Dietary Protein Source Determines the Degree of Hypertension and Renal Disease in the Dahl Salt-Sensitive Rat

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Abstract—Previous studies demonstrated that a whole-grain diet attenuated sodium-dependent hypertension and renal disease in Dahl salt-sensitive rats from the colony at the Medical College of Wisconsin (Dahl SS/Mcw rats) compared with rats maintained on a purified AIN-76A diet. The present experiments determined which component(s) of the grain diet prevented renal and cardiovascular disease. Male SS/Mcw rats were maintained on isocaloric diets identical to AIN-76A, except the source of protein (wheat gluten for casein), carbohydrate (wheat flour for sucrose), or fat (soybean oil for corn oil) was substituted in separate diets. Rats were maintained on the different diets from weaning and studied after 3 weeks on a high-salt (4.0% NaCl) diet. Substitution of the carbohydrate in the diet did not affect body weight, arterial pressure, or renal disease. Replacement of casein with wheat gluten significantly reduced body weight (258±7 versus 353±3 grams), mean arterial pressure (133±2 versus 153±2 mm Hg), and albumin excretion (9±1 versus 50±7 mg/d) to levels of rats fed the whole-grain diet (n=7 to 16/group). Replacement of the fat in the diet increased arterial pressure without affecting body weight or albumin excretion. The results of the present study indicate that dietary components other than sodium play an important role in the development of hypertension and renal disease in the Dahl SS/Mcw rat. (Hypertension. 2005;45[part 2]:736-741.)

Key Words: hypertension, sodium-dependent ■ rats ■ blood pressure ■ diet

The composition and quantity of dietary nutrients can have profound effects on arterial blood pressure. In humans, increased consumption of food containing cholesterol, saturated fats, or carbohydrates is associated with an elevation of arterial blood pressure, whereas a high-protein diet is linked to a decrease in blood pressure.1–4 Experimental studies have also examined the influence of changes in dietary protein, fat, and carbohydrates on arterial blood pressure in animal models. The development of hypertension is accelerated in genetic models of hypertension depending on the source and composition of dietary protein, carbohydrates,5–9 and fat.9,10 These clinical and experimental results indicate that different components of the diet, independent of sodium chloride content, can have a profound influence on the long-term level of arterial blood pressure.

Studies from our laboratory demonstrated that sodium-sensitive changes in blood pressure and kidney disease in inbred Dahl salt-sensitive rats from the colony at the Medical College of Wisconsin (Dahl SS/Mcw rats) were modified depending on the chow fed to the rats. Rats fed chow formulated following the guidelines established by the American Institute of Nutrition in 1976 (AIN-76A)11,12 had a greater degree of sodium-dependent hypertension and renal disease in comparison to rats fed a diet derived largely from grain products.13 The AIN-76A and the grain diet (Teklad 3075S) are composed of approximately the same percentage of protein, carbohydrates, and fat. Differences between the 2 diets, however, include the source of protein (casein in the AIN-76A versus corn and wheat protein in the grain diet), carbohydrates (sucrose and corn starch in AIN-76A versus corn and wheat flour in the grain diet), and fat (corn oil in the AIN-76A diet versus soybean oil in the grain diet).

The present study was specifically designed to test the hypothesis that the source of dietary protein, fat, or carbohydrates can lead to the modulation of hypertension and renal disease in the Dahl SS/Mcw rat. Because characteristics of sodium-dependent hypertension and renal disease in the SS/Mcw rat are similar to those observed in human populations,14,15 the elucidation of environmental modifiers of the disease phenotype in these animals may provide important clues into the influence of diet on human disease. To address this question, rats were fed custom diets throughout their lives from weaning. The custom diets used the identical formulation as the AIN-76A diet with the exception that the source of protein, carbohydrates, or fat in the custom diets was similar to that used in the grain diet.

Methods

Experimental Animals

Experiments were performed on inbred Dahl SS/Mcw. Breeders were fed the purified AIN-76A rodent diet (Dyets, Inc, Bethlehem, Pa) containing 0.4% NaCl. Weanlings were randomly placed and
maintained on 1 of the 5 different diets described. The salt content of each diet was 0.4% NaCl from weaning until 9 weeks of age. At 9 weeks, the salt content of the chow was increased to 4.0% NaCl, and the rats were studied at 12 weeks of age. The MCW Institutional Animal Care and Use Committee approved all experimental protocols.

Diet
The formulation of the purified AIN-76A diet follows guidelines recommended by the American Institute of Nutrition. \( ^{11,12} \) The whole-grain diet was obtained from Harlan Teklad (3075S; Madison, Wis) and is made from ground wheat and corn. The AIN-76A and the Teklad 3075S diets are composed of approximately the same percentage of protein (18% to 20%), carbohydrates (60% to 65%), fat (5%), and fiber (4% to 5%), with similar amounts of vitamins and minerals. To determine which component of the whole-grain diet is important in the attenuation of the hypertension and renal disease in the SS/Mcw rats, 3 isocaloric diets (Dyets, Inc) were produced that were maintained on the individual diets containing 4.0% NaCl. After surgical procedures were performed on the first day of the 2-week experimental protocol, the rats were deeply anesthetized with an intraperitoneal injection of ketamine (35 mg/kg), xylazine (10 mg/kg), and acepromazine (0.5 mg/kg), with supplemental anesthesia administered when needed. Using aseptic technique, polyvinyl catheters were implanted in the femoral artery, tunneled subcutaneously, and exteriorized at the back of the neck in a lightweight tethering spring. Both antibiotic (100 000 U/kg penicillin G, intramuscular) and analgesic (0.1 mg/kg Buprenex, subcutaneous) were administered after surgery, and the rats were allowed to fully awaken from anesthesia on a temperature-controlled pad. After recovery from anesthesia, all rats were placed in individual stainless steel cages that permit daily measurement of arterial blood pressure and overnight urine collection.

Experimental Protocol
The rats recovered for 1 week after surgery. During this time they were maintained on the individual diets containing 4.0% NaCl. After the recovery period, high-salt blood pressure measurements were obtained from 9:00 AM to 12:00 PM on 3 consecutive days. After the second day of blood pressure measurement, an overnight urine collection (from 4:00 PM to 8:00 AM) was obtained for measurement of urinary sodium, creatinine, and albumin excretion rates. After the blood pressure recording obtained the next morning, arterial plasma samples were obtained for measurement of plasma creatinine concentration and plasma renin activity while the rats were maintained on a high-NaCl diet.

Subsequent to the high-salt blood sampling protocol, the rats were administered furosemide (10 mg/kg, intraperitoneal), and the diet was switched to a low salt content (0.4% NaCl). The rats were then maintained on the low-salt diet for 2 days. On the next night, an overnight urine collection (from 4:00 PM to 8:00 AM) was obtained to quantify sodium and creatinine excretion; blood pressure was measured after 2 days on the low-NaCl diets; and arterial blood was sampled to measure plasma creatinine concentration and plasma renin activity on low NaCl.

Urine electrolytes were measured by flame photometry (IL-943; Instrumentation Laboratories, Lexington, Mass). Plasma and urine creatinine values were measured with an assay based on the Jaffe Reaction by autoanalyzer (ACE; Alfa Wasserman, Fairfield, NJ). Urine albumin was quantified with a fluorescent assay that used Albumin Blue 580 dye (Molecular Probes, Eugene, Ore) and a fluorescent plate reader (FL600; Bio-Tek, Winooski, Vt). Plasma renin activity (PRA) was measured using a modification of the method of Sealey and Laragh. \( ^{16} \)

Histological Analysis of Kidney Tissues
Kidneys (n=4/group) were obtained for histological analysis from non-instrumented Dahl SS/Mcw rats maintained on each of the diets as described. The rats were deeply anesthetized with sodium pentobarbital (50 mg/kg, IP); the kidneys were then removed, bisected along the mid sagittal plane, and placed in a 10% formaldehyde solution in phosphate buffer. The tissue was paraffin-embedded in an automatic tissue processor (Microm HMP 300), cut in 3-μm sections (Microm HM355S), mounted on silanized/charged slides, and stained with Gomori One-Step Trichrome. Slides were photographed using a Nikon E-400 fitted with a Spot Insight camera; digital micrographs were taken at different magnifications. Individual glomeruli (30 to 40 per rat) were evaluated using the semiquantitative index method of Raj et al; \( ^{17} \) glomeruli were scored from 0 (best) to 4 (worst) on the basis of glomerulosclerosis and mesangial expansion as we previously described. \( ^{13} \) The percentage of the outer medullary tissue containing blocked tubules filled with protein was quantified by determining the proportion of red-stained structures in this region using MetaMorph Image Analysis software (version 4.6; Universal Imaging Systems Corp) as we previously described. \( ^{13} \) The grading of glomerular and medullary damage was performed in a blinded manner.

Statistical Analysis
All data are presented as the mean±SE. A 1-way analysis of variance was used to determine the differences in parameters between the rats maintained on the different diets. A Tukey post-hoc test was used when appropriate. The 95% confidence interval was considered significant.

Results
The level of mean arterial blood pressure (MAP) in rats maintained on the different diets containing high salt (4.0% NaCl, n=7 to 16/group) and low salt (0.4% NaCl, n=5 to 16/group) is illustrated in Figure 1. MAP averaged 154±2 mm Hg on the 4.0% NaCl diet and was significantly reduced to 132±4 mm Hg after sodium depletion in the SS/Mcw rats fed the AIN-76A chow from weaning. The MAP on high and low salt was not significantly different from these values in SS/Mcw rats fed the purified diet with wheat flour substituted as the carbohydrate. Substitution of the casein with wheat gluten as the protein in the diet led to a significant reduction in MAP on either the high (133±2 mm Hg) or low (115±3 mm Hg) NaCl intake compared with rats on the AIN-76A diet. In contrast, the substitution of corn oil with soybean oil resulted in the highest levels of MAP observed when the diet contained either high (168±5 mm Hg) or low (155±4 mm Hg) NaCl. Finally, as we previously reported, \( ^{13} \) the rats fed the Teklad 3075S whole-grain diet had significantly lower levels of MAP in comparison to the rats fed the AIN-76A chow regardless of the sodium intake. Heart rate (data not shown) averaged 393±8 beats/min in the rats fed the AIN-76A diet containing 4.0% NaCl, and 390±6 beats/min in the rats fed the AIN-76A diet containing 0.4% NaCl. The average heart rate was not different from this value in the groups fed any of the other diets, regardless of sodium content.

Body weight was significantly lower in rats maintained from weaning on the grain diet or the diet with wheat gluten substituted for casein in the AIN-76A formulation in comparison to the group maintained from weaning on the AIN-
76A diet (Figure 2; n=12 to 18/group). Paralleling the differences in body weight, the total kidney weight averaged 3.5±0.1 grams in the rats maintained on the AIN-76A diet (data not shown). The total kidney weight was not significantly different from that value in the rats fed the purified AIN-76A diet with wheat flour substituted for sucrose (flour), the AIN-76A diet with wheat gluten substituted for casein (gluten), the AIN-76A diet with soybean oil substituted for corn oil (soybean oil), or the grain diet (grain). *P<0.05 vs rats on the AIN-76A diet.

Albumin excretion, as an index of kidney disease, averaged 50±7 mg/d in rats fed the AIN-76A chow (Figure 2; n=9 to 16/group). The albumin excretion rate was not significantly different from that obtained from rats fed the diet substituted with either carbohydrate or fat, but it was significantly greater than the plasma cholesterol in rats fed the gluten (70±3 mg/dL) or the grain diet (87±3 mg/dL).

Plasma cholesterol and protein were quantified in noninstrumented rats (n=4 rats/group). Total plasma protein was not different in any of the groups of rats when compared, with the average of 5.7±0.1 g/dL in the rats fed the AIN-76A diet. Plasma cholesterol averaged 129±13 mg/dL in rats fed the AIN-76A diet; this value was not different from that obtained from rats fed the diet substituted with either carbohydrate or fat, but it was significantly greater than the plasma cholesterol in rats fed the gluten (70±3 mg/dL) or the grain diet (87±3 mg/dL).

Representative histological slides of kidneys are presented in Figure 3. Consistent with previous reports in the Dahl SS/Mcw rat,13,18 severe glomerular sclerosis (blue fibrotic tissue and collapsed capillary structure) and blocked tubules in the outer medulla (red protein deposition casts) are apparent in the SS/Mcw fed the purified AIN-76A diet. Less glomerular and tubular injury is evident in the kidneys of the SS/Mcw rats fed the whole-grain diet or the gluten-substituted diet. The glomerular injury index (n=4/group) was significantly greater in the Dahl SS/Mcw rats fed the
purified AIN-76A diet (2.5±0.2) than in the rats fed the whole grain (1.6±0.2) or the gluten diet (1.2±0.3). There was no difference in the glomerular injury index between the rats fed the AIN-76A diet and those receiving the carbohydrate-substituted or fat-substituted diets. Moreover, 17.4±2.5% of the area of the outer medulla stained for protein casts (indicating blocked tubules, n=4/group) in the kidneys of rats fed the AIN-76A diet. This percentage was not different in the kidneys of rats fed the fat-substituted diet (18.6±3.0%), but it was significantly lower in rats fed the grain diet (4.1±0.5%), the carbohydrate-substituted diet (9.1±0.7%), and the protein-substituted diet (7.1±1.2%).

PRA averaged 0.4±0.1 ng angiotensin I/mL per hour in the SS/Mcw rats fed the AIN-76A diet containing 4.0% NaCl (n=16). The PRA significantly increased to 2.5±0.6 ng angiotensin I/mL per hour when the NaCl content of the diet was decreased to 0.4%. The PRA values in the other groups of rats were not significantly different from those observed in the rats fed the AIN-76A diet at either level of sodium intake (n=5 to 11). The sodium excretion rate of rats on the high-salt diet averaged 5.5±1.1 mEq/d in rats fed the AIN-76A diet containing 4.0% NaCl (n=16). The sodium excretion rates were significantly greater in the other groups consuming the different diets containing 4.0% NaCl, ranging from 8.0±0.7 mEq/d in the flour-substituted diet (n=16) to 11.7±0.9 mEq/d in the diet containing gluten (n=14). The sodium excretion rates in the rats maintained on the low-sodium (0.4% NaCl, n=10 to 16) diets were not different from the average excretion rate of 0.7±0.2 mEq/d in the group fed the AIN-76A chow.

**Discussion**

The present study demonstrates a significant influence of the composition of the diet, independent of NaCl content, on salt-sensitive hypertension and renal disease in Dahl SS/Mcw rats. As we previously demonstrated, arterial blood pressure, urinary albumin excretion, the degree of glomerular damage, and the percentage of necrotic renal tubules were all significantly greater in the Dahl SS/Mcw rats fed the purified AIN-76A than in rats fed the whole-grain chow. To determine which component of the whole-grain chow was responsible for the attenuation of hypertension and renal disease in the SS/Mcw, additional groups of rats were fed diets based on the AIN-76A formulation with simple substitutions of the carbohydrate, fat, or protein present in the whole grain chow. Substitution of the carbohydrate had a minimal influence on the renal disease and hypertension-related phenotypes, but replacement of the casein with wheat gluten led to a reduction in arterial blood pressure on low or high salt, a decrease in body weight, and a significant reduction in the severity of the kidney disease. In contrast, substitution of the fat source from corn to soybean oil led to a greater level of arterial blood pressure but did not affect body weight or kidney damage in comparison to rats fed the AIN-76A chow. The source of the nutrients in the diet, in particular the source of the protein, has a significant impact on the level of blood pressure and renal disease in the Dahl SS/Mcw rat.

The AIN-76A diet and the derivatives of this diet used in the present study contain identical amounts of protein, carbohydrate, and fat; as such, these diets are isocaloric. In addition, the percentage of sodium, potassium, vitamins, minerals, and fiber are identical. Because the steady-state sodium excretion rate was not greater in rats fed the AIN-76A diet in comparison to rats fed the other diets, an elevated consumption of the AIN-76A diet is not a likely explanation for the observed differences in phenotypes. The only difference between the different diets is the source of protein, carbohydrate, or fat. Casein is the protein source for the purified AIN-76A diet, which led to the greatest degree of hypertension and renal disease in the present study, in contrast to ground corn and wheat that serve as the source of protein in the whole-grain diet. Although casein-based diets have not been demonstrated to be pro-hypertensive, the development of hypertension in the spontaneously hypertensive rat (SHR) model is accentuated in rats fed a casein-based diet in comparison to SHR fed a soy protein-based diet. Furthermore, a greater
degree of renal disease and a shorter life span was observed in normal rats fed diets that used casein as the protein source compared with rats fed soy-based diets. In the present study, salt-sensitive hypertension and renal disease, judged by interstitial fibrosis, tubular necrosis, albuminuria, and increased renal mass, were attenuated when wheat gluten was substituted for casein in the diet. The mechanisms by which the protein and the fat source alter the severity of renal and cardiovascular disease remain to be elucidated.

The substitution of corn oil with soybean oil led to an elevation of arterial pressure in the conscious SS/Mcw compared with rats fed the AIN-76A diet on either high or low salt. This observation was unexpected because soybean oil is a source of linolenic acid, which has been shown to lower arterial blood pressure and increase the lifespan of stroke-prone SHR compared with rats fed a diet enriched in linoleic acid. Because soybean oil and gluten are both components of the grain diet, the hypertensive effects of soybean oil are apparently masked by the hypotensive effects of the gluten in the grain diet. Alternatively, there may be an unknown interaction between the protein, carbohydrate, and the fat sources, which leads to a reduction in arterial pressure in rats fed the grain diet. Interestingly, the degree of renal disease was not increased in rats fed the soybean oil diet despite the elevation of MAP. This observation indicates that the level of MAP and the severity of renal disease are not necessarily directly related. The mechanisms mediating the increase in MAP when soybean oil was substituted for corn oil in the AIN-76A diet remain to be determined.

The absence of effects after carbohydrate substitution was also surprising in this study. The carbohydrate in the AIN-76A diet is two-thirds sucrose and one-third corn starch, whereas the carbohydrate source in the grain diet is primarily starch. Because sucrose-feeding to Dahl salt-sensitive rats, SHRs, and Sprague Dawley rats leads to an increase in arterial blood pressure, we expected to observe that the diet with wheat flour substituted for sucrose would normalize blood pressure in the SS/Mcw rats. The lack of any difference in the disease phenotypes between the rats fed these 2 diets indicates that the carbohydrate source does not affect the degree of hypertension or renal disease in the Dahl SS/Mcw rat.

The mechanisms by which alterations in the source of dietary protein lead to the dramatic changes in body weight, MAP, and kidney disease observed in the present study are not presently known. One possibility is that there is altered absorption of nutrients in rats fed the diet containing gluten. Gluten induces celiac disease in ~1% of the human population; associated with this disease process is malabsorption, malnutrition, and a decreased risk of hypertension or hypercholesterolemia. The decrease in body weight and reduction in plasma cholesterol in the SS/Mcw rats fed the gluten or grain diets are consistent with such an interpretation. Differences in intestinal absorption were not reflected in the plasma protein concentration, which was not different between the rats fed different diets. Moreover, urinary sodium excretion was

the same or greater in the rats fed the gluten or grain diets in comparison to the other rats, indicating that the steady-state consumption and absorption of sodium, and therefore food, was the same or greater in these rats. In addition, although gluten-derived polypeptides have been demonstrated to induce morphological changes in the intestinal mucosa of germ-free rats, these types of changes are not apparent in rats maintained under standard conditions. The potential role of alterations in intestinal nutrient absorption in rats fed gluten or whole-grain diets, as well as alterations in hormonal, autocrine, or paracrine factors remain to be examined.

Perspectives

The substitution of casein with equal amounts of wheat gluten significantly attenuated the development of sodium-sensitive hypertension and renal disease in the SS/Mcw rat. Indices of renal disease in the SS/Mcw rat were not altered by substitution of the dietary carbohydrate or fat source, although the degree of hypertension was accentuated by the substitution of soybean oil for corn oil. Because the characteristics of sodium-dependent hypertension and renal disease in the SS/Mcw rat are similar to those observed in human populations, the source of dietary protein and fat may also affect the severity of renal and cardiovascular disease in patients.

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References

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