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Effects of Genotype and Diet on Age-Related Lesions in Ad Libitum Fed and Calorie-Restricted F344, BN, and BNF3F1 Rats

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The effects of calorie restriction (CR) on age-related lesions in Brown Norway, Fischer 344, and BNF3F1 hybrid rats are presented. A logistic regression analysis of data from histologic samples from rats of each genotype, sex, and diet at 12, 18, 24, 30, and 36 months of age demonstrated the effects of age, diet, and sex on lesion prevalence in all three genotypes. CR reduced the prevalence of neoplastic, nonneoplastic proliferative, and degenerative lesions. All genotype-sex-age cohorts demonstrated a reduced average lesion burden with CR. Importantly, some lesions common to Brown Norway rats seldom occurred in Fischer 344 rats and vice versa. Some lesions that occurred in only one parental strain also occurred in BNF3F1 rats. Many traits occurred in all three genotypes but at significantly different prevalence rates. We suggest that the diseases and lesions that rats develop as they age are controlled by genes and environmental factors such as CR.

A GENERAL consensus among gerontologists is that calorie restriction (CR) slows the aging process, at least in the species examined to date (1–3). The mechanism by which CR achieves this remains to be established. Among its many effects on aging, CR has been shown to reduce the prevalence of both spontaneously occurring and induced neoplastic diseases (1–4). Arguably, this is an important reason why rodents on a CR diet live longer than those fed ad libitum (AL). This study was designed to determine the effect of lifelong CR on the prevalence of all lesions in cohorts of approximately 30 rats of both sexes and of three genotypes, Fischer 344 (F344), Brown Norway (BN), and the BN × F344 F1 hybrid (BNF3F1), necropsied at 12, 18, 24, 30, and 36 months of age. The effects of age, diet, and sex on the prevalence of every common lesion within each genotype were assessed by fitting linear logistic regression models. Lesions were designated as neoplastic, nonneoplastic proliferative, or degenerative based on their known or assumed pathology, and the effect of CR on each lesion category was determined. The effect of genotype on lesion prevalence was assessed in 24- and 30-month-old rats. Finally, the effects of age, genotype, diet, and sex on the average number of lesions per rat were assessed.

Materials and Methods

All of the rats used in this study were obtained from the specific pathogen-free, barrier-maintained colony at the National Center for Toxicologic Research (NCTR), Jefferson, AR. The rats were weaned at 3 weeks and were subsequently single housed in polycarbonate cages. Rats allocated to the AL cohorts received NIH-31 open formula diet (Purina Mills, Inc., Richmond, IN), and CR rats were fed a modified version of NIH-31 that was supplemented with 1.67× the fat-soluble and B-complex vitamins as the AL chow. The introduction of reduced caloric allotment began at 12 weeks and was reduced stepwise until 14 weeks, when rats were receiving 40% fewer calories than age-matched AL animals. The CR cohorts were maintained at this level of caloric restriction relative to age-matched AL cohorts throughout their lifespan.

The rats were shipped to the U.S. Department of Agriculture (USDA) Human Nutrition Research Center on Aging at Tufts University by air (Delta Air Cargo, Atlanta, GA) and underwent necropsy within 12 hours of receipt. The original experimental design included 30 male and 30 female rats of each genotype at each age from both the AL and CR diet groups. Table 1 presents the actual number of animals in each age-diet-sex-genotype group in this study. Older age cohorts of AL animals usually had fewer than 30 animals, because few animals lived to these advanced ages (4).

Within 12 hours after arrival each rat was placed in a chamber filled with pure CO_2, resulting in loss of consciousness in 30 seconds and death in 5 minutes. The left side of the atrium was cut and a 19 × 7/8 gauge butterfly needle (Abbott Hospitals, Inc., North Chicago, IL) was placed in the right ventricle. Each rat was perfused with saline followed by Tellyesniczky's fixative, a mixture of formalin, glacial acetic acid, and 70% ethanol at a ratio of 2:1:20. The rats were then postfixed for several weeks. The tissues analyzed from each animal included the salivary and lacrimal glands, submandibular lymph node, trachea, thyroid and parathyroid glands, heart, lung, stomach, duodenum, jejunum, ileum, cecum, mesenteric lymph node, colon, adrenal gland, pituitary, kidney, urinary bladder, liver, spleen, pancreas, knee, muscle of the leg, spine with spinal cord in situ, eye, cerebrum, thalamus, midbrain, medulla, cerebellum, and either testes and accessory male sex organs or ovary and uterus. Samples of any grossly visible lesions were also taken. After the tissues were dehydrated and infiltrated with paraffin, 6-μm sections were cut and stained with hematoxylin and eosin (H&E). Histologic sections were examined and lesion data for each rat were entered into a relational database (FoxPro, Microsoft, Redmond, WA). No quantitative

Table 1. Number of Rats, Listed for Each Genotype-Diet-Sex-Age Group

Genotype	Diet	Age (mo.)	Female	Male
F344	AL	12	30	26
		18	29	29
		24	28	35
		30	15	5
	CR	12	32	24
		18	29	27
		24	28	30
		30	18	27
		36	21	3
BN	AL	12	25	24
		15	1	4
		18	33	32
		24	29	38
		29	1	0
		30	30	47
		33	0	1
		36	1	0
	CR	12	30	29
		15	0	2
		18	30	25
		24	40	40
		27	0	1
		29	3	1
		30	23	26
		33	0	8
		36	28	8
		42	6	6
BNF3F1	AL	12	25	29
		18	28	30
		24	45	27
		30	28	30
		36	19	30
		42	1	1
	CR	12	30	27
		18	29	28
		24	42	29
		30	36	27
		36	30	18
		42	26	16

measure for the severity of the lesion was made in this study. If any particular lesion occurred more than once in any animal, the animal was scored only once for that lesion.

An analysis of the data began with a calculation of the prevalence of lesions in each genotype-age-diet-sex cohort. Table 2 lists all rare and common lesions observed in each of the three genotypes, including both sexes and diet groups in this study. Arbitrarily, common lesions were defined as those occurring in at least 10% of the animals of any genotype-diet-sex cohort. For comparison of lesion prevelance in older animals, percentages rather than raw numbers are presented because the size of the various cohorts varied (see Table 4 below). Many lesions occurred too infrequently to be analyzed statistically.

The data were analyzed by the use of SYSTAT version 5.2 for the Macintosh, SYSTAT version 7.0.1 for Windows (SPSS Inc., Chicago, IL), and SAS for Windows version 6.12 (SAS Institute, Cary, NC). The effects of age, diet, and sex on the

Table 2. Lesion Type and Frequency of Occurrence

Organ	Lesion	BN* (n = 572)	F344* (n = 436)	BNF3F1* (n = 631)
Adipose tissue	steatitis	0	1	1
Adrenal gland	adenoma	10	9	12
	adrenal ectasia	1	0	1
	ectopic bone	1	0	0
	focal cortical hyperplasia	48	1	16
	focal cytoplasmic vacuolization	88	5	8
	focus of enlarged pale cells	202	1	67
	ganglioneuroma	0	0	1
	mineralization	0	0	1
	pheochromocytoma	11	10	29
Any site	fibroadenoma	3	6	5
	fibroma	0	3	3
	fibrosarcoma	1	1	4
	hemangiosarcoma	2	0	0
	leiomyoma	3	0	3
	mesothelioma	0	3	3
	neurofibroma	0	0	2
	rhabdomyosarcoma	0	1	0
Blood vessel	lymphoid nodule	1	1	0
	mineralization	0	3	1
	polyarteritis nodosa	3	1	4
Bone	chondroma	1	0	0
	cyst	0	1	0
	osteoma	1	0	0
Brain	astrocytoma	0	0	1
	glioma	0	0	2
	hydrocephelus	4	1	1
	infarct	2	0	0
	myoblastoma	5	0	4
Epididymus	adenoma	0	1	0
	granuloma	3	0	7
Exobital gland	Harderian gland metaplasia	24	57	0
Eye	cataract	31	20	0
	melanoma	2	0	0
	mineralization	0	3	0
	retinal degeneration	8	125	1
Harderian gland	adenoma	0	0	1
	cyst	1	0	0
	lymphoid nodule	0	4	2
Heart	endocardial fibrosis	13	0	7
	fibrosis	36	146	90
	infarct	1	0	0
	leukocyte nodule	0	0	3
	lymphoid nodule	2	0	3
	myocardial degeneration	11	1	6
	myocarditis	0	12	18
	thrombosis	0	1	0
Kidney	adenoma	1	1	0
	calculi	7	0	0
	carcinoma	1	0	1
	cysts	1	1	0
	focal cortical atrophy	1	0	9
	focal mineralization	16	205	254
	glomerulonephropathy	0	65	5
	hydronephrosis	257	0	6

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Table 2. Lesion Type and Frequency of Occurrence (*continued*)

Organ	Lesion	BN* ($n = 572$)	F344* ($n = 436$)	BNF3F1* ($n = 631$)
Kidney (*continued*)	hyperlplasia of urothelium	62	34	103
	lymphoid nodule	20	12	69
	metaplastic bone	2	0	2
	mineralization of ureter	5	0	15
	pigment	3	1	3
	protein casts in renal tubules	41	56	52
Liver	basophilic nodule	0	0	3
	bilary cyst	12	1	2
	bile duct hyperplasia	6	107	36
	diffuse fatty change	4	6	4
	ectasia	1	6	0
	fibrosis	3	0	0
	focal fatty change	1	15	3
	granuloma	0	6	0
	hepatocarcinoma	1	0	3
	leukocytic nodule	3	1	13
	liver necrosis	0	23	4
	lymphoid nodule	21	37	58
Liver or spleen	leukemia	2	69	1
Lung	adenocarcinoma	2	2	12
	alveolar epithelial hyperplasia	2	0	1
	alveolar histiocytosis	134	0	24
	granuloma	4	4	0
	lymphoid nodule	304	245	398
	metastasis to the lung	3	0	2
	mineralization	2	4	0
	pigment	3	0	0
Lymph node	cyst	8	5	1
	pigment	2	4	28
Mammary gland	adenocarcinoma	0	2	0
	adenoma	1	0	3
	hyperplasia	0	2	2
Ovary	atrophy	0	0	2
	cyst	2	1	8
	ectasia	0	3	1
	pigment	1	0	24
Pancreas	adenoma	0	2	1
	exocrine atrophy	77	39	49
	islet cell adenoma	7	12	13
	islet cell carcinoma	1	0	1
	islet cell hyperplasia	62	6	23
	lymphoid nodule	2	1	2
	lymphoid nodule in islet	1	0	3
Pituitary	adenoma	10	39	92
	cyst	0	0	2
	ectasia	0	2	0
	pigment	3	0	2
Prostate	adenoma	1	0	4
	atrophy	5	1	2
	hyperplasia	0	0	10
	inflammation	1	2	2
	lymphoid nodule	2	0	0
	mineralization	1	0	8
Salivary gland	atrophy	1	6	1
	lymphoid nodule	6	0	3

Table 2. Lesion Type and Frequency of Occurrence (*continued*)

Organ	Lesion	BN* ($n = 572$)	F344* ($n = 436$)	BNF3F1* ($n = 631$)
Skin	epidermal inclusion cyst	0	0	1
	hair matrix tumor	0	0	3
	melanoma	5	0	3
	papilloma	1	0	2
	sebaceous adenoma	0	2	0
	sebaceous gland cyst	0	0	6
Spinal root	degeneration	142	45	210
Stomach	cystic glands	9	33	80
	papilloma	0	0	1
	squamous cell carcinoma	0	1	0
	ulcer	3	1	1
Testis	atrophy	166	25	36
	Leydig cell hyperplasia	4	78	18
	Leydig cell tumor	0	60	21
	mineralization	1	2	14
Thyroid	adenoma	1	1	6
	carcinoma	0	0	3
	cystic follicle	21	3	51
	C-cell adenoma	1	13	17
	C-cell carcinoma	0	1	0
	C-cell hyperplasia	0	57	7
Trachea	tracheal gland cyst	20	28	112
Urinary bladder	adenoma	0	0	1
	carcinoma	0	0	1
	edema	0	0	6
	lymphoid nodule	2	4	15
	papilloma	0	0	1
Uterus	cystic endometrium	2	1	23
	endometrial hyperplasia	0	2	3
	fibrosis	48	30	16
	polyp	6	9	16

*The two diet and two sex groups have been combined for each genotype.

prevalence of every common lesion within a genotype were assessed by fitting linear logistic regression models as follows:

$$\log(\text{odds}) = \mu + \beta_{age}\text{age} + \beta_{diet}\text{diet} + \beta_{sex}\text{sex},$$

where age is the age in months, diet is coded 1 for AL and 2 for CR, and sex is coded 1 for males and 2 for females; here μ is the constant and β_i is the parameter estimate for i. No rats older than 30 months were included in this analysis because there were too few older AL animals to obtain reliable models. Sex effects were not assessed for lesions in sex-specific organs such as the ovary, uterus, and testis. Wald statistics were used to assess the statistical significance of the coefficients.

A positive value of β_{diet} indicates a greater prevalence in CR animals, whereas a negative value indicates a greater prevalence in AL animals. A positive value of β_{sex} indicates a greater prevalence in females, whereas a negative value indicates a greater prevalence in males. The logistic regression models and derived odds ratios [exp(β)] are presented in Table 3. The odds ratio gives the (multiplicative) change in odds of having the lesion resulting from a unit change in the predictor.

Diet-by-linear age interactions, $\beta_{age\text{-}diet}$age $\times$ diet, were considered and allowed to remain in the model when their contri-

Table 3. Linear Regression Analysis

Genotype	Organ	Lesion	Type*	Statistic†	Parameter‡				
					Age	Diet	Sex	Age × Diet	*y* Intercept
F344	exorbital gland	Harderian gland metaplasia	p	*p*	0.001	0.002	0.001		
				β	0.10	−1.09	2.91		−7.61
				OR	1.10	0.34	18.30		
	heart	fibrosis	d	*p*	0.001	0.001	0.001		
				β	0.20	−1.86	−0.64		−1.35
				OR	1.22	0.16	0.53		
	kidney	glomerulonephropathy	p	*p*	0.001	—	0.001	0.016	
				β	0.79	1.50	−4.38	−0.33	−5.76
				OR	2.20	4.30	0.01	0.72	
		mineral deposition	d	*p*	0.001	0.329	0.001		
				β	−0.08	−0.40	7.44		−10.61
				OR	0.92	0.67	999.00		
		protein casts	d	*p*	—	0.001	0.001		
				β	0.04	−3.09	1.16		−0.06
				OR	1.04	0.05	3.18		
	liver	bile duct hyperplasia	p	*p*	0.001	0.001	0.001		
				β	0.15	−1.97	−1.41		0.47
				OR	1.16	0.14	0.24		
		leukemia	n	*p*	0.001	0.001	0.013		
				β	0.25	−1.64	−0.81		−4.20
				OR	1.29	0.20	0.44		
		lymphoid nodule	p	*p*	0.524	0.034	0.001		
				β	0.02	−0.78	1.39		−3.91
				OR	1.02	0.46	4.03		
		necrosis	d	*p*	0.001	0.061	0.001		
				β	−0.17	−0.90	−1.84		3.84
				OR	0.85	0.41	0.16		
	lung	lymphoid nodule	p	*p*	0.013	0.033	0.732	0.037	
				β	−0.13	−1.40	−0.07	0.06	3.14
				OR	0.88	0.25	0.94	1.07	
	pancreas	atrophy	d	*p*	0.002	0.001	0.069		
				β	0.09	−1.76	−0.67		−0.98
				OR	1.10	0.17	0.51		
	retina	degeneration	d	*p*	0.001	0.540	0.191		
				β	0.11	0.15	0.31		−4.71
				OR	1.12	0.54	0.19		
	stomach	cysts	d	*p*	0.001	0.005	0.002		
				β	0.13	−1.25	1.73		−6.80
				OR	1.14	0.29	5.64		
	testis	Leydig cell adenoma	n	*p*	0.001	0.001			
				β	0.10	−2.27			−1.09
				OR	1.11	0.10			
		Leydig cell hyperplasia	p	*p*	0.001	0.001			
				β	−0.82	−7.86			14.52
				OR	0.44	0.00			
	thyroid	C-cell hyperplasia	p	*p*	0.001	0.001	0.562		
				β	0.12	−2.56	0.18		−1.26
				OR	1.12	0.08	1.20		
	trachea	cysts	d	*p*	0.029	0.001	0.691		
				β	0.08	−2.60	0.18		−1.41
				OR	1.08	0.08	1.19		
BN	adrenal gland	focal cortical hyperplasia	p	*p*	0.001	0.001	0.001		
				β	0.09	−0.96	−1.99		1.50
				OR	1.09	0.38	0.14		
		focal cytoplasmic vacuolization	d	*p*	0.001	0.001	0.019		
				β	0.11	−1.14	0.65		−4.14
				OR	1.12	0.32	1.92		

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Table 3. Linear Regression Analysis (*continued*)

Genotype	Organ	Lesion	Type*	Statistic†	Parameter‡				
					Age	Diet	Sex	Age × Diet	*y* Intercept
BN (*continued*)									
	heart	fibrosis	d	*p*	0.001	0.001	0.001		
				β	0.18	–2.38	–2.93		–0.70
				OR	1.20	0.09	0.05		
	kidney	hydronephrosis	d	*p*	0.105	0.001	0.001		
				β	0.02	–0.75	–0.58		3.67
				OR	1.02	0.47	0.56		
		lymphoid nodule	p	*p*	0.201	0.016	—		
				β	0.05	–1.60	–13.51		11.72
				OR	1.05	–0.44	0.00		
		protein casts	d	*p*	0.001	—	0.001		
				β	0.37	–17.54	1.45		4.12
				OR	1.44	0.00	4.28		
	lung	lymphoid nodule	p	*p*	0.003	0.100	0.665		
				β	0.03	–0.29	0.08		–0.26
				OR	1.03	0.75	1.08		
	pancreas	atrophy	d	*p*	0.001	0.002	0.017	0.001	
				β	0.64	5.64	–0.75	–0.27	–15.32
				OR	1.89	281.80	0.47	0.76	
		islet cell hyperplasia	p	*p*	0.002	0.001	0.001		
				β	0.08	–3.56	–2.45		3.67
				OR	1.08	0.03	0.09		
	renal pelvis	urothelial hyperplasia	p	*p*	0.028	0.002	0.001	0.035	
				β	–0.13	–2.99	–1.72	0.08	4.72
				OR	0.88	0.05	0.18	1.09	
	spinal root	degeneration	d	*p*	0.001	0.001	0.800	0.001	
				β	1.30	12.17	–0.08	–0.50	–10.01
				OR	3.66	999.00	0.92	0.61	
	testis	atrophy	d	*p*	0.001	0.001		0.001	
				β	0.32	2.83		–0.13	–8.08
				OR	1.37	16.92		0.88	
	thyroid	follicular cysts	d	*p*	0.005	—	—		
				β	0.14	–13.58	–13.03		21.56
				OR	1.15	0.00	0.00		
	uterus	fibrosis	d	*p*	0.026	0.001			
				β	0.05	–2.81			–0.03
				OR	1.06	0.06			
BNF3F1	adrenal gland	foci of pale staining cells	d	*p*	0.003	0.001	0.002		
				β	0.05	–1.45	–0.95		–0.38
				OR	1.05	0.24	0.39		
	heart	fibrosis	d	*p*	—	0.007	0.001	0.020	
				β	0.00	–3.89	–1.41	0.10	1.18
				OR	1.00	0.02	0.25	1.10	
	kidney	lymphoid nodule	p	*p*	0.021	0.001	0.001		
				β	0.04	–1.29	–1.15		0.04
				OR	1.04	0.28	0.32		
		mineral deposition	d	*p*	0.005	0.933	0.001		
				β	0.04	–0.02	6.00		–11.95
				OR	1.04	0.98	403.19		
		protein casts	d	*p*	0.001	0.001	0.593		
				β	0.14	–1.34	–0.18		–4.58
				OR	1.16	0.26	0.84		
	liver	bile duct hyperplasia	p	*p*	0.003	0.001	0.001		
				β	0.08	–1.67	–1.83		–0.64
				OR	1.08	0.19	0.16		

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Table 3. Linear Regression Analysis (*continued*)

Genotype	Organ	Lesion	Type*	Statistic†	Parameter‡				*y* Intercept
					Age	Diet	Sex	Age × Diet	
BNF3F1 (*continued*)									
	liver (*continued*)	lymphoid nodule	p	*p*	0.469	0.098	0.398		
				β	0.01	–0.47	–0.24		–1.54
				OR	1.01	0.62	0.79		
	lung	lymphoid nodule	p	*p*	0.001	0.006	0.034	0.006	
				β	–0.11	–1.40	0.35	0.05	2.59
				OR	0.90	0.25	1.42	1.06	
	pancreas	atrophy	d	*p*	0.001	0.760	0.001		
				β	0.08	–0.11	–1.74		–2.51
				OR	1.09	0.89	0.18		
		islet cell hyperplasia	p	*p*	0.340	0.006	0.005		
				β	–0.03	–2.08	–2.14		2.59
				OR	0.97	0.12	0.12		
	pituitary gland	adenoma	n	*p*	0.001	0.001	0.001		
				β	0.09	–1.64	1.97		–5.17
				OR	1.09	0.19	7.19		
	renal pelvis	urothelial hyperplasia	p	*p*	0.017	0.001	0.001	0.001	
				β	–0.11	–4.23	1.16	0.12	0.74
				OR	0.90	0.02	3.19	1.12	
	spinal root	degeneration	d	*p*	—	0.001	0.004	0.001	
				β	–0.03	–5.35	–0.70	0.18	1.30
				OR	0.97	0.01	0.50	1.20	
	stomach	cysts	d	*p*	—	0.017	0.629	0.014	
				β	–0.02	–2.56	–0.13	0.08	–0.93
				OR	0.98	0.08	0.88	1.09	
	trachea	cysts	d	*p*	0.001	0.476	0.525		
				β	0.04	0.16	–0.14		–2.79
				OR	1.04	1.17	0.87		

Note: This analysis is for each common lesion for the parameters of age, diet, sex, and age-by-diet interactions. The analysis included only those rats 12, 18, 24, and 30 months of age.

*The lesions were categorized as either degenerative (d), neoplastic (n), or proliferative nonneoplastic (p).

†The parameter estimate, β, and the odds ratio, OR, and the significance value, *p*, for each variable are those of the corresponding Wald statistics.

‡When the value presented for *p* is .001, then $p \leq .001$. Where no *p* value is listed, —, there was computational difficulty in establishing the significance level of the given parameter because of an absence of cases in one group.

bution reached statistical significance ($p \leq .05$). When an interaction is present in the model, the odds ratio corresponding to a change in one of the predictors depends on the value of the other predictor. For example, the odds of a 12-month-old animal having a particular lesion are

$$\exp(\mu + 12\beta_{age} + \text{diet} \times \beta_{diet} + \text{sex} \times \beta_{sex} + 12 \times \text{diet} \times \beta_{age\text{-}diet}),$$

and the odds of an 18-month-old AL animal having this same lesion are

$$\exp(\mu + 18\beta_{age} + \text{diet} \times \beta_{diet} + \text{sex} \times \beta_{sex} + 18 \times \text{diet} \times \beta_{age\text{-}diet}),$$

so that the odds ratio corresponding to this 6-month age difference is obtained by subtracting the first expression from the second:

$$\exp(6\beta_{age} + 6 \times \text{diet} \times \beta_{age\text{-}diet}).$$

The observed significance levels in Table 3 are provided for every common lesion in the three genotypes for the parameters of age, diet, and sex. The age-by-diet interaction was included in this equation only when significant ($p \leq .05$). An exception to this was those cases in which one group of animals had no cases of the lesion of interest. In these instances, a significance value could not be calculated because of the inability to divide by zero.

Utilizing the data in Table 3, we find it possible to estimate the odds that a rat in a specific genotype-age-diet-sex group will have the lesion of interest. How to arrive at such an estimate is demonstrated by the use of adrenal gland cortical hyperplasia in BN rats as an example: The *y* intercept for adrenal gland focal cortical hyperplasia in BN rats is 1.50, the β_{age} is 0.09, the β_{diet} is (–0.96), and the β_{sex} is (–1.99). Thus, the log odds that a 12-month-old female AL fed BN rat will have this lesion are 1.50 + 0.09(12) – 0.96(1) –1.99(2) or –2.36, and the odds that a 12-month-old female AL BN rat will have focal cortical hyperplasia are $e^{-2.36} = 0.09$. This information is valuable for estimating the numbers of animals for future studies.

The significance of genotype on differences in the prevalence for each common lesion at 24 and 30 months of age between the two inbred strains, BN and F344 rats, and between BNF3F1 rats and each parental strain, was examined by Pearson chi-square analysis of 2 × 2 contingency tables. Only

the data from animals that were 24 and 30 months old were used for these comparisons, because the inclusion of younger animals with only a few lesions would serve only to dilute differences between genotypes by augmenting the numbers of unaffected individuals in the groups to be compared (Tables 4a and 4 b).

The number of distinct lesions in each animal in the study was counted, from which the mean ± *SE* average lesion burden within each genotype-age-diet-sex cohort was calculated. These data are presented in Figures 1–3.

Results

When the data from all three genotypes, of both sexes and diets, were combined, a total of 141 distinct lesions were observed (Table 2). A description of each common lesion follows. Most of these lesions have been previously described (4–22).

Adrenal Gland

Three lesions, focal cortical hyperplasia, focal cytoplasmic vacuolization, and foci of enlarged pale cells, occurred commonly in the cortex of the adrenal gland. All of these showed a significant increase in prevalence with age within the genotype in which it was common. All three occurred significantly less frequently in CR than in AL rats. Foci of cortical hyperplasia, common in female BN rats, were characterized as rounded proliferations of well differentiated, slightly enlarged cortical cells. Focal cytoplasmic vacuolization, common in male and female BN rats, was characterized by clusters of cortical cells with vacuolated cytoplasm. Foci of enlarged pale cells, clusters of pale staining cortical cells in the zona glomerulosa, were common in female BN and male BNF3F1 rats. Few adrenal lesions of any kind were seen in F344 rats.

Exorbital Gland

This is one of the two lacrimal glands of the rat; the other is the Harderian gland, located behind the eye. The exorbital gland of both F344 female and male BN rats often had areas that were histologically similar to the Harderian gland; this lesion was termed Harderian gland metaplasia of the exorbital gland.

Heart

One of the most frequently observed lesions was cardiomyopathy, characterized by focal areas of fibrosis and degenera-

Table 4a. Percentage of 24- and 30-Month-Old AL Rats With Each Common Lesion: Females

					χ^2 Analysis*		
Organ	Lesion (% Affected)	BN ($n = 43$)	F344 ($n = 59$)	BNF3F1 ($n = 73$)	BN vs F344 ($p =$)	BN vs BNF3F1 ($p =$)	F344 vs BNF3F1 ($p =$)
Adrenal gland	cortical hyperplasia	44.2	0	1.4	0.001	0.001	0.367
	cytoplasmic vacuolization	67.4	3.4	1.4	0.001	0.001	0.439
	enlarged pale cells	72.1	0	15.1	0.001	0.001	0.002
Breast	fibroadenoma	2.3	6.8	6.8	0.478	0.288	0.673
Exorbital gland	Harderian gland metaplasis	0	28.8	0	0.001		0.001
Heart	degeneration	20.9	0	8.2	0.001	0.049	0.024
	fibrosis	4.7	52.5	4.1	0.001	0.890	0.001
Kidney	glomerulonehropathy	0	22.0	0	0.003		0.001
	hydronephrosis	69.8	0	0	0.001	0.001	
	mineralization	18.6	61.0	94.5	0.001	0.001	0.001
	protein casts	58.1	40.7	12.3	0.015	0.001	0.003
Liver	bile duct hyperplasia	0	23.7	0	0.002		0.001
	fatty nodule	0	6.8	1.4	0.133	0.441	0.216
	leukemia	0	25.4	0	0.010		0.001
	lymphoid nodule	18.6	16.9	5.5	0.489	0.025	0.108
Lung	lymphoid nodule	81.4	33.9	65.8	0.001	0.071	0.001
Pancreas	atrophy	48.8	10.2	0	0.001	0.001	0.011
Pituitary gland	adenoma	16.3	33.9	53.4	0.141	0.001	0.004
Renal pelvis	urothelial hyperplasia	14.0	10.2	37.0	0.378	0.008	0.001
Retina	degeneration	0	23.7	0	0.002		0.001
Spinal root	degeneration	76.7	15.3	32.9	0.001	0.001	0.010
Stomach	cysts	4.7	20.3	24.7	0.057	0.006	0.281
Thyroid	C-cell adenoma	0	10.2	2.7	0.050	0.274	0.144
	C-cell hyperplasia	0	28.8	4.1	0.001	0.178	0.001
	cysts	0	0	24.7		0.001	0.001
Trachea	cysts	11.6	16.9	15.1	0.773	0.604	0.806
Uterus	fibrosis	72.1	22.0	8.2	0.001	0.001	0.075

*The results of a χ^2 analysis of prevalence in the parental inbred and the BNF3F1 are shown. When the value presented for p is .001, then $p \leq .001$.

Table 4b. Percentage of 24- and 30-Month-Old AL Rats With Each Common Lesion: Males

Organ	Lesion (% Affected)	BN ($n = 85$)	F344 ($n = 62$)	BNF3F1 ($n = 85$)	χ^2 Analysis* BN vs F344 ($p =$)	BN vs BNF3F1 ($p =$)	F344 vs BNF3F1 ($p =$)
Adrenal gland	cytoplasmic vacuolization	24.7	4.8	0	0.001	0.001	0.040
	enlarged pale cells	72.9	0	32.9	0.001	0.001	0.001
Exorbital gland	Harderian gland metaplasis	21.2	1.6	0	0.001	0.001	0.240
Heart	fibrosis	30.6	51.6	31.8	0.010	0.868	0.015
Kidney	glomerulonehropathy	0	56.5	1.2	0.001	0.316	0.001
	hydronephrosis	61.2	0	0	0.001	0.001	
	lymphoid nodule	15.3	9.7	29.4	0.316	0.027	0.004
	protein casts	17.6	3.2	15.3	0.007	0.679	0.017
Liver	bile duct hyperplasia	3.5	43.5	22.4	0.001	0.001	0.006
	leukemia	1.2	32.3	0	0.001	0.316	0.001
	lymphoid nodule	12.9	1.6	16.5	0.013	0.516	0.003
Lung	lymphoid nodule	63.5	30.6	44.7	0.001	0.014	0.084
Pancreas	atrophy	36.5	19.4	12.9	0.024	0.001	0.290
	islet cell hyperplasia	45.9	0	7.1	0.001	0.001	0.033
Pituitary gland	adenoma	1.2	12.9	8.2	0.003	0.030	0.356
Renal pelvis	urothelial hyperplasia	17.6	3.2	7.1	0.007	0.036	0.312
Retina	degeneration	1.2	19.4	0	0.001	0.316	0.001
Spinal root	degeneration	45.9	9.7	42.4	0.001	0.643	0.001
Stomach	cysts	1.2	3.2	9.4	0.385	0.017	0.141
Testes	atrophy	81.2	3.2	8.2	0.001	0.001	0.211
	Leydig cell adenoma	0	48.4	0	0.001		0.001
Thyroid	C-cell hyperplasia	0	22.6	2.4	0.001	0.155	0.001
	cysts	21.2	3.2	4.7	0.002	0.001	0.654
Trachea	cysts	2.4	14.5	18.8	0.006	0.001	0.492

*The results of a χ^2 analysis of prevalence in the parental inbred and the BNF3F1 are shown. When the value presented for p is .001, then $p \leq .001$.

tion in the myocardium. This lesion was usually most severe in papillary muscles. As seen in Table 3, the odds of having this lesion increased significantly with age in BN and F344 rats. The odds were significantly greater for AL rats than for CR rats in all three strains. Cardiomyopathy was observed in female F344 rats and in male rats of both the F344 and BNF3F1 genotypes. It occurred significantly more frequently in F344 than in BNF3F1 rats ($p \leq .005$). In a separate study of 13 F344 and 14 BN male rats that were 24 months of age, in which the severity of cardiomyopathy in the papillary muscle was graded on a scale from 0 to 4, we found that this lesion was significantly more severe in F344 than in BN rats ($p \leq .0001$; data not shown).

Kidney

Six lesions occurred commonly in the kidney: glomerulonephropathy, protein casts in tubules, hydronephrosis, lymphoid nodules, mineral deposition, and hyperplasia of the urothelium. Glomerulonephropathy is a well-known lesion of F344 rats (5–10). It is characterized by the presence of numerous severely dilated tubules in the cortex and medulla containing brightly eosinophilic protein casts. In F344 rats, the odds of having this lesion increased significantly with age; that is, old rats were 2.2 times more likely to have this lesion than younger rats. Because there was a significant age-by-diet interaction, the effects of diet are obscured, as described above. Glomerulonephropathy was common in F344 rats, never observed in BN rats, and occurred in only 5 BNF3F1 rats (Table 2).

Occasional protein casts in nondilated renal tubules were commonly observed in BN and BNF3F1 rats. Affected kidneys never had more than a few dilated tubules, in contrast to the more numerous protein casts in dilated tubules observed in the F344 rats with glomerulonephropathy. The odds of its occurrence were decreased by the CR diet (Table 3).

Hydronephrosis, grossly visible as dilation of the pelvis of either the left, right, or both kidneys, was common in BN rats. This lesions, which may be secondary to renal calculi (11), was observed in a few BNF3F1 rats, but it was always very mild. This was one of the few lesions that demonstrated no age association (Table 3).

Another common kidney lesion was deposition of small granules of mineral, often at the corticomedullary junction. This lesion was commonly observed in female F344 and BNF3F1 rats in both diet groups, but it occurred in only three males.

Hyperplasia of the urothelium of the renal pelvis occurred commonly in BN and BNF3F1 rats. This lesion consisted of proliferation of urothelium that extended as cords into the renal medulla. The prevalence of this lesion did not differ between BN and BNF3F1 female rats, but male BNF3F1 rats had signifi-

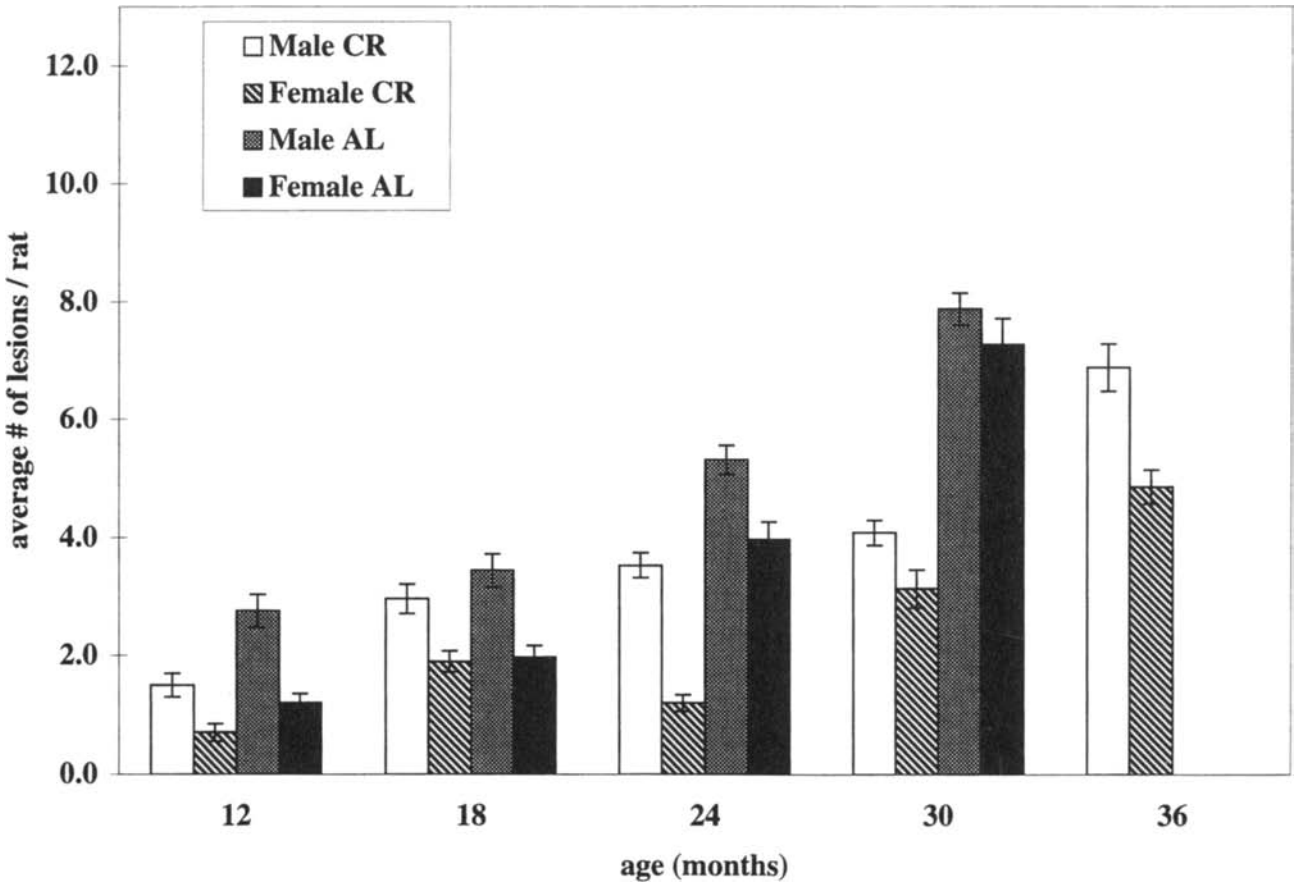

Figure 1. Age-associated lesion burden in BN rats.

cantly fewer of these lesions than did BN rats. Although these areas of proliferation were often associated with mineral deposition, this lesion, unlike mineral deposition, was common to both males and females.

Liver

Bile duct hyperplasia, characterized as the focal proliferation of bile ducts, often associated with fibrosis, was commonly observed in both male and female F344 rats and only rarely in male BNF3F1 rats. In both genotypes, the odds of having bile duct hyperplasia increased with age and were decreased by CR diet.

Large granular cell leukemia has been reported as the most common neoplasm in F344 rats (4,7,12–15). Leukemia was often associated with a grossly enlarged spleen, but in this study a diagnosis was based on the presence of neoplastic cells in sinusoids of liver. This disease was nearly exclusively restricted to F344 rats. It occurred in two BN and one BNF3F1 rats. The odds that a F344 rat had this lesion increased with age and were decreased in the CR diet group.

Focal mild coagulative necrosis of liver was common in male F344 rats. There was no significant effect of diet.

Lung

Alveolar histiocytosis was characterized by areas of pulmonary parenchyma in which alveoli were filled with macrophages containing large amounts of foamy cytoplasmic material that appeared gray in H&E sections. Alveolar histiocytosis occurred predominantly in CR BN rats. A second common lung lesion observed was small nodules of lymphocytes adjacent to the bronchi. It was common to all genotypes, and prevalence was not affected by diet, age, or sex.

Pancreas

Atrophy of the exocrine pancreas was characterized by focal or diffuse areas of exocrine tissue loss and replacement by fat. In such areas the islets were usually intact, though surrounded by adipocytes. Necrosis and inflammation were not associated with this pancreatic atrophy. This lesion was common in the AL cohorts of all six genotype-sex groups except for female BNF3F1 rats. All three genotypes showed an age-associated increase in the odds of having this lesion. The effects of diet on this lesion differed by genotype: in the BN rats, CR was associated with increased odds of having this lesion; in BNF3F1 rats, there was no significant affect of diet on the odds; and in the F344 rats, the odds of having this lesion were decreased by CR (Table 3).

Islet cell hyperplasia was commonly observed only in male BN and BNF3F1 rats, with a significantly greater prevalence in BN rats (Table 4b). In both genotypes in which it was observed, the odds that a rat had this lesion were decreased if the rat had been CR fed. The aberrant, hyperplastic islets were larger than normal and often had fingerlike projections extending into surrounding parenchyma. This morphology is distinct from the rounded configuration of hyperplastic islets seen in obese mice.

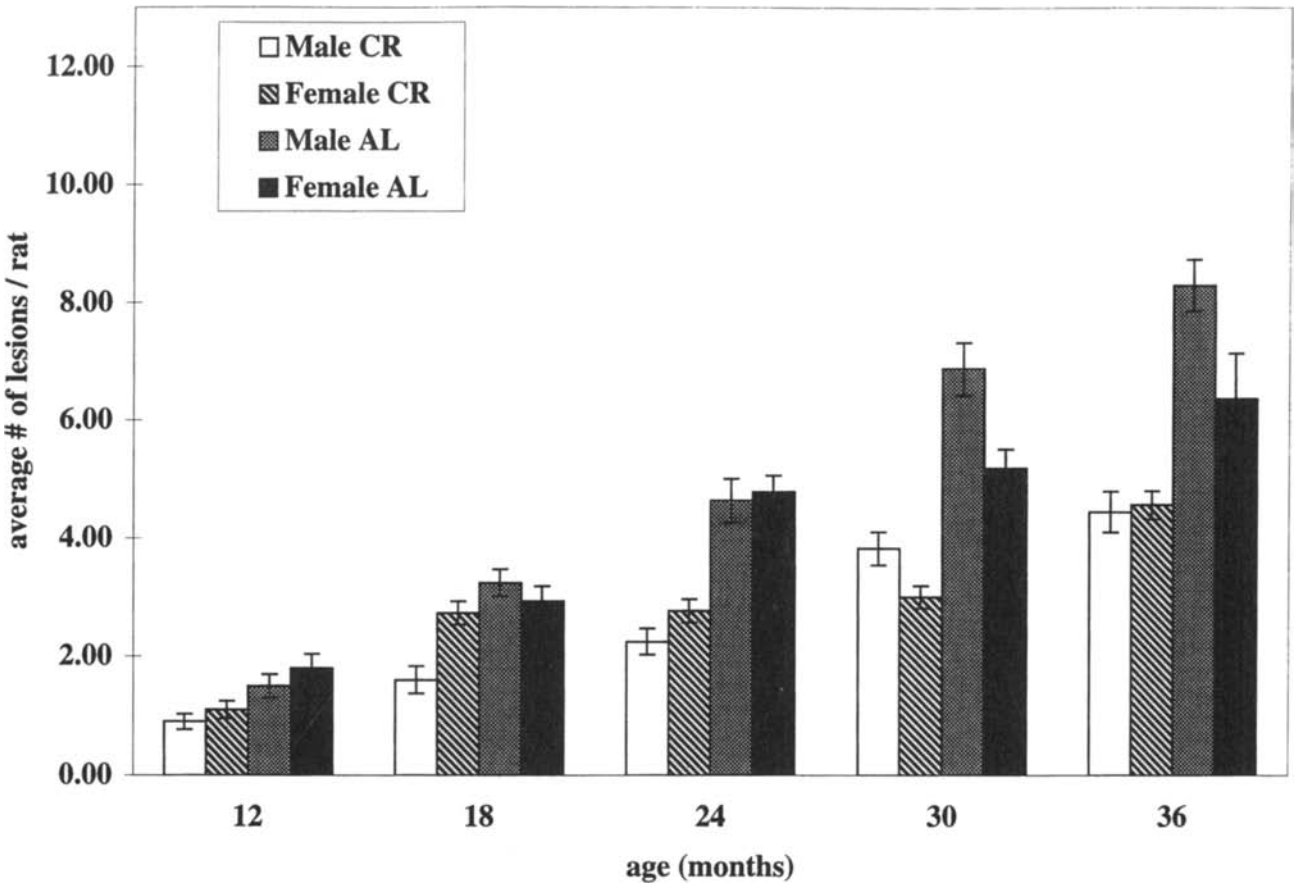

Figure 2. Age-associated lesion burden in BNF3F1 rats.

Pituitary

Pituitary adenoma was frequently observed in F344 and BNF3F1 female rats (Table 4a). In this lesion, the pituitary gland was often hemorrhagic and grossly visible upon dissection of the brain. In our study this lesion was commonly found only in female F344 rats; its overall prevalence was not great enough in the combination of males and females for it to be considered a common lesion for the genotype overall. However, among BNF3F1 rats, the odds of having this lesion were greater for females than males; the odds increased with age and decreased with CR.

Retina

Degeneration of the outer nuclear layer of retina was common in F344 rats, which are albino. The odds of a F344 rat having retinal degeneration increased with age, but they were unaffected by diet, as has previously been reported (16). A few BN and BNF3F1 CR rats at least 36 months old also had a severe loss of outer nuclear cells.

Spinal Roots

The diagnosis of the degeneration of spinal roots was based on the observance of large vacuoles in spinal roots containing cellular debris and macrophages. Affected rats often had similar changes in the sciatic nerve (data not shown). This lesion was observed in all three genotypes and both sexes at 30 months of age or older. It was significantly more common in female AL BN rats than in either F344 or BNF3F1 female AL rats. For the male AL cohorts, the prevalence was greater in BN than in F344 rats, but there was no significant difference between BN and BNF3F1 rats. CR had a significant sparing effect on this lesion in BN and BNF3F1 rats; it occurred too infrequently in F344 rats for a diet effect to be detected.

Stomach

Cystic crypts in the stomach deep mucosa was an unusual lesion in that it was observed in every genotype-sex-diet cohort of rats. It occurred commonly in female AL F344 and BNF3F1 rats and significantly less frequently in female BN rats. The odds that a F344 rat had this lesion increased with age, female gender, and AL diet. The effect of diet was also seen in BNF3F1 rats, in which there was also an age-by-diet interaction. This age-by-diet interaction resulted in decreased odds that CR BNF3F1 rats at every age had this lesion as compared with age-matched AL BNF3F1 rats.

Testis

Three testicular lesions, Leydig or interstitial cell hyperplasia, Leydig cell adenoma, and testicular atrophy, were commonly observed in this study. Leydig cell hyperplasia appeared as clusters of approximately 30 or more Leydig cells. Several distinct areas of hyperplastic Leydig cells were often observed

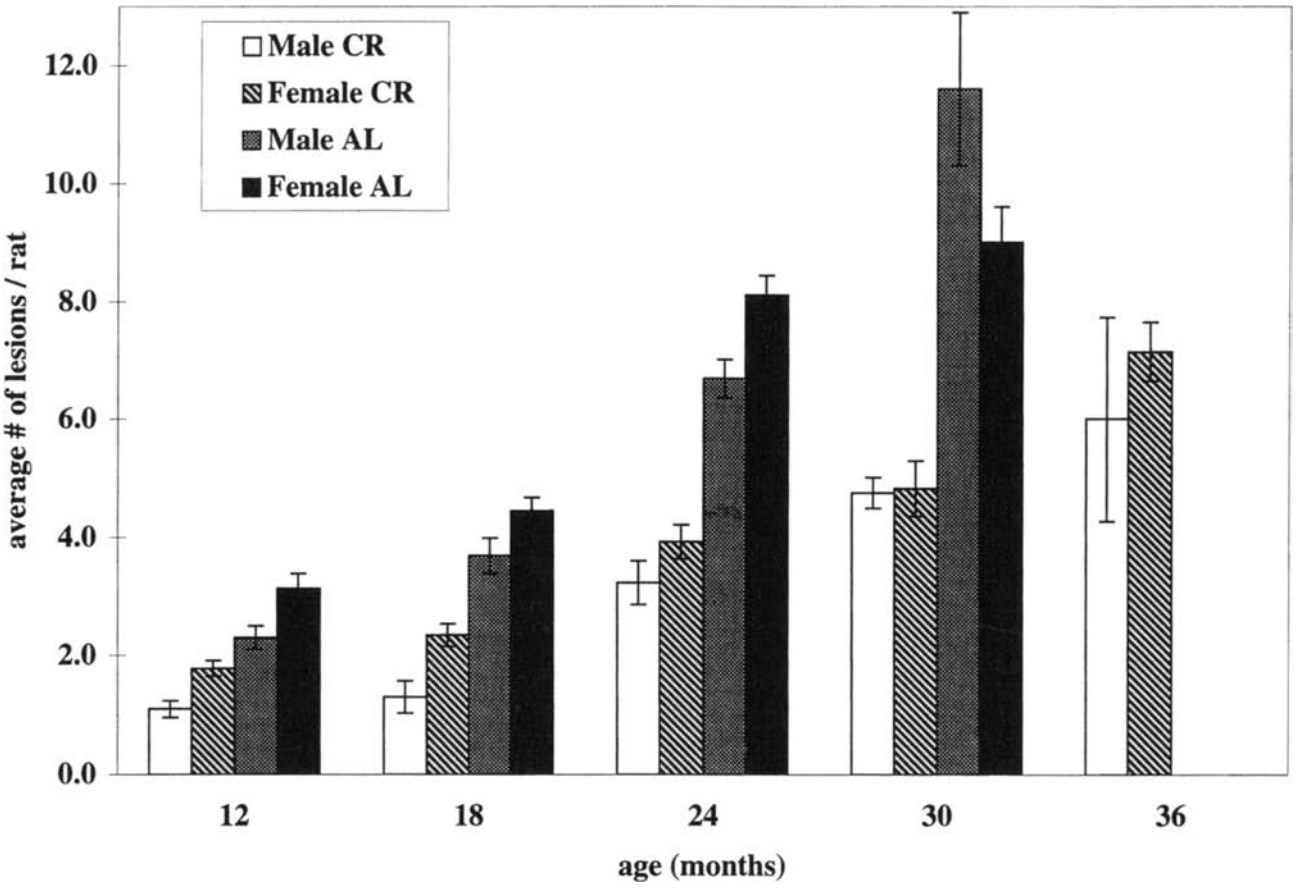

Figure 3. Age-associated lesion burden in F344 rats.

within a single section of testis. This lesion was commonly observed in the F344 genotype and significantly less frequently in the other genotypes (Table 2). This lesion showed significant effects of diet and age (Table 3) and was observed more frequently in rats ≤18 months of age than in those that were 24 or 30 months of age.

Leydig cell adenoma has previously been reported as particularly common in F344 rats (4–6,14,17–19). Nearly all testes from F344 rats ≥24 months of age were completely replaced by tumor tissue. The few remaining seminiferous tubules were often atrophic. This lesion was never observed in BN rats, but it was observed in 13 BNF3F1 hybrids (Table 2). In the F344 genotype, the odds of an animal having this lesion increased with age and AL feeding (Table 3).

Testicular atrophy, in which some or most seminiferous tubules were found to be thin, and often devoid of sperm and all precursor sperm cells, was observed in all three genotypes studied. It was significantly more common in BN rats than in either F344 or BNF3F1 rats. This is the only common lesion that occurred significantly more frequently in CR than in AL rats. This was particularly evident in BN rats, but it also was true of F344 rats. It may be that CR F344 rats had a higher prevalence of atrophy than AL F344 rats simply because their testicular tissue was not replaced by interstitial cell tumors at an earlier age.

Thyroid

In the thyroid, three lesions, C-cell hyperplasia, C-cell adenoma, and cystic thyroid follicles, were commonly observed. The incidence of C-cell hyperplasia demonstrated a significant inverse relationship with age in F344 rats. This lesion was significantly more common among the AL animals, and there was no effect of sex in the F344 rats (Table 3). The incidence of C-cell hyperplasia in male 24- and 30-month-old rats was significantly greater in F344 than in BNF3F1 hybrids (Table 4b), and no cases of this lesion were observed in BN rats (Table 2). A comparison of the incidence of C-cell hyperplasia among the 24- and 30-month-old females demonstrated that its incidence was significantly greater in F344 than in BN rats, but, unlike the males, it was not significantly different from the incidence in the BNF3F1 hybrid.

The C-cell adenoma did not occur commonly enough to be considered a common lesion in any of the genotypes. It was, however, possible to compare the incidence in the 24- and 30-month-old females, in which the incidence was observed to be significantly greater in F344 than BN rats, while at the same time demonstrating comparable incidences in F344 and BNF3F1 rats.

Thyroid follicular cysts were commonly observed in BN rats, in which a significant effect of age and diet was observed only in the AL fed animals. Although in the males this lesion was

observed in all genotypes, among the 24- and 30-month-old animals, its occurrence was observed significantly more frequently in the BN rats than in either the F344 or BNF3F1 rats (Table 4b). However, in the 24- and 30-month-old females, this lesion was found only in the BNF3F1 hybrid, in which its incidence was significantly greater than in either the F344 or BN strains.

Effect of Diet on Lesion Prevalence

Table 3 shows that CR significantly reduced the odds of rats' having nearly all common lesions in all three genotypes and in both sexes. This reduction generally occurred in all three types of lesion, neoplastic, nonneoplastic proliferative, and degenerative, as indicated by either a negative value for β_{diet} or by the presence of a significant age-by-diet interaction. The prevalence of all three types of common tumors, pituitary adenoma in the BNF3F1, and leukemia and Leydig cell adenoma in F344 rats, were each reduced by CR. Of the proliferative lesions observed, all such lesions common in the BN or F344 rats showed a significant effect of diet; five out of the six proliferative lesions in the BNF3F1 hybrid also showed a significant effect of diet on prevalence. The single proliferative lesion that showed no effect of diet was the lymphoid nodules in the livers of BNF3F1 rats. The prevalence of all nine degenerative lesions in BN rats was significantly reduced by CR; in F344 and BNF3F1 rats, seven of eight and three of eight degenerative lesions, respectively, were similarly affected by CR. The degenerative lesions unaffected by diet were as follows: kidney mineral deposition, pancreatic atrophy, and tracheal cysts in the BNF3F1 rats; and kidney mineral deposition, liver necrosis, and retinal degeneration in the F344 rats.

Effect of Age on Lesion Prevalence

Age was significantly associated with an increased prevalence of the majority of common lesions in this study. Many of these lesions seldom or never occurred in rats younger than 18 months of age. The only common lesions that failed to show a significant age-associated increase in prevalence were the lymphoid nodules in the livers of the F344 and BNF3F1 rats, kidney hydronephrosis and lymphoid nodules in the kidney of BN rats, and pancreatic islet cell hyperplasia in the BNF3F1 hybrids. The following lesions showed a significant negative correlation with age (i.e., they occurred more commonly in younger animals): kidney mineral deposition, liver necrosis, and lymphoid nodules in the lung in F344 rats; urothelial hyperplasia in BN rats; and lymphoid nodules in the lung, urothelial hyperplasia, spinal root degeneration, and cystic glands in the stomach of BNF3F1 hybrid rats.

Effects of Sex on Lesion Prevalence

The data in Table 3 demonstrate that the prevalence of the majority of lesions present in both males and females was significantly influenced by sex. For the F344, of the 15 commonly occurring lesions found in both males and females, only lung lymphoid nodules, pancreatic atrophy, retinal degeneration, thyroid C-cell hyperplasia, and tracheal cysts showed no effect of sex on prevalence. Five lesions, Harderian gland metaplasia, stomach cysts, kidney protein casts, mineral deposition, and liver lymphoid nodules, were significantly more common in females than males. The remaining four lesions, kidney glomerulonephropathy, bile duct hyperplasia, leukemia, and liver necrosis were significantly more common in the males than females. For the BN rats, only the lung lymphoid nodule and spinal root degeneration showed a significant effect of sex on prevelance. Other than protein casts in the kidneys and foci of cytoplasmic vacuolization in the liver, which were significantly more often observed in females than in males, the prevelance of the other eight common lesions was significantly greater in the BN males than in the females. For the BNF3F1, the four lesions comparably distributed among males and females were kidney protein casts, lymphoid nodules in the liver, stomach cysts, and tracheal cysts. The four common lesions for which prevelance was significantly greater in females than in males were mineral deposition in the kidney, lung lymphoid nodules, pituitary gland adenoma, and urothelial hyperplasia. The remaining seven common lesions, adrenal gland foci of pale cells, heart fibrosis, kidney lymphoid nodule, bile duct hyperplasia, pancreatic atrophy, islet cell hyperplasia, and spinal root degeneration, were more common in BNF3F1 males than in females. Thus the pattern of the effects of sex on the prevelance of common lesions varied among the three genotypes.

Effect of Genotype on Lesion Prevalence

The data in Tables 4a and 4b demonstrate that genotype affected the prevalence of many lesions as profoundly as did diet. Nine commonly occurring lesions in the female rats occurred significantly more frequently in BN rats than in either F344 or BNF3F1 female rats. These were cortical hyperplasia, cytoplasmic vacuolization and foci of enlarged pale cells in the adrenal gland, heart degeneration, protein casts in kidney, hydronephrosis, pancreatic atrophy, spinal root degeneration, and uterine fibrosis. Similarly, the eight lesions that occurred significantly more often in female F344 rats than in female BN and female BNF3F1 rats were Harderian gland metaplasia in the exorbital gland, heart fibrosis, glomerulonephropathy, mineralization of the kidney, bile duct hyperplasia, leukemia, retinal degeneration, and thyroid C-cell hyperplasia. Lastly, the three lesions that occurred significantly more often in female BNF3F1 rats than in female rats of either parental strain were kidney mineralization, pituitary gland adenoma, and thyroid cysts.

For the males, the 10 lesions that occurred significantly more often in BN rats than in F344 and BNF3F1 were foci of cytoplasmic vacuolization, foci of enlarged pale adrenal cells, Harderian gland metaplasia, hydronephrosis, lymphoid nodules in the lung, islet cell hyperplasia, pancreatic atrophy, urothelial hyperplasia, testicular atrophy, and thyroid cysts. The six lesions for which prevelance was significantly greater in F344 males than in BN or BNF3F1 males were glomerulonephropathy, bile duct hyperplasia, leukemia, retinal degeneration, Leydig cell adenoma, and thyroid C-cell hyperplasia. In the males, the only lesion for which the prevelance was significantly greater in the BNF3F1 hybrid than in either parental strain was the lymphoid nodules in the kidney.

Effect of Age and Diet on Lesion Burden

The effects of age and CR on individual lesions were studied; in addition, the effects of both age and diet on the lesion burden were examined. When the number of lesions in each animal was simply totaled, it was possible to calculate a mean value for lesion burden for each cohort. Figures 1–3 present the

mean number of lesions per rat ± *SE* for each genotype and demonstrate that the mean number of lesions observed in each animal increased linearly with age in all genotype-sex-diet cohorts. In every age group, CR rats had significantly fewer total lesions, on average, than did AL rats.

Discussion

This experiment was designed to examine the range of histopathologic changes that occur during the life span of rats, to calculate the prevalence of each lesion at 6-month intervals during the life span, and to determine the effects of CR on the prevalence of neoplastic, proliferative, and degenerative lesions, as well as its effect on lesion burden. The data support the widely held view that "laboratory rats not only live longer but also have fewer age-associated diseases when their food intake is restricted" (20). In all three genotypes and both sexes, most kinds of lesions increased in prevalence with age. CR brought about a decrease in the prevalence of most lesions, whether neoplastic, proliferative, or degenerative. (Exceptions, such as retinal and testicular degeneration and pancreatic atrophy, simply demonstrate that CR does not beneficially modify every disease process). We conclude that in cross-sectional terminal studies, most lesions can be viewed as biomarkers of aging because it is possible to estimate the physiologic age of an animal by determining its lesion burden. CR rats nearly always appear younger than AL rats of the same chronological age because their lesion burdens are smaller.

The age-related lesions of F344, BN, and BNF3F1 rats have been reported previously (5–7,11,21). An important finding of this work is that the three strains of rats differed significantly in the set of age-related lesions that they developed. These lesion sets can be viewed as the aging phenotypes of these strains. Some lesions such as leukemia, glomerulonephropathy, and Leydig cell adenoma occurred commonly in F344 rats and very rarely in the other strains. These phenotypes behaved genetically rather like recessive traits. Other lesions such as hydronephrosis occurred in one parental strain, BN, and in BNF3F1 rats but not in the other parental strain, F344. Such phenotypes behaved as dominant traits with penetrance that was seldom complete because not all animals developed every lesion to which they were presumably susceptible. Other lesions occurred in both parental genotypes, but more commonly in one than in the other. It is most likely that these lesions have a complex inheritance involving several genes.

It could be argued that the apparent genetic basis for many of these lesions is spurious. Many other rat strains also develop many of these lesions (5), suggesting that these lesions simply reflect how all rats age. Although this point must be granted in part, it should be understood that many of the commonly utilized rat strains were derived from common ancestor strains such as the Wistar (22). The BN strain probably did not share this common ancestry, as it has been demonstrated to be genetically distinct from other inbred rat strains (23), supporting the argument that BN and F344 rats develop different sets of age-related lesions because their age-related lesion genes are distinct.

Another objection to the conclusion that age-related lesions have a genetic basis is that lesion prevalence within inbred and hybrid strains has been highly variable from study to study. For example, the prevalence of leukemia in control F344 rats in eighty 2-year carcinogenicity studies, conducted by the National Toxicology Program, ranged from 10% to 72%. Similarly, the range for pheochromocytomas was 6–65% (13). These data are so highly variable that it seems unlikely that they could be under genetic control. However, the fact that there is variability in expression of a trait as a result of environmental variability does not in any way preclude that the traits' expression is also controlled by genes.

The best way to explain the genetics of many of the traits described here is to assume that they are controlled by a number of genes, as well as by the environment. To understand how these putative genes interact, one would have to breed hybrid rats together to produce an F2 generation. In that generation all of the genes would segregate independently, and the population of F2 rats would be composed of genetically distinct individuals (26). The environmental contribution to each lesion phenotype would be held constant by maintaining all rats under identical conditions. The genetics of each trait could be analyzed by the use of a quantitative trait locus (QTL) analysis, which is predicated on traits' being controlled by more than one gene. This approach has been used to analyze the genetics of longevity and age-related diseases in mice (24), and its application has been extended to phenotypes that are not normally distributed (25), such as the age-related lesions found in rats. As applied to F2 populations, QTL analysis is capable of defining genetic loci even in the face of environmentally-related phenotypic variability (26).

An examination of the data in this paper also reveals another important aspect of the pathology of aging rats. Given that all individuals of any inbred or hybrid strain are genetically identical or very nearly so, it is reasonable to view all the rats within any genotype-sex cohort essentially as replicates or clones. Moreover, in this and comparable studies, the animals were raised under highly controlled, uniform environmental conditions. Despite near identity of nature and nurture, however, animals within each genotype-sex-diet cohort exhibited greater and greater diversity as they aged, in that one 24-month-old BN rat, for example, had 10 lesions whereas another had only 2. Part of this variability may be due to sampling artifact; i.e., the liver section from one rat happened to include several hyperplastic bile ducts but the liver section from another rat happened not to. However, the prevalence of many lesions, such as glomerulonephropathy, which is readily diagnosed in any section of kidney, is not affected by sampling artifact.

Because the genotype and environment were held constant and because sampling artifact did not play a significant role, the variability in prevalence of most lesions comes from chance alone. Moreover, in view of the fact that the prevalence of most lesions increased with age, it can next be argued that the effect of aging on lesion development is to increase the odds that an individual will develop any of the lesions to which it is genetically predisposed. Given the stochastic nature of lesion development, one rat will develop one set of lesions and another identical rat will develop a different set, though both will be subsets of the total repertoire of lesions that the genotype is capable of developing. Rats of another genotype will also differ from one another for the same reasons. Some of their lesions will be the same as those of the other rat genotype, because both genotypes share some common age-related lesion genes. The more similar two genotypes are, the more similar will be the set of lesions that they are capable of devel-

oping. However, if two genotypes are quite genetically different, as are the F344 and BN strains, then their sets of age-related lesions will be distinct. How does the effect of CR fit in this context? We propose that the effect of CR is simply to reduce the odds of developing most of the lesions to which the individual is susceptible. The mechanism of that effect remains a complete mystery.

It is not feasible to study aging without regard to age-related lesions. It is also not appropriate to consider lesions as mere epiphenomena of aging, because aging cannot be measured as an entity apart from the lesions that are found associated with it. Future studies of aging should focus on those things that change in individuals as they age that makes them more likely to develop specific lesions. The results from this study suggest strongly that the set of lesions that any genotype of rat develops is regulated by both genes and diet. It should be possible through QTL analysis to identify genes controlling age-related lesions. Once the genes are identified, it will then be possible to determine how their expression is modified by caloric restriction.

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REFERENCES

1. Arking R. Altering aging: interventions known to modify longevity and aging. In: Arking, R., ed. *Biology of Aging: Observations and Principles.* Sunderland: Sinauer Associates; 1998:314–327.
2. Merry BJ, Holehan AM. Effects of diet on aging. In: Timiras PS, ed. *Physiological Basis of Aging and Geriatrics.* Boca Raton, FL: CRC Press; 1994:171–178.
3. Weindruch R, Walford RL. *The Retardation of Aging and Disease by Dietary Restriction.* Springfield, IL: CC Thomas; 1989.
4. Thurman J, Bucci T, Hart RW, Turturro A. Survival, body weight and spontaneous neoplasms in ad libitum and food-restricted Fischer 344 rats. *Tox Pathol.* 1994;22:1–9.
5. Bronson RT. Rate of occurrence of lesions in 20 inbred and hybrid genotypes of rats and mice sacrificed at 6 month intervals during the last years of life. In: Harrison DE, ed. *Genetic Effects on Aging II.* Caldwell, NJ: Telford Press; 1990:279–358.
6. Coleman GL, Barthold SW, Obaldistan SJ, Foster S, Jonas AM. Pathological changes during aging in barrier-reared Fischer 344 male rats. *J Gerontol.* 1977;32:258–278.
7. Maeda H, Gleiser CA, Masoro EJ, Murata I, McMahan CA, Yu BP. Nutrition influences on aging of Fischer 344 rats: II. Pathology. *J Gerontol.* 1985;40:671–688.
8. Masoro EJ, Yu BP, Bertrand HA. Action of food restriction in delaying the aging process. *Proc Nat Acad Sci USA.* 1981;79: 4239–4241.
9. McDermott GF, Ingram A, Scholey J, Kirkland JL, Whiteside CI. Glomerular dysfunction in the aging Fischer 344 rat is associated with excessive growth and normal mesangial cell function. *J Gerontol.* 1996;51:M80–M85.
10. Yu BP, Masoro EJ, McMahan CA. Nutrition influences on aging of Fischer 344 rats: I. Physical, metabolic, and longevity characteristics. *J Gerontol.* 1985;40:657–670.
11. Burek JD. *Pathology of Aging Rats.* Boca Raton, FL: CRC Press; 1978.
12. Allen J, Ashby R. Preliminary communication investigations on leukemia in the F344 rat. *Arch Toxicol.* 1982;5:307–309.
13. Haseman JK, Arnold J, Eustis SL. Thirty-five tumor incidences in Fischer 344 rats: NTP historical data. In Boorman GA, ed. *Pathology of the Fischer Rat.* San Diego, CA: Academic Press; 1990.
14. Rao GN, Haseman JK, Grumbein S, Crawford DD, Eustis SL. Growth, body weight, survival and tumor trends in F344/N rats during an eleven-year period. *Tox Pathol.* 1990;18:61–70.
15. Ward JM, Reynolds CW. Large granular lymphocyte leukemia: a heterogeneous lymphocytic leukemia of F344 rats. *Am J Pathol.* 1983;111:1–10.
16. O'Steen WK, Landfield PW. Dietary restriction does not alter retinal aging in the Fischer 344 rat. *Neurobiol Aging.* 1991;12:455–462.
17. Lipman RD. Pathobiology of aging rodents: Inbred and hybrid models. *Exp Gerontol.* 1997;32:215–228.
18. Seng JE, Gandy J, Turturro A, et al. Effects of caloric restriction on expression of testicular cytochrome P450 enzymes associated with the metabolic activation of carcinogens. *Arch Bioch Biophys.* 1996;335:42–52.
19. Thurman JD, Moeller RB, Turturro A. Proliferative lesions of the testis in ad libitum-fed and food-restricted Fischer-344 and F3BNF1 rats. *Lab Anim Sci.* 1995;45:635–640.
20. Weindruch R, Sohal RS. Seminars in medicine of the Beth Israel Deaconess Medical Center: caloric intake and aging. *New Eng J Med.* 1997;337:986–994.
21. Lipman RD, Chrisp CE, Hazzard DG, Bronson RT. Pathologic characterization of Brown Norway, Brown Norway × Fischer 344, and Fischer 344 × Brown Norway rats with relation to age. *J Gerontol.* 1996;51A: B54–B59.
22. Lindsey JR. Historical foundations. In: Baker HJ, Lindsey JR, Weisbroth SH, eds. *The Laboratory Rat, Vol. I. Biology and Diseases.* San Diego, CA: Academic Press; 1979.
23. Canzian F. Phylogenetics of the laboratory rat Rattus norvegicus. *Genome Res.* 1997;7:262–267.
24. Jackson AU, Fornes A, Galecki A, Miller RA, Burke, DT. Multiple-trait quantitative trait loci analysis using a large mouse sibship. *Genetics.* 1999;151:787–795.
25. Kruglyak L, Lander ES. A nonparametric approach for mapping quantitative trait loci. *Genetics.* 1995;139:1421–1428.
26. Schork NJ, Krieger JE, Trolleit MR, et al. A biometrical genome search in rats reveals the multigenic basis of blood pressure variation. *Genome Res.* 1995;5:164–172.

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