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Influence of diet and genetics on hypertension and renal disease in Dahl salt-sensitive rats

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Mattson, David L., Mary Pat Kunert, Mary L. Kaldunski, Andrew S. Greene, Richard J. Roman, Howard J. Jacob, and Allen W. Cowley, Jr. Influence of diet and genetics on hypertension and renal disease in Dahl salt-sensitive rats. *Physiol Genomics* 16: 194–203, 2004. First published November 4, 2003; 10.1152/physiolgenomics.00151.2003.—Experiments examined the influence of diet and genetics on hypertension and renal disease in inbred Dahl salt-sensitive (SS/Mcw) rats and consomic rats in which chromosomes 16 (SS.BN16) or 18 (SS.BN18) of the normotensive Brown Norway rat were inserted into the genetic background of the SS/Mcw. Dahl SS/Mcw breeders and offspring were randomly placed on a purified AIN-76A diet or a grain-based diet, and male offspring were screened for cardiovascular and renal phenotypes following 3 wk on a 4.0% NaCl diet. High-salt arterial blood pressure (162 ± 5 mmHg, $n = 10$), urinary protein excretion (147 ± 16 mg/day, $n = 14$), and albumin excretion (72 ± 9 mg/day, $n = 14$) were significantly elevated in the Dahl SS/Mcw maintained on the purified diet compared with rats fed the grain-based diet. Rats fed the purified diet also exhibited significantly more renal glomerular and tubular damage than rats fed the grain diet. Moreover, feeding the purified diet to the parents led to a significant increase in blood pressure in the offspring, regardless of offspring diet. Similar dietary effects were observed in SS.BN16 and SS.BN18 rats. In rats fed the purified diet, substitution of chromosomes 16 or 18 led to a significant decrease in arterial blood pressure, albumin excretion, and protein excretion compared with the SS/Mcw. Chromosomal substitution did not, however, affect albumin or protein excretion in the consomic rats compared with the SS/Mcw when the rats were maintained on the grain diet. These data demonstrate a significant influence of diet composition on salt-induced hypertension and renal disease in the Dahl SS/Mcw rat.

sodium-dependent hypertension; blood pressure; consomic

EPIDEMIOLOGICAL STUDIES HAVE indicated that arterial blood pressure can be strongly influenced by dietary consumption of both macro- and micronutrients. The level of arterial blood pressure in human populations is directly correlated to consumption of sodium and inversely related to dietary calcium, potassium, and magnesium intake (15, 19, 22). Moreover, dietary intake of cholesterol, saturated fats, or carbohydrates is associated with elevated blood pressure, whereas individuals consuming a high-protein diet tend to have a lower level of arterial blood pressure (15, 16, 19, 34). In addition to the studies in human populations, a number of experimental studies have examined the influence of changes in the protein, fat, and carbohydrate composition of the diet on arterial blood pressure in animal models. Notably, it has been demonstrated that the development of hypertension is accentuated in genetic models of hypertension including the spontaneously hypertensive rat (SHR) and the Dahl salt-sensitive (SS) rat depending on the source and composition of dietary protein (27), carbohydrates (21, 26, 38, 39), and fat (33, 39). These clinical and experimental results indicate that the composition of the diet, independent of sodium chloride intake, has a profound influence on the long-term level of arterial blood pressure.

Our laboratory recently began a large phenotyping project to examine the genetic factors leading to NaCl-sensitive hypertension in an inbred colony of Dahl SS rats maintained at the Medical College of Wisconsin (SS/Mcw). A systematic phenotyping protocol was designed to assess NaCl-sensitive changes in blood pressure and kidney-related phenotypes in Dahl SS/Mcw rats and consomic rats in which individual chromosomes from the normotensive Brown Norway rat are introgressed by marker-assisted breeding into the genetic background of the Dahl SS/Mcw (12). During the course of these studies, we observed that a seemingly minor adjustment in the diet was associated with a marked decrease in the degree of hypertension and renal-associated disease in the Dahl SS/Mcw and the consomic rats.

The present study was specifically designed to determine whether it was the diet fed to the breeders, to the male offspring, or both that was of importance in the development of hypertension and renal disease in the offspring. Dahl SS/Mcw breeders and male offspring were maintained throughout life on a purified diet or a grain-based diet containing 0.4% NaCl. The male offspring of each group of parental rats were randomly placed on either of the two diets at weaning to create four groups of rats that were examined for hypertension and renal disease. Additional studies were then performed to evaluate the influence of the different diets on hypertension and renal disease in consomic strains of rats in which chromosomes 16 (SS.BN16) or 18 (SS.BN18) of the normotensive Brown Norway rat were inserted into the genetic background of the SS/Mcw and to determine whether the genetic effects of chromosomal substitution would be altered by the composition of the diet.

METHODS

Experimental Animals

Experiments were performed on inbred lines of Dahl salt-sensitive rats (SS/Mcw), consomic SS.BN16 rats, and consomic SS.BN18 rats maintained as inbred colonies at the Medical College of Wisconsin (MCW). The MCW Institutional Animal Care and Use Committee approved all experimental protocols. The rats were maintained on either the purified AIN-76A rodent diet purchased from Dyets (Beth-

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lehem, PA) or a whole grain diet obtained from Harlan Teklad (3075S; Madison, WI) containing 0.4% NaCl with tap water.

The consomic rat lines were derived as previously described (12, 28) by using inbred normotensive BN/Mcw and SS/Mcw rats. Residual heterozygosity and genetic contamination were eliminated by using a set of 182 microsatellite markers for genotyping that provided even coverage of the 21 chromosomes. The progenitor rats used for the present study were homozygous for all regions tested, and each of these parental strains has since undergone a periodic total genomic scan to ensure allelic homogeneity. Our preliminary data indicated that the maximal effect of the diet occurred in the male Dahl SS/Mcw rats, the animals with the greatest degree of hypertension and renal disease, although we did observe dietary effects in female SS.BN16 rats. To maximize the statistical power of our experimental design, the present studies were carried out in male rats.

Diets

The formulation of the purified AIN-76A diet follows the guidelines recommended by the American Institute of Nutrition in 1977 (7). The whole grain diet used in the present study was obtained from Harlan Teklad (3075S), is made from ground wheat and corn, and does not contain alfalfa or soybean meal or any food products of animal origin. The Teklad 3075S diet is custom-made for MCW and is similar in composition to Teklad diet 2016S (**http://www.teklad.com/**) but is extruded rather than pelleted and contains 18% protein. The AIN-76A and the Teklad 3075S diets are composed of approximately the same percentage of protein (18–20%), carbohydrate (60–65%), fat (5%), and fiber (4–5%) with similar amounts of vitamins and minerals. Differences between the two diets, however, include the source of protein (casein in the AIN-76A vs. corn and wheat protein in the grain diet), carbohydrate (sucrose and corn starch in AIN-76A vs. corn and wheat flour in the grain diet), and fat (corn oil in the AIN-76A diet vs. soybean oil in the grain diet).

Group 1: Influence of Parental and Offspring Diet on Hypertension and Renal Disease in Male Dahl SS/Mcw Rats

Experiments were performed on male SS/Mcw rats to determine whether the dietary effects observed could be attributed to the diet upon which the parental rats were maintained or whether the phenotypic effects of the different diets were due strictly to the diet provided to the male offspring. Dahl SS/Mcw breeders and offspring were maintained throughout life on the purified AIN-76A diet (A, parental diet; a, offspring diet from weaning) or the grain-based diet (G, parental diet; g, offspring diet from weaning) containing 0.4% NaCl. The male offspring of each group of parental rats were placed randomly on either of the two diets at weaning and maintained on that particular dietary background throughout the study. This experimental design produced four groups of male Dahl SS/Mcw offspring: Aa, Ag, Gg, and Ga. As described above, the salt content of the diet for the breeders and the weanlings was 0.4% NaCl in both diet types. At 9 wk of age, the salt content of the diets for the offspring was switched to 4.0% NaCl, and the rats were maintained on this diet for the 3 wk leading up to the experimental protocol.

Group 2: Influence of Diet and Chromosomal Substitution on Hypertension and Renal Disease in Dahl SS/Mcw Rats

A second set of experiments was performed in male Dahl SS/Mcw rats, consomic SS.BN16 rats, and consomic SS.BN18 rats that were maintained on the same diet as their parents. The diet was either the purified AIN-76A diet or the grain-based diet (Teklad 3075S). The salt content of the diet for the breeders and the weanlings was 0.4% NaCl in both diet types. At 9 wk of age, the salt content of the diet for the male offspring was switched to 4.0% NaCl, and the rats were maintained on this diet for the 3 wk leading up to the experimental protocol.

Surgical Preparation

All surgical procedures were performed on the first Monday of the 2-wk 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, we implanted polyvinyl catheters in the femoral artery, tunneled subcutaneously, and exteriorized these at the back of the neck in a lightweight tethering spring. Both antibiotic (100,000 U/kg penicillin G im) and analgesic (0.1 mg/kg Buprenex sc) were administered postsurgically, and the rats were allowed to fully awaken from anesthesia on a temperature-controlled pad. Following 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 were permitted to recover for a week following surgery. During this time they were maintained on the high-salt (4.0% NaCl) diet. Near the end of the recovery period, urine was collected overnight to measure the urinary excretion of protein and albumin. Daily blood pressure measurements were also made on postsurgical *days 3–5* to acclimate the rats to the daily measurement of blood pressure.

High-salt blood pressure measurements were obtained from 9:00 AM to 12:00 PM on three 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, potassium, and creatinine excretions. Following the blood pressure recording obtained the following morning, arterial plasma samples were obtained for measurement of plasma creatinine concentration and plasma renin activity (PRA) while the rats were maintained on a high-NaCl diet.

Subsequent to the high-salt blood sampling protocol, the rats were administered an intraperitoneal injection of furosemide (10 mg/kg), and the diet was switched to a low salt content (0.4% NaCl) to volume deplete the animals. The rats were then maintained on the low-salt diet for 2 days. On the following night, an overnight urine collection (from 4:00 PM to 8:00 AM) was obtained to quantify sodium, potassium, and creatinine excretion; blood pressure following NaCl depletion was measured; and a sample of arterial blood was collected to measure plasma creatinine concentration and PRA when the rats were maintained on the low-NaCl diet.

Histological analysis of kidney tissues. Kidneys were obtained for histological analysis from Dahl SS/Mcw rats maintained on either the purified AIN-76A or the whole grain diet in *group 2*. The rats were deeply anesthetized with pentobarbital sodium (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's 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 (20–25 per rat) were graded from 0 (best) to 4 (worst) on the basis of glomerulosclerosis and mesangial expansion as described previously (12, 29). 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) as previously described (12). The grading of glomerular and medullary damage was performed in a blinded manner.

Statistical analysis. All data are presented as the mean ± 1 SE. A two-way analysis of variance was utilized to determine the differences in parameters between the Dahl SS/Mcw, the SS.BN16, and the SS.BN18 rats on the different diets. To determine whether there was an influence of the parental vs. the weanling diet in the Dahl SS/Mcw

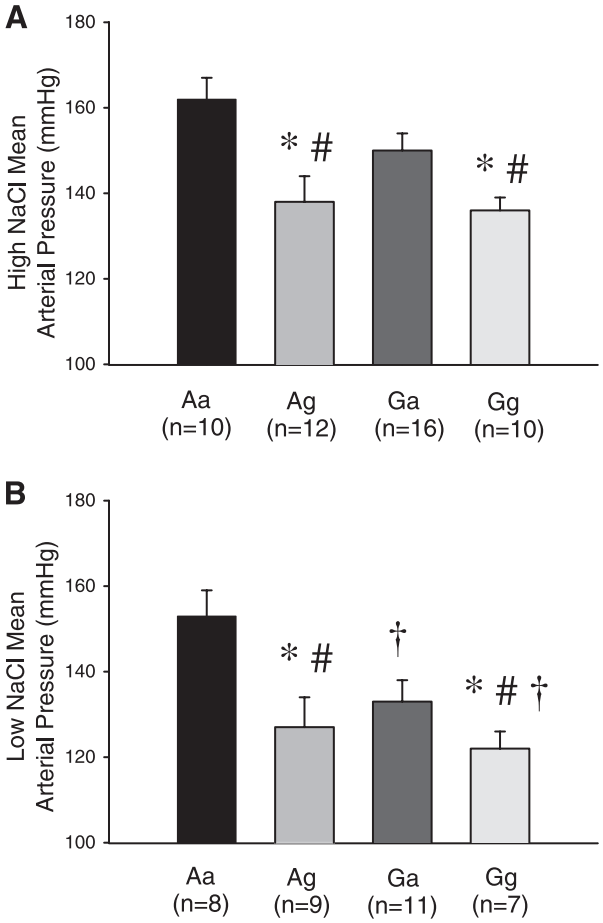

Fig. 1. Mean arterial blood pressure (MAP) in Dahl SS/Mcw rats maintained on different dietary backgrounds. Dahl SS/Mcw breeders and offspring were maintained throughout life on the purified AIN-76A diet (A, parental diet; a, offspring diet from weaning) or the grain-based diet (G, parental diet; g, offspring diet from weaning). The male offspring of each group of parental rats were randomly placed on either of the two diets at weaning to provide four groups of male SS/Mcw offspring: Aa, Ag, Gg, and Ga. Arterial pressure was measured in the rats when maintained on a high salt intake (4.0% NaCl, *A*) for 3 wk or following volume depletion with furosemide and placement on a low-salt diet (0.4% NaCl, *B*). *$P < 0.05$ vs. Aa. †$P < 0.05$ A vs. G (parental diet). #$P < 0.05$ a vs. g (offspring diet).

rats, the data were also analyzed with a two-way analysis of variance. The differences in individual values between the four different dietary treatment groups were evaluated using a one-way analysis of variance followed by a Tukey post hoc test when appropriate. The 95% confidence interval was considered significant.

RESULTS

Group 1: Influence of Parental and Offspring Diet on Hypertension and Renal Disease in Male Dahl SS/Mcw Rats

The level of mean arterial blood pressure (MAP) in rats on both high and low salt intake was significantly greater in the Aa group of SS/Mcw than in the Ag or Gg groups of rats (Fig. 1). The two-way ANOVA revealed a significant effect of the offsprings' diet on high-salt MAP, with the purified diet leading to more severe hypertension. A comparison of the blood pressure measured in these same male rats following NaCl depletion demonstrated a significant influence of both the parental and offspring diet on MAP; the maintenance of either the parents or the offspring on the purified AIN-76A diet significantly increased MAP measured in the SS/Mcw rats after volume depletion. The differences in MAP measured in the rats fed a high-NaCl intake and following NaCl depletion occurred in the absence of any differences in heart rate in any of the groups from the heart rate average of 398 ± 12 and 390 ± 4 beats/min in the Aa rats on high and low salt, respectively. Interestingly, the absolute decrease in MAP following NaCl depletion was not different between the groups administered the different diets; MAP decreased 13 ± 3 in the Aa group, 12 ± 3 in the Ag group, 13 ± 1 in the Ga group, and 14 ± 5 mmHg in the Gg group following NaCl depletion.

Urinary excretion of albumin and protein in the different groups is illustrated in Fig. 2. Albumin excretion averaged 70 ± 9 mg/day in the Aa group, which was significantly greater than the albumin excretion measured in any of the other groups. Similarly, protein excretion averaged 164 ± 31 mg/day in the Aa group, a value significantly greater than observed in

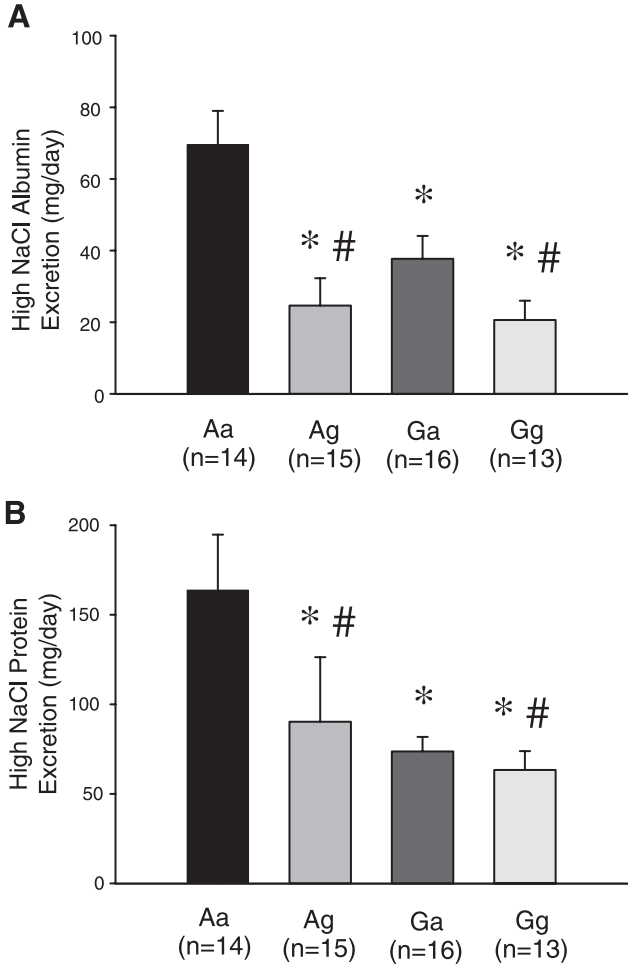

Fig. 2. Albumin excretion (*A*) and protein excretion (*B*) in Dahl SS/Mcw rats maintained on different dietary backgrounds. Dahl SS/Mcw breeders and offspring were maintained throughout life on the purified AIN-76A diet (A, parental diet; a, offspring diet from weaning) or the grain-based diet (G, parental diet; g, offspring diet from weaning). The male offspring of each group of parental rats were randomly placed on either of the two diets at weaning to provide four groups of male SS/Mcw offspring: Aa, Ag, Gg, and Ga. Excretion rates were measured when the rats had been maintained on a high salt intake (4.0% NaCl) for 3 wk. *$P < 0.05$ vs. Aa. #$P < 0.05$ a vs. g (offspring diet).

Table 1. *Comparison of experimental parameters in groups of SS/Mcw rats with different dietary backgrounds*

	SS/Mcw			
	Aa	Ag	Ga	Gg
Body wt, g	347±5 (16)	309±8*‡ (15)	318±7*† (16)	302±5*†‡ (16)
LS PRA, ng ANG I·ml^{-1}·h^{-1}	3.4±0.4 (7)	1.7±0.4* (8)	2.6±0.2 (12)	2.2±0.4 (7)
HS PRA, ng ANG I·ml^{-1}·h^{-1}	1.8±0.4 (8)	0.56±0.08‡ (7)	1.6±0.7 (13)	0.9±0.2‡ (6)
LS plasma creatinine, mg/dl	0.42±0.03 (7)	0.37±0.01 (8)	0.39±0.02 (12)	0.39±0.02 (7)
HS plasma creatinine, mg/dl	0.32±0.01 (8)	0.24±0.02 (7)	0.29±0.03 (13)	0.28±0.03 (7)
LS sodium excretion, meq/day	1.2±0.2 (13)	1.6±0.1 (13)	1.3±0.1 (13)	1.3±0.1 (13)
HS sodium excretion, meq/day	11.6±1.3 (13)	15.0±0.9 (14)	11.9±1.2 (15)	12.9±1.4 (13)

Values are means ± SE; no. of animals are in parentheses. HS, high salt; LS, low salt; PRA, plasma renin activity; A and a, purified AIN-76A parental and offspring diet, respectively; G and g, grain-based parental and offspring diet, respectively. *$P < 0.05$ vs. Aa. †$P < 0.05$ A vs. G (parental diet). ‡$P < 0.05$ a vs. g (offspring diet).

the other groups of rats. There was a significant influence of the offspring diet on both albumin and protein excretion, with the purified AIN-76A diet leading to increased albumin and protein excretion rates.

Body weight and the values for plasma creatinine, PRA, and sodium excretion measured in rats fed a high-salt diet and after volume depletion are presented in Table 1. Body weight was significantly greater in the Aa group than in any of the other groups of rats; a significant effect of the purified AIN-76A diet to increase body weight was observed in groups in which this diet was provided to either the breeders or the male offspring. There was also a significant effect of diet on PRA. The PRA was significantly lower in the Ag than in the Aa group after volume depletion, and placement of the offspring on the purified AIN-76A diet led to a significant increase in PRA under conditions of elevated NaCl intake. No significant differences were detected in low- or high-salt diet sodium excretion or plasma creatinine concentration between the dietary groups. Urinary potassium excretion was not significantly different from the excretion rate in the Aa group (1.1 ± 0.1 meq/day) in the Ag, Ga, and Gg groups of SS/Mcw.

Group 2: Influence of Diet and Chromosomal Substitution on Hypertension and Renal Disease in Dahl SS/Mcw Rats

Groups of age-matched, male Dahl SS/Mcw, SS.BN16, and SS.BN18 rats were studied in which both the breeders and male offspring were fed the purified AIN-76A diet or the grain-based diet. The influence of both genetic (chromosome substitution) and environmental factors (diet) on blood pressure before and after NaCl depletion is illustrated in Fig. 3. In the rats fed the high-NaCl diet, MAP was significantly elevated in the SS/Mcw, SS.BN16, and SS.BN18 rats maintained on the purified AIN-76A diet compared with the same strains maintained on the grain-based diet. Substitution of chromosome 16 from the normotensive BN rat led to a significant reduction in MAP compared with the SS/Mcw when the rats were maintained on either diet. Substitution of chromosome 18 also led to a reduction in MAP compared with the Dahl SS/Mcw when the rats were fed the purified AIN-76A diet, although there was no significant difference in high-salt MAP between the SS.BN18 and SS/Mcw when the rats were fed the grain-based diet. High-salt heart rate averaged 400 ± 10, 365 ± 3, and 384 ± 8 beats/min in the SS/Mcw, SS.BN16, and SS.BN18 rats, respectively, when the rats were maintained on the purified AIN-76A diet and was not different in the rats fed the grain diet. Illustrated in Fig. 3*B*, the low-salt MAP (measured following NaCl depletion with furosemide and maintenance on a low-NaCl diet) was significantly lower than the high-salt level of MAP for each strain with each diet. In addition, MAP was elevated in the rats fed the purified AIN-76A diet compared

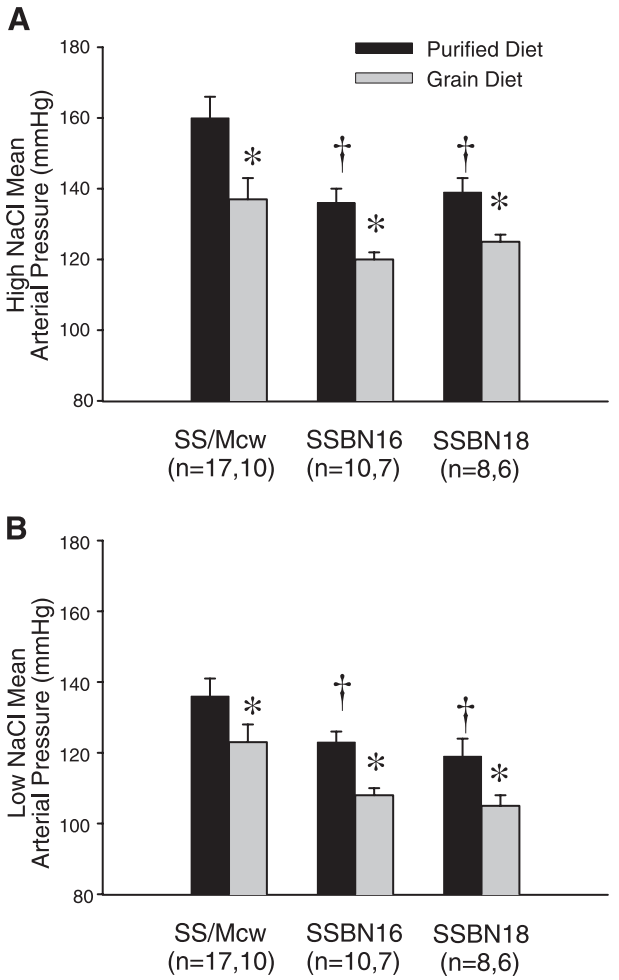

Fig. 3. MAP in Dahl SS/Mcw rats and in consomic rats in which chromosome 16 (SS.BN16) and chromosome 18 (SS.BN18) from the Brown Norway rat have been substituted on the SS/Mcw genetic background. The rats were maintained throughout life on the purified AIN-76A diet or the grain-based diet. Arterial pressure was measured in the rats when maintained on a high salt intake (4.0% NaCl, *A*) for 3 wk or following volume depletion with furosemide and placement on a low-salt diet (0.4% NaCl, *B*). *$P < 0.05$ vs. same strain on AIN-76A diet. †$P < 0.05$ vs. Dahl SS/Mcw on same diet.

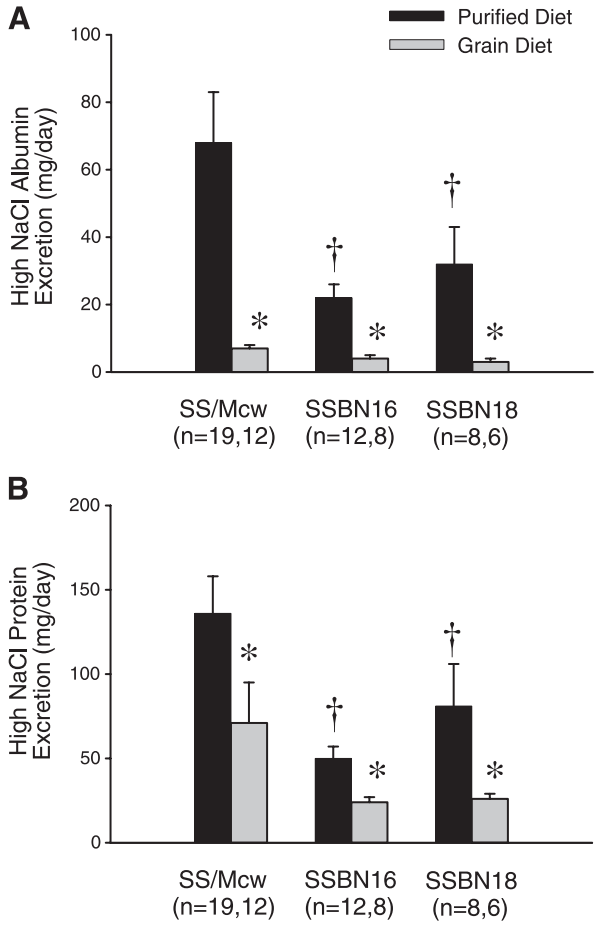

Fig. 4. Albumin excretion (*A*) and protein excretion (*B*) in Dahl SS/Mcw rats and consomic rats in which chromosome 16 (SS.BN16) and chromosome 18 (SS.BN18) from the Brown Norway rat have been substituted on the SS/Mcw genetic background. The rats were maintained throughout life on the purified AIN-76A diet or the grain-based diet. Excretion rates were measured when the rats had been maintained on a high sodium intake (4.0% NaCl) for 3 wk. *$P <$ 0.05 vs. same strain on AIN-76A diet. †$P < 0.05$ vs. Dahl SS/Mcw on same diet.

with the same strains maintained on the whole grain diet after volume depletion. Moreover, the level of MAP was significantly greater after volume depletion in the Dahl SS/Mcw than was measured in either the SS.BN16 or SS.BN18 rats when fed either diet. Heart rates after volume depletion averaged 385 ± 8 in SS/Mcw, 387 ± 12 in SS.BN16, and 383 ± 8 beats/min in SS.BN18 rats maintained on the purified diet.

Urinary protein and albumin excretion were also significantly affected by both diet and substitution of chromosomes 16 and 18 (Fig. 4). The excretion of albumin and protein was significantly greater in each strain when fed the purified AIN-76A diet compared with rats fed the whole grain diet. Substitution of chromosome 16 or 18 also led to a significant decrease in protein excretion compared with the Dahl SS/Mcw rat when the rats were maintained on the purified diet. When the rats were fed the grain diet, however, albumin excretions in the Dahl SS/Mcw, SS.BN16, and SS.BN18 rats were not significantly different.

Body weight, sodium excretion, plasma creatinine concentration, and PRA are presented in Table 2. Body weight was significantly higher in the age-matched Dahl SS/Mcw, SS.BN16, and SS.BN18 rats fed the purified diet compared with the rats fed the grain diet. In addition, the body weights of the SS.BN16 and SS.BN18 were significantly greater than in the SS/Mcw on the same diet. There were no significant differences in PRA between the congenic and Dahl SS/Mcw fed the same type of diet. No significant differences were detected in plasma creatinine concentration between the strains or within the strains maintained on the purified AIN-76A or the whole grain diet.

Sodium excretion was not affected by the purified or grain diet when comparing within the SS/Mcw or SS.BN18 groups, indicating that the SS/Mcw and SS.BN18 rats consumed the same amount of food when maintained on either diet. In contrast, sodium excretion in the SS.BN16 rats maintained on high or low salt was significantly higher in the rats fed the purified diet compared with the SS.BN16 rats fed the whole grain diet, indicating that the SS.BN16 consumed more of the purified AIN-76A food than the whole grain diet and possibly explaining the differences in body weight between the groups. In addition, when comparing between strains, both the SS.BN16 and SS.BN18 rats excreted significantly more sodium than the SS/Mcw when maintained on the purified diet.

Representative histological images of kidneys obtained from SS/Mcw rats maintained on the purified or whole grain diet are presented in Fig. 5. Consistent with previous reports in the Dahl SS/Mcw rat (12), severe glomerular sclerosis (blue fibrotic tissue and collapsed capillary structure) and blocked tubules in the outer medulla (red protein deposition casts) are evident in the kidney of the SS/Mcw rat maintained on the purified AIN-76A diet. Less glomerular and tubular injury is visibly evident in the kidney of the SS/Mcw maintained on the whole grain diet. The glomerular injury score and the percent-

Table 2. *Comparison of experimental parameters in SS/Mcw, SS.BN16, and SS.BN18 rats maintained on a purified or a grain-based diet*

	SS/Mcw		SS.BN16		SS.BN18	
	Purified diet	Grain diet	Purified diet	Grain diet	Purified diet	Grain diet
Body wt, g	340±9 (19)	303±6* (13)	360±5† (12)	331±4*† (8)	365±8† (10)	292±8* (7)
LS PRA, ng ANG I·ml^{-1}·h^{-1}	2.8±0.6 (14)	2.0±0.6 (5)	3.0±0.2 (10)	2.3±0.7 (6)	2.5±0.6 (7)	2.8±1.0 (5)
HS PRA, ng ANG I·ml^{-1}·h^{-1}	0.84±0.13 (13)	0.68±0.30 (7)	1.13±0.11 (10)	0.77±0.36 (6)	0.70±0.16 (8)	0.30±0.03 (5)
LS plasma creatinine, mg/dl	0.39±0.06 (14)	0.32±0.02 (7)	0.35±0.01 (7)	0.30±0.06 (6)	0.35±0.01 (7)	0.33±0.02 (4)
HS plasma creatinine, mg/dl	0.33±0.03 (13)	0.28±0.04 (9)	0.33±0.01 (10)	0.30±0.01 (6)	0.31±0.02 (7)	0.32±0.01 (6)
LS sodium excretion, meq/day	1.5±0.2 (19)	1.3±0.10 (12)	1.6±0.2 (10)	1.1±0.2 (8)	1.3±0.2 (8)	1.2±0.3 (6)
HS sodium excretion, meq/day	9.5±1.3 (19)	9.5±1.5 (10)	18.3±0.6† (10)	12.5±1.3* (8)	13.6±0.5† (8)	11.8±1.8 (6)

Values are means ± SE; no. of animals are in parentheses. *$P <$ 0.05 vs. same strain on AIN-76A diet. †$P < 0.05$ vs. Dahl SS/Mcw on same diet.

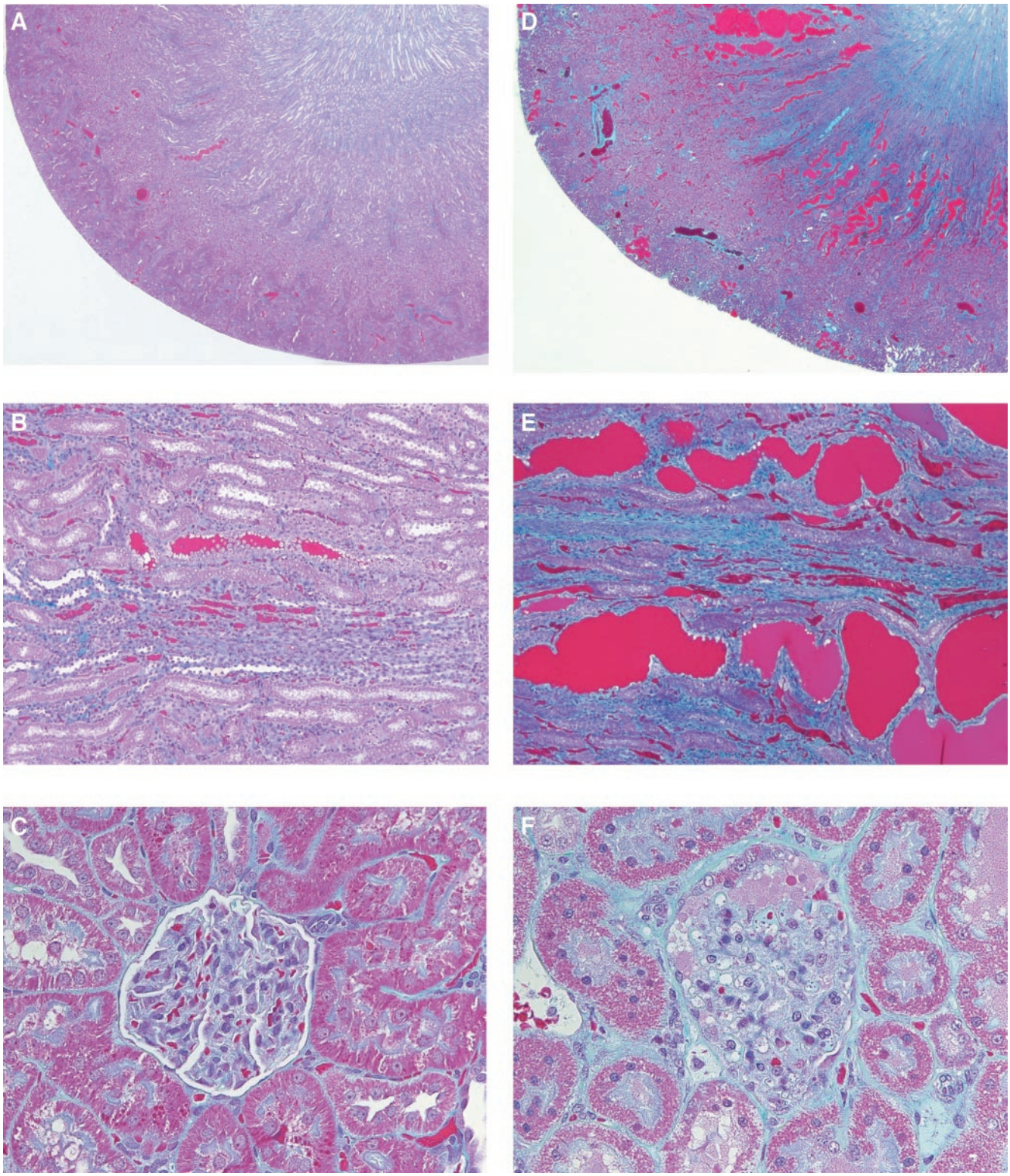

Fig. 5. Light microscopy images of whole kidneys (1×, original magnification; *A* and *D*), renal outer medullary regions (10×, original magnification; *B* and *E*), and glomeruli (40×, original magnification; *C* and *F*) of Dahl SS/Mcw rats maintained on whole grain diet (*A–C*) or the purified AIN-76A diet (*D–F*). Severe glomerular sclerosis (blue fibrotic tissue and collapsed capillary structure) and blocked tubules (red protein deposition casts) are evident in the kidneys of SS/Mcw rats maintained on the AIN-76A diet. Visibly less glomerular and tubular injury is evident in the kidney of the SS/Mcw maintained on the whole grain diet.

age of the outer medulla consisting of blocked tubules are presented in Fig. 6. The glomerular injury index was significantly greater in the Dahl SS/Mcw rats fed the purified AIN-76A diet (3.1 ± 0.1, $n = 6$ rats, 171 total glomeruli) than in the rats fed the whole grain diet (1.6 ± 0.2, $n = 6$ rats, 158 total glomeruli). Moreover, 11.4 ± 2.9% percent of the area of the outer medulla of the rats fed the AIN-76A diet stained for protein casts (indicating blocked tubules) compared with only 0.6 ± 0.3% of the area of the outer medulla of the rats maintained on the whole grain diet.

DISCUSSION

The results of the present study indicate that there is a significant influence of the composition of the diet, independent of NaCl content, on the development of salt-sensitive hypertension and renal disease in Dahl SS/Mcw rats. This effect was also observed in the consomic rats in which chromosome 16 or 18 of the Brown Norway rat was introgressed into the Dahl SS/Mcw genetic background. Arterial blood pressure, albumin and protein 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 diet. Moreover, there was a significant influence of the diet fed to the breeders on the blood pressure and degree of renal disease observed in the offspring. Thus the nutrient composition of the diet, in addition to the NaCl content, has a significant impact on the level of blood pressure and renal disease in the Dahl SS/Mcw rat.

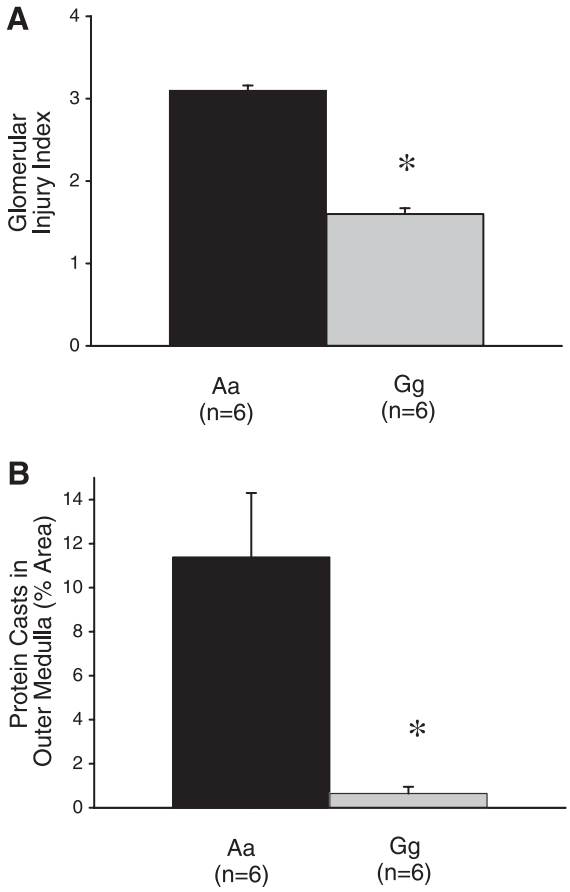

Fig. 6. Glomerular injury score (*A*) and percentage of renal outer medulla consisting of necrotic tubules (*B*) in kidneys of Dahl SS/Mcw rats maintained on purified AIN-76A or whole grain diets. *$P < 0.05$ vs. AIN-76A diet.

Influence of Dietary Composition on Hypertension and Renal Disease in the Dahl SS/Mcw Rat

The two diets employed in the present study contain similar amounts of protein (18–20%), carbohydrate (60–65%), fat (5%), and fiber (4–5%). Moreover, the percentage of sodium, potassium, vitamins, and minerals are nearly identical. The major difference between the two diets is the source of the protein, carbohydrate, and fat used to make up the diets. 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 are the source of protein in the whole grain diet. Although casein-based diets have not been demonstrated to be prohypertensive, the development of hypertension in the SHR model is accentuated in rats fed a casein-based diet compared with SHR fed a soy protein-based diet (27). Interestingly, the degree of interstitial fibrosis and tubular necrosis accompanied by marked proteinuria and salt-sensitive hypertension observed in the Dahl SS/Mcw fed the purified AIN-76A diet is similar to that observed in normal rats overloaded with protein by exogenous administration of bovine serum albumin (1). The protein content and composition of protein in the diet may therefore have an important influence on the development of hypertension and renal disease.

A second difference between the two diets used in the present study is the source of carbohydrate. The carbohydrate mixture of the AIN-76A diet consists of primarily simple sugar (2/3 sucrose) and starch (1/3 corn starch) compared with the complex carbohydrates in the grain diet. This may also play a role in the hypertensive effects of the purified diet since sucrose feeding to Dahl salt-sensitive rats (21, 26, 39), SHR (38), and Sprague-Dawley rats (8, 18, 30) leads to an increase in arterial blood pressure. This effect has typically been attributed to increased insulin resistance due to the rapid conversion of simple sugars consumed in the diet to glucose with the subsequent enhanced demand for insulin (2); the increased insulin resistance may then increase sodium retention or enhance vascular resistance. The marked differences in renal interstitial fibrosis and renal damage seen in the Dahl SS/Mcw rats fed the purified AIN-76A diet, however, suggest that other factors may contribute to this response.

The third major difference between the diets is that the source of fat in the purified AIN-76A diet is corn oil, whereas soybean oil provides the fat source in the whole grain diet. Soybean oil is a rich source of linolenic acid, whereas the corn oil is deficient in this fatty acid (31), which is the precursor for the synthesis of arachidonic acid and eicosanoids (24). The source of dietary fat has also been demonstrated to affect blood pressure in rats. A diet combining linoleic acid and sucrose was prohypertensive in Dahl SS rats (39), and stroke-prone SHR rats fed a diet rich in linolenic acid had lower blood pressure and a longer lifespan compared with stroke-prone SHR fed a diet enriched in linoleic acid (33). Although the exact constituent(s) of these two diets which accounts for the phenotypic differences observed in the present study is not known, the differing sources of protein, carbohydrate, and fat in the diets

indicate potential sources that could account for the observed phenotypic differences in the Dahl SS/Mcw rats.

One of the more striking findings in the present study is that the diet fed to the parents affects the severity of the disease in the offspring. Feeding the purified AIN-76A diet to the breeders led to a significant elevation in body weight and arterial blood pressure after volume depletion, independent of the diet of the offspring. Moreover, there was a tendency for the offspring of parents fed the AIN-76A diet to have a higher MAP and urinary albumin excretion when fed a high-salt diet. The potential for maternal influences to alter blood pressure in human populations and in animal models has been well recognized and documented (4, 5, 40). It has been reported that human infants who are either small at birth, small in relation to placental size, short at birth, or who fail to gain weight in infancy have increased rates of cardiovascular disease and non-insulin-dependent diabetes (4, 5). These effects are often blamed upon maternal undernutrition. More recently, it has been recognized that a maternal nutritional imbalance may also be a predisposing factor for cardiovascular and other diseases in humans (3). Furthermore, a recent report demonstrated that feeding pregnant rats a diet supplemented with 25% lard led to hypertension in normally fed female offspring (20). Although the present study did not address birth weight or birth size of the pups from the breeders maintained on the different diets, we observed that litter size was not different between the groups of rats with parents maintained on the AIN-76A or the whole grain diet. Moreover, the adult body size was greater in the rats whose parents were maintained on the AIN-76A diet, which led to increased hypertension and renal disease. Additional studies will be required to elucidate the influence of the diets on the size of the pups at birth as well as any nutritional imbalance that may be present in either diet.

Influence of Chromosomal Substitution on Hypertension and Renal Disease in the Dahl SS/Mcw Rat

The present studies also examined the effect of substitution of chromosomes 16 or 18 from the normotensive BN rat into the Dahl SS/Mcw on hypertension- and renal disease-related phenotypes. Substitution of either chromosome 16 or 18 from the BN to the Dahl SS/Mcw rat led to a reduction in MAP compared with the SS/Mcw parental rat when maintained on the AIN-76A diet. Furthermore, as an indicator of renal disease, urinary protein and albumin excretion rates were also reduced in the two consomic strains when fed the purified diet. These data indicate that genes important in salt-sensitive hypertension and renal disease are located on chromosomes 16 and 18 of the Dahl SS/Mcw rat. Our group recently published a linkage analysis of an intercross between the Dahl SS/Mcw and Brown Norway salt-insensitive rats from inbred colonies at MCW (10, 35). Total genome scans were performed using 217 polymorphic genetic markers on 113 male F2 rats phenotyped for 219 measured or derived traits. This linkage analysis indicated the existence of a broad range of traits related to pathways of functional importance in hypertension that mapped to 19 different chromosomes. Of particular significance was a cluster of blood pressure-related traits that mapped to chromosome 18. The present data obtained in the consomic SS.BN18 rat confirm that blood pressure- and renal disease-related genes are located on chromosome 18.

The substitution of chromosome 16 also partially attenuated the degree of hypertension and renal disease in the SS/Mcw rats, indicating that important disease genes also reside on this chromosome. This result was somewhat unexpected since the previous genetic linkage study of an intercross between the Dahl SS/Mcw and Brown Norway rats did not identify quantitative trait loci for blood pressure or renal disease on chromosome 16 (10, 35). This observation is not completely surprising since the heterogeneous genetic background of the F2 may mask the effects of the alleles found on chromosome 16. Both phenotypic noise and heterogeneous genetic backgrounds may make the detection of weak or complex quantitative trait loci (QTLs) difficult. Moreover, our group has previously reported that substitution of chromosome 13 from the BN rat into the SS/Mcw background also had a profound effect on hypertension and renal disease (12), despite a lack of QTLs identified in the F2 cosegregation study (10, 35). The data obtained from the SS.BN16 consomic rats therefore reveal the presence of additional genes of hypertension and/or renal disease on chromosome 16 that were not revealed in the F2 linkage study.

Interaction between Environment (Diet) and Genetics in Hypertension and Renal Disease in the Dahl SS/Mcw Rat

An additional interesting observation from the present studies is the interaction between environment and genetics in the determination of the final disease phenotype. Substitution of chromosomes 16 and 18 from the BN to the Dahl SS/Mcw rat led to a significant reduction in arterial blood pressure as well as a significant reduction in albumin and protein excretion in the rats maintained on the purified diet. Interestingly, the influence of chromosomal substitution was diminished or eliminated when the rats were maintained on the grain diet despite the same level of NaCl intake. The effect of diet on the disease phenotypes in the consomic rats demonstrates that environmental factors can have a profound impact on hypertension and renal disease. It is important to recognize that a host of other factors can also affect these disease phenotypes; the age of the rats, the period of time maintained on the high-salt diet, and the NaCl content of the diet are just a few of any number of factors that can potentially affect the final disease phenotype in the SS/Mcw rats. The present data therefore demonstrate the importance of both genetic and environmental factors in the development of hypertension and renal disease in the Dahl SS/Mcw rat.

The results of these studies in experimental animals may have important implications to human health and dietary effects in hypertensive and renal disease patients. The hypertensive disease process in Dahl SS rats exhibits many similarities to that observed in hypertensive African Americans (9, 11, 13, 14, 23). This inbred strain of rats has a low-renin, sodium-sensitive form of hypertension that is associated with a progressive decline in renal function that often results in end-stage renal disease (32, 36). The present data, indicating that the source of protein, carbohydrate, fat, and/or other dietary components can have a significant impact on disease in experimental models of hypertension and renal disease, are consistent with human epidemiological data that have demonstrated an association between protein, carbohydrate, and fat intake and the level of arterial blood pressure in humans (15, 16, 19).

Further studies to discern the dietary components leading to accentuation of the disease phenotypes, along with the dissection of the genes of hypertension, may provide important clues regarding the interactions between diet and genes that affect hypertension and renal disease.

Although the mechanisms leading to hypertension and renal disease in the present study remain to be determined, it is interesting that the groups of rats with the greatest body weight tended to have the highest blood pressure, greatest albumin and protein excretion rates, and also tended to have the highest PRA levels on low or high NaCl intake. This trend is consistent with the observations made in obese patients (37), in which a weight reduction was associated with a lowering of arterial pressure and decreased PRA; and epidemiological data which have demonstrated a positive association between obesity and high blood pressure (25). Moreover, PRA is elevated in obesity-induced hypertension in dogs (17). Although additional studies need to be performed, the present data support a role of PRA in the elevation in blood pressure in these rats.

In summary, the present data indicate that there is a significant influence of the composition of the diets fed the parents as well as the offspring on the development of hypertension and renal disease in Dahl SS rats. The severity of the hypertensive and renal disease associated phenotypes was greater in Dahl SS/Mcw, SS.BN16, and SS.BN18 rats fed the purified AIN-76A diet compared with the whole grain diet. These results indicate that the source of protein, carbohydrate, and fat in the diet can have a profound impact on the severity of hypertension and renal disease in the Dahl SS genetic model of hypertension. Although the mechanism of the effect of diet on the severity of hypertension and renal disease in susceptible individuals is not yet understood, dietary and other environmental influences can clearly have a large impact on the results of genetic studies.

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