0060), protein type (P = 0.0056), df: diabetes (P = 0.0463), protein type (P = 0.0326) (by regression).

**FIGURE 2** Plasma glucose concentration in BKS.cg-m + Lepr°/°/Lepr°/° (DB) and m+/ +/+ (CON) mice consuming casein or soy protein at 12 or 20% of energy. Data are means ± pooled SEM, n = 5 or 6, df, final day. Significant effects: time (P < 0.0001); diabetes (P < 0.0001); time × diabetes (P < 0.0001) (by repeated-measures ANOVA); diabetes (P < 0.0001) (by regression on df).
There was no effect of protein type or quantity on PGLUC and they continued so throughout the study (P = 0.0144; protein quantity (P = 0.0036) (by regression on df). Food intake. DB mice ate more than CON mice throughout the study (P < 0.0001), and there was no effect of protein type or quantity (data not shown). In DB mice, FI increased from d 0 to 30 (~5.9 ± 0.1 to 7.7 ± 0.2 g/d) and stabilized until d 150 (~8.3 ± 0.6 g/d). In CON mice, FI was approximately the same throughout the study (2.6 to 2.8 ± 0.2 g/d).

Blood. All DB mice were diabetic upon arrival, as indicated by urine glucose concentrations (659–1458 mmol/L; data not shown). At d 0, PGLUC concentrations in fed mice were ~97% higher in DB than in CON mice (P < 0.0001), and they continued so throughout the study (P < 0.0001). There was no effect of protein type or quantity on PGLUC concentrations in either DB or CON mice that had been fed or deprived of food for 7 h (Fig. 2).

Overall, SU concentrations (Fig. 3) were ~49% higher in DB than in CON mice (P = 0.0312). In addition, SU concentrations were higher in the 20C groups (protein quantity, P = 0.0036; protein type, P = 0.0214). TC concentrations (data not shown) were ~88% higher in DB than in CON mice (P = 0.0085). No effect of protein type or quantity was found. For SALB concentrations, no effects of diabetes, protein type or quantity were found (data not shown).

Urine. DB mice had ~255% higher UAE than CON mice at d 0 (P < 0.0001) (Table 3). Overall, DB mice also had larger UAE than CON mice (P < 0.0001), but the differences were dependent on dietary treatment (protein × protein type P = 0.0200; diabetes × protein quantity, P < 0.0001). The effects of diet on UAE (Fig. 4) varied with protein type, P < 0.0001; protein quantity, P = 0.0242; protein type × protein quantity, P = 0.0111). At later time points, the rest of the DB mice (i.e., those consuming 20C, 20S and 12C diets) also started losing weight; thus, the differences among the dietary groups were not as great.

![FIGURE 3](https://academic.oup.com/jn/article-abstract/133/3/673/4688040)

**FIGURE 3** Serum urea concentrations in BKS.cg-m +Leprdb/+Leprdb (DB) and m+/-m+ (CON) mice consuming casein or soy protein at 12 or 20% of energy. Data are means ± pooled SEM, n = 5 or 6. df, final day. Significant effects: diabetes (P = 0.0312); protein type (P = 0.0214); protein quantity (P = 0.0036) (by regression on df).

![FIGURE 4](https://academic.oup.com/jn/article-abstract/133/3/673/4688040)

**FIGURE 4** Change in urinary albumin excretion from d 0 (ΔUAE) in BKS.cg-m +Leprdb/+Leprdb (DB) and m+/-m+ (CON) mice consuming casein or soy protein at 12 or 20% of energy. Data are means ± pooled SEM, n = 5 or 6. df, final day. Significant effects: time × diabetes × protein quantity (P = 0.015); time × protein quantity (P = 0.006); time (P = 0.02); diabetes × protein type (P = 0.0200); diabetes × protein quantity (P < 0.0001); UAE at d 0 – mean UAE at d 0 (P < 0.0001); diabetes (P < 0.0001); protein type (P = 0.0510); protein quantity (P < 0.0001) (by repeated-measures ANOVA); d 30: diabetes (P = 0.0035); protein quantity (P = 0.0155); d 60: diabetes × protein quantity (P < 0.0001); diabetes (P < 0.0001); protein quantity (P < 0.0001); d 90: diabetes × protein quantity, diabetes (P < 0.0001); protein type (P = 0.0065); protein quantity (P < 0.0001); d 120: diabetes × protein type (P = 0.003); diabetes × protein quantity (P < 0.0001); diabetes (P < 0.0001); protein type (P = 0.004); protein quantity (P < 0.0001); d 150: diabetes × protein quantity (P = 0.0140); diabetes (P = 0.0010); protein quantity (P = 0.0025); df: diabetes × protein quantity (P = 0.0010); diabetes (P = 0.0008); protein quantity (P < 0.0001) (by regression).

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**TABLE 3**

| Urinary albumin excretion (UAE) in BKS.cg-m +Leprdb/+Leprdb and m+/-m+ mice consuming casein (C) or soy protein (S) at 12 or 20% of energy |
|-----------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                             | n     | d0      | d30      | d60      | d90      | d120     | d150     |
| Diabetic                    |       |         |          |          |          |          |          |
| 20C                         | 6     | 0.56 ± 0.09 | 0.57 ± 0.11 | 1.14 ± 0.12 | 1.56 ± 0.20 | 1.87 ± 0.24 | 1.02 ± 0.15 | 1.24 ± 0.23 |
| 20S                         | 5     | 1.00 ± 0.20 | 0.62 ± 0.25 | 1.13 ± 0.18 | 0.80 ± 0.20 | 1.16 ± 0.05 | 0.81 ± 0.22 | 1.01 ± 0.11 |
| 12C                         | 6     | 0.93 ± 0.10 | 0.39 ± 0.16 | 0.37 ± 0.12 | 0.54 ± 0.18 | 0.51 ± 0.18 | 0.56 ± 0.20 | 0.60 ± 0.02 |
| 12S                         | 5     | 0.99 ± 0.27 | 0.26 ± 0.06 | 0.37 ± 0.10 | 0.40 ± 0.08 | 0.18 ± 0.03 | 0.41 ± 0.10 | 0.32 ± 0.12 |
| Control                     |       |         |          |          |          |          |          |
| 20C                         | 6     | 0.24 ± 0.07 | 0.07 ± 0.01 | 0.05 ± 0.01 | 0.08 ± 0.03 | 0.06 ± 0.01 | 0.07 ± 0.02 | 0.10 ± 0.02 |
| 20S                         | 5     | 0.23 ± 0.04 | 0.14 ± 0.02 | 0.12 ± 0.03 | 0.11 ± 0.03 | 0.12 ± 0.03 | 0.14 ± 0.03 | 0.16 ± 0.03 |
| 12C                         | 5     | 0.24 ± 0.11 | 0.08 ± 0.01 | 0.07 ± 0.03 | 0.08 ± 0.02 | 0.07 ± 0.02 | 0.08 ± 0.03 | 0.06 ± 0.01 |
| 12S                         | 6     | 0.26 ± 0.10 | 0.08 ± 0.01 | 0.08 ± 0.02 | 0.09 ± 0.01 | 0.08 ± 0.03 | 0.08 ± 0.03 | 0.08 ± 0.03 |

1 Values are mean ± SEM.

2 Final day.
time and were different between DB and CON mice (time $\times$ protein quantity, $P = 0.0060$; time $\times$ diabetes $\times$ protein quantity, $P = 0.0150$).

In CON mice, UAE decreased after 30 d of dietary intervention ($\Delta$UAE = $-0.14 \pm 0.04$ at d 30) and was maintained approximately constant thereafter. No effects of diet were seen in CON mice. In DB mice, $\Delta$UAE varied over time and was dependent on diet. Protein quantity began to have an effect at d 30 ($P = 0.0155$), which became stronger at d 60 ($P < 0.0001$). It was maintained thereafter, but was reduced somewhat at d 150 ($P = 0.0025$). Protein type had a detectable effect at d 90 ($P = 0.0065$), but no effect by d 150. This effect of protein type was attributed mainly to the effect seen at high protein intake. The smaller effect of diet toward the end of the study was caused by a reduction of UAE for the 20C group, whereas the other DB mice maintained approximately the same UAE as in the previous months. Comparing the final time point (df) to the initial one (d 0), UAE in DB mice was $\sim$100% higher for the 20C group, approximately the same for the 20S group, $\sim$33% lower for 12C group, and $\sim$67% lower for the 12S group ($\Delta$UAE = $+0.7 \pm 0.38$, $+0.01 \pm 0.38$, $-0.33 \pm 0.23$ and $-0.47 \pm 0.19$, respectively).

**Kidneys.** Kidney weights (Fig. 5) were $\sim$46% higher in DB than in CON mice ($P < 0.0001$). Protein quantity had a significant effect ($P < 0.0013$), which was dependent on diabetes ($\times$ protein quantity; $P < 0.0358$). In DB mice, kidney weights were $\sim$19% lower in the 12% protein groups (12C and 12S), but no differences were seen among the CON mice.

**DISCUSSION**

The present study used male BKS.cg-m $+$Lepr$^{db}$/+Lepr$^{db}$ diabetic mice to study the effects of protein type and quantity on urinary albumin excretion, which is a good predictor of diabetic nephropathy progression (17,18,27).

In the control mice, UAE decreased slightly by 2 mo of age, and leveled off thereafter, independently of dietary treatment. In contrast, UAE of the diabetic mice was already elevated at $35 \pm 4$ d of age. When a high animal protein diet (20% casein) was fed, UAE of the diabetic mice increased with time to macroalbuminuric concentrations, reaching a maximum at $\sim$5 mo of age, and then slightly decreased subsequently. Similar results were reported by Brouhard et al. (28), who found a maximum UAE at 4.25 mo of age and a decline at 5 mo in diabetic mice fed 27 or 50% protein diets. The later decline in UAE may be related to a reduction in the glomerular filtration rate (GFR) to a level below that of the control mice; this has been shown to occur in male BKS.cg-m $+$Lepr$^{db}$/+Lepr$^{db}$ diabetic mice at $\sim$5 mo of age as well (29). In these diabetic mice, lower protein intake (12% protein) led to a decrease in UAE, which indicated an improved macromolecular permselectivity, suggesting a slower progression or even protection from diabetic nephropathy. This UAE reduction with the lower protein diets was seen after 30 d and was maintained for the next 5 mo until the end of the study. This finding agrees with the extensive literature, which shows that low protein diets protect against nephropathy in animal models and in humans [see Maroni and Mitch (3) for a recent review].

At a high protein intake (20%), our results showed that the increase in UAE that occurred when casein was fed was absent when soy protein was consumed. The diabetic mice consuming the high soy protein diet even had a reduction in UAE after 30 d of intervention, which agreed with the UAE reduction seen when the lower protein diets (with either soy or casein) were consumed. However, when the high soy diet was fed, UAE levels returned to baseline values after 60 d of the dietary intervention and stayed at that microalbuminuric level thereafter, showing only minor fluctuations. This maintenance of UAE at microalbuminuric level suggests that a high soy protein diet may maintain macromolecular permselectivity and slow the progression of diabetic nephropathy. Moreover, we found that the effects of protein type and quantity were independent of glycemic control because no differences were seen in plasma glucose concentrations among the different diabetic groups that had been fed or deprived of food. In summary, our results suggest that soy protein diets may confer some protection against diabetic nephropathy in male BKS.cg-m $+$Lepr$^{db}$/ +Lepr$^{db}$ diabetic mice.

Other studies in the literature have shown that soy protein has a beneficial effect on nondiabetic nephropathy. In the late 1980s, Williams and Walls showed that in rats subjected to unilateral nephrectomy and partial infarction of the contralateral kidney, replacing casein with soy protein for 3 mo resulted in less proteinuria, less renal hypertrophy, less histological damage and increased survival (10). Soy protein was also shown to markedly reduce the progression of nephropathy in the aging rat model Fisher 344, which has a high incidence of old-age nephropathy (30). More recently, several studies on animal models of PKD have shown that soy protein is effective in retarding cyst development (31), reducing tubular and interstitial pathology (32) and ameliorating epithelial and interstitial changes (33). The beneficial effect of soy protein on cyst score and kidney weight in the pcy PKD mouse model was shown to be dependent on protein quantity and mouse gender, with the strongest effect seen in female mice consuming low soy protein diets (6 g/100 g) (34). This dependence on protein quantity was noted in the present study, with a stronger effect seen in diabetic mice consuming the low soy protein diet than in those consuming the high soy protein diet. More recently, Maddox and collaborators found that soy protein consumption also prevented an increase in UAE in Zucker rats (35).

Several interesting observations from the present study deserve further investigation. First, we found that mice consuming the high soy protein diets or the lower protein diets had lower SU concentrations than those consuming the high casein diet. This difference was likely to be associated with the protein composition of the diet because it was seen in both diabetic and control mice. The lower SU concentrations may be important. As proposed by Bankir et al. (36), lower SU
concentration could affect kidney hemodynamics and reduce hyperfiltration in the diabetic kidney. The second observation concerns the amount of bioavailable phosphorus. All diets in the present study had the same total amount of phosphorus. However, there was less bioavailable phosphorus in the soy protein because the majority of phosphorus in soy (~70%) is found in the form of phytic acid (37). It is thought that low phosphorus intake (independent of protein quantity) slows the progression of renal failure (38). Thus, the lower amount of bioavailable phosphorus in soy may contribute to the observed effects of the soy protein diet. Third, we found that diabetic mice consuming 12% soy protein had a lower growth rate and a faster weight loss than diabetic mice consuming other diets. However, no such differences were seen among the control mice. This may suggest a differential requirement for certain nutrients between diabetic and control mice. Further research is required to investigate these issues.

In conclusion, the data from this study show that diets rich in soy protein prevent an increase in UAE, which is typically produced by partial nephrectomy. V. Diets containing whole dried meat. Arch. Hydrolyzates for amino acid analysis. Anal. Biochem. 56: 66–73.


