Longevity in obese and lean male and female rats of the Zucker strain: prevention of hyperphagia[1–3]

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ABSTRACT Zucker obese (*fa/fa*) and lean (*Fa/Fa*) rats were fed a soy protein diet ad libitum under barrier conditions from 4 wk of age until death. Obese rats were also pair fed with lean controls to prevent hyperphagia. Time of death was determined and tissues collected at necropsy for histologic examination. Lean rats had longer 10th percentile survivorship (males 966 compared with 667 d, females 983 compared with 620 d) and maximum life spans (males 1067 compared with 803 d, females 1163 compared with 744 d) than did obese rats. Preventing hyperphagia increased maximum life span in both males (1010 d) and females (975 d). Pathologies in lean rats were similar to those reported for other rodent strains. For obese rats fed ad libitum, end-stage renal disease (ESRD) was the major cause of mortality (males: 91.1%, females: 93.3%). Prevention of hyperphagia decreased deaths attributable to ESRD (males: 64.4%, females: 51.1%). A smaller restriction in energy intake (8–18%) required to prevent hyperphagia compared with the 35–40% in most other studies produced similar increases in longevity, suggesting that obese Zucker rats are particularly sensitive to energy restriction. Amelioration of early onset of renal disease is a likely explanation. Percentage body fat in food-restricted obese rats did not differ from that of animals fed ad libitum; thus, reduced longevity is not the result of obesity per se, but rather is influenced by other metabolic pathologies occurring in this strain of rats homozygous for the *fa* gene. Because microalbuminuria with progression to ESRD is a complication in human obesity, the Zucker strain offers the opportunity to investigate initiating mechanisms of this pathology. *Am J Clin Nutr* 1997;66:890–903.

KEY WORDS Zucker rats, hyperphagia, sex, aging, renal disease, obesity, energy restriction

INTRODUCTION

It has been proposed that when we feed laboratory rodents nutritious, palatable food ad libitum and limit their physical activity we are "overdosing" them, inducing an earlier onset of degenerative diseases and decreased survival (1, 2). We have proposed that people in the United States are also exposed to a "toxic" environment of tasty, plentiful food and "forced" inactivity resulting in obesity and premature death (3). In obese humans, small decreases of body weight (≈5–10%) significantly improve comorbid conditions such as non-insulin-dependent diabetes mellitus (NIDDM) and dyslipidemia (4). Diabetes, the leading cause of end-stage renal disease (ESRD) in the United States, accounts for about one-third of all ESRD (5). Of diabetic persons receiving renal dialysis, 60% have NIDDM (5). If this analogy holds for animal models, it is likely that prevention of overeating in obese rats, ie, hyperphagia, will delay the onset of degenerative diseases and extend longevity. To test this hypothesis, we conducted a longevity study of genetically obese Zucker rats in which ESRD is consistent with that seen in NIDDM in humans. The Zucker strain is often considered an animal model for human obesity associated with metabolic disease.

Obesity in Zucker rats is inherited as a single Mendelian recessive trait (*fa*) (6). Zucker rats homozygous for the *fa* gene are morbidly obese and characterized by fat cell hypertrophy and hyperplasia (7), increased adipose tissue lipoprotein lipase (LPL) activity (8), hyperinsulinemia, hypertriglyceridemia, hyperphagia (9), insulin resistance (10), and decreased energy expenditure (11). Obesity is not visually apparent before weaning, but as early as 2 d of age thermogenesis is lower than in lean rats (11). By 7 d of age, both fat cell size and adipose tissue LPL activity are elevated (12). Increased pancreatic insulin release and body fat are detected by 2 wk (13) and there is some evidence of hyperinsulinemia in utero (14).

Hyperphagia develops at weaning and persists until the rats are ≈6–8 mo of age. Pair feeding does not ameliorate obesity; pair-fed obese rats, although smaller than ad libitum–fed obese rats have similar percentages of body fat (8). Pair feeding does not decrease hyperinsulinemia nor does it decrease the activity of adipose tissue LPL (8). These findings are consistent with results in some postobese adult humans who continue to have elevated adipose tissue LPL activity (15).

The *fa* mutation itself is a defect in the leptin receptor gene (*Ob-R*) that produces a single amino acid substitution of a glutamine for a proline (16–18). The defect is present in all three receptor isoforms—Ob-Ra, Ob-Rb, and Ob-Re—and is expressed in tissues of Zucker obese rats at concentrations similar to those for the wild-type isoform in lean Zucker and Sprague-Dawley rats (19). How this genetic defect translates into the obese syndrome remains to be elucidated; however, the

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fact that the receptor is present in a variety of tissues, eg, hypothalamus, pancreas, liver, kidney, and gonads (20), indicates that multiple sites of action could influence development of the metabolic disease seen in these genetically obese rodents. For example, leptin resistance in Zucker rats resulting from either a nonfunctional receptor or down-regulation of the receptor may influence satiety, hunger, or a variety of metabolic signals downstream from Ob-R.

Only a small number of longevity studies that used genetically obese rodent strains have been reported. The design of these studies typically restricted body weight to that of lean controls. Food intake is typically less in weight-restricted genetically obese animals than in ad libitum–fed genetically lean controls. In one such study, Lane and Dickie (21) showed that the life span of body weight–restricted obese hyperglycemic mice (*ob/ob*) was significantly increased. Koletsky and Puterman (22) reported similar results for the spontaneously hypertensive (SHR) rat. Neither of these studies reported body composition, however, and it is likely that these food-restricted obese mice and rats were fatter than weight-matched nonobese controls. The study by Cleary et al (8), which established that food restriction (≈22%) from birth did not normalize percentage body fat in 33-wk-old male obese Zucker rats, supports this statement.

Harrison et al (23) reported that in *ob/ob* mice, the effects of food restriction on longevity could be separated from the effects of adiposity. In that study, *ob/ob* mice restricted by 34% had a median life span similar to wild type (*Ob/Ob*) mice, although their percentage body fat remained significantly higher (48% compared with 22%). Similarly, Bertrand et al (24) reported that the decrease in body fat content caused by food restriction does not appear to be causally related to extension of life span; these studies used male Fischer 344 rats, not generally considered to be obese (22% body fat) (25). In morbid obesity, > 50% of body weight is body fat (twice as much as in the Fischer 344 rat) and could play a role in decreased life span. The CD-COBS-VAF substrain of Sprague-Dawley rats that has been widely used in toxicologic studies since its introduction in 1988 (26) has a higher percentage body fat at 1 y of age (males: 26.5%; females: 35.1%) when fed ad libitum than did earlier strains of Sprague-Dawley rats, eg, CD-COBS (27), and are considered by some to be obese. Dietary restriction results in increased survivorship for males, but not females, compared with animals fed ad libitum (28, 29).

Harrison and Archer (30) argued that genetic effects have significant influences on the aging process, even with respect to the established role of food restriction in altering longevity. These authors consider it important to study a wide variety of genotypes to dissect out interactions between the physiologic aging process and the effects of food restriction.

To date, the only attempt to establish longevity for the Zucker strain occurred in the 1970s. Zucker and Seronde reported that when obese Zucker rats were fed a diet containing 24% casein, the average life span was 412 ± 15 d compared with 659 ± 48 d for lean rats. This work was communicated to us in draft manuscript form (J Stern, personal communication, 1975), but the data were never published. These data are confounded because specific pathogen-free (SPF) rats were not used, nor were known homozygous lean rats (*Fa/Fa*). Because the presence of microbial and viral infection can dramatically alter the aging process (31), these results must be considered preliminary. Zucker and Seronde did not assess the contribution of increased food intake to mortality in obese Zucker rats. Here we report longevity and pathology data for both obese (*fa/fa*) and lean (*Fa/Fa*) males and females of the genetically obese Zucker strain fed ad libitum, and the effects on longevity and pathology of food-restricting obese rats to amounts eaten by their homozygous lean counterparts.

METHODS

Animals

The Zucker rats used in this study were from the original cross between Sherman and Merck 3M strains as originally described by Zucker and Zucker (6). All rats were bred and housed under barrier conditions in the Animal Model Core of the Clinical Nutrition Research Unit at the University of California Davis. Zucker SPF heterozygous females (*Fa/fa*) were mated with SPF homozygous obese males (*fa/fa*). SPF homozygous lean control rats were bred from known SPF homozygous lean breeders. One day after birth, litters were standardized to 8–10 pups/dam. At 3 wk of age, one rat per litter was killed, necropsied, and examined for pathology. Nasal and fecal smears and serologic tests were run for specific pathogens including ecto- and endoparasites, mycoplasma, Sendai virus, PVM virus, and RCC/SDA virus. No rats tested positive for any of the aforementioned parasites. Rats were weaned at 4 wk of age and housed individually in stainless steel hanging cages (20 cm × 25 cm × 18 cm) in laminar-flow bioclean units (≤ 300 rats/room). These units are self-contained environmental barriers that circulate nonrecyclable air through high-efficiency particulate arrestor filters at a constant rate (providing class 100 laminar-flow clean air). Ambient temperature was maintained at 25–26 °C and the light-dark cycle was set at 12 h-12 h with lights on at 0600 and off at 1800. The study protocol was approved by the University of California Davis Animal Care and Use Committee.

The following procedures prevented contamination of the barrier facility with known pathogens. All personnel donned sterilized uniforms, masks, hoods, shoe covers, and gloves before entering the facility. All material that entered the barrier facility was autoclaved. Water was acidified by addition of hydrochloric acid to pH 3.5. The excretion absorbent was changed three times per week. Cages and cage support racks were washed in a mechanical cage washer (with detergents and water at 180 °C) weekly. In addition, a broad survey of potential microbiological contamination was carried out weekly by technical staff.

Experimental design

Six groups of Zucker rats (45 per group) were followed from weaning at 4 wk of age until spontaneous death: ad libitum–fed obese males, pair-fed obese males, ad libitum–fed lean males, ad libitum–fed obese females, pair-fed obese females, and ad libitum–fed lean females. Food intake of ad libitum–fed rats was measured twice per week in 4- and 3-d periods. Food intake of ad libitum–fed lean rats was used to determine the precise amount of food to be fed to the pair-fed groups. From weaning until spontaneous death, pair-fed rats were fed once per day 1 h before the onset of the dark cycle as described by Yu et al (32). To minimize diet-induced nephropathy associ-

ated with feeding rats casein-based diets, soy protein was used as described by Iwasaki et al (33). Pair-fed obese rats received the same absolute amount of vitamins and minerals as ad libitum–fed obese rats. Their diet was adjusted bimonthly. Diet composition (g/100 g diet) for ad libitum–fed rats included soy protein (21.00), DL-methionine (0.35), hydrolyzed starch (43.45), sucrose (15.00), corn oil (10.00), vitamin mix (2.0), and mineral mix (5.00) from Bertrand et al (24), choline chloride (0.20), and microcrystalline cellulose (3.00). Energy from macronutrients was ≈21% from protein, 22% from fat, and 57% from carbohydrate.

Body weights were monitored weekly. Rats were followed in this study until all had died. Moribund rats, characterized by rapid loss in body weight and inanition, were killed when death within 48 h could be reliably predicted.

Necropsy and pathology

Cages were monitored twice a day for deaths, dead animals were necropsied promptly, and a gross pathologic examination was conducted within 12 h of death to determine the cause of death. Standardized procedures for determination of the cause of death of rats were used (34). Tissues routinely collected and preserved in neutral buffered formalin (10%) at necropsy included adrenal glands, brain, heart, kidneys, liver, lung, pancreas, spleen, and stomach. In addition, any tumors or other tissues with evidence of pathology on gross examination were collected and fixed. A subset of the tissue collected consisting of liver, heart, kidneys, adrenal glands, and pancreas, was examined in each animal. Sections of grossly abnormal tissue observed by the prosector at necropsy were examined in every case in which they occurred. Tissues to be examined were embedded in paraffin, sectioned to 4–5 μm, and stained with hematoxylin and eosin.

Histologic examination and grading of lesions in each tissue was performed by Robert E Harris Jr, a veterinary pathologist. Routine histopathologic examination was performed by using a light microscope with calibrated oculars, and observations were recorded on a separate form for each animal. Certain commonly occurring lesions in the liver, heart, kidneys, and pancreas were scored or measured. All other lesions were described briefly. In the liver, hepatic fatty degeneration was scored as focal, multifocal, or diffuse and whether the lesion was absent, mild, moderate, or severe. Chronic cardiac muscle degeneration and mineralization were scored as none, mild, moderate, or severe on the basis of subjective examination. In the pancreas, changes in islets of Langerhans were scored as islet hypertrophy (none, mild, moderate, or severe) or islet cell adenoma. When a proliferative lesion was determined to be an adenoma, its approximate diameter in micrometers was measured at 100 × power by using calibrated microscope oculars. Chronic degenerative renal disease was scored as absent, grade 1 (≤ 33% of renal parenchyma affected), grade 2 (34–67% affected), or grade 3 (> 67% affected). When there was reasonable supporting evidence, a presumptive cause of death was given. Scored data were collected and entered into an electronic spreadsheet, whereas all other data were tabulated based on the number of occurrences of each type of lesion, and the days of age of the animal in which the lesion occurred. The number of days until death from renal failure was subjected to statistical analysis (SAS; SAS Institute Inc, Cary, NC), which included an F test of multiple means to determine whether significantly differing means were present, followed by multiple pooled t tests to determine which pairs of means were significantly different at $P \leq 0.001$. Two rats were excluded from analyses. No necropsy data were available for one ad libitum–fed obese male. One lean male was killed prematurely because of an uncorrectable jaw occlusion.

Analysis of survival data

Longevity data are presented as survivorship curves with calculations of 50th percentile, 10th percentile, and maximum life span in days. The Kaplan-Meier test (35) was used for statistical analysis of these data. In addition, Gompertz analysis was used to calculate the mortality rate doubling time (MRDT) (36). Analysis of variance (ANOVA) with Neuman-Keuls post hoc analysis was used for body-composition data.

RESULTS

Food intake

Obese male rats fed ad libitum ate significantly more than lean male rats from weaning (4 wk of age) until 48 wk of age (**Figure 1**). Thus, in pair-fed obese rats, average food restriction for males was 8.7%. Food intake from 48 wk until 95 wk was comparable in obese and lean male rats. After 95 wk, food intake of ad libitum–fed obese male rats became variable and less than that of lean rats. This pattern continued until all rats had died. Food intake of pair-fed obese rats was not significantly different from that of lean rats. In obese female rats (Figure 1), food intake was elevated above that of lean rats from weaning until 62 wk of age; the average food restriction in pair-fed obese females was 19.8%. Mean weekly food intake became variable for ad libitum–fed obese females from 62 wk of age until all rats had died, and obese females ate significantly less than lean and pair-fed females.

The degree of hyperphagia in obese females was greater than that in obese males (Figure 1). A 7.2% restriction for the period between 4 and 60 wk of age achieved a 47% increase in median life span in obese males. For obese females, a 45% increase in median life span was achieved by an 18.2% restriction over the same period. The maximum restriction produced by pair feeding obese to lean rats occurred between 4 and 20 wk of age (males: 17.7%; females: 28.7%).

The variability in food intake that occurred for both male and female obese rats fed ad libitum is likely related to deterioration in physiologic function as rats near death. This variability appeared earlier (62 wk compared with 95 wk) for female than for male rats, corresponding to earlier mortality in females than in males. Some variability also occurred in both male and female lean rats, but at a much later time point, ≈130 wk.

Body weight

Body weights of obese males (**Figure 2**) increased until 60 wk of age (780 g) and then declined with advancing age. Pair-fed obese rats reached their average maximum weight by 65 wk (615 g); average weight remained constant until 82 wk and then declined with advancing age. Lean rats reached their average maximum weight by 100 wk of age (550 g); it remained constant until 120 wk, after which it declined with advancing age. The average body weights of pair-fed obese rats were higher than those of lean rats and lower than those of

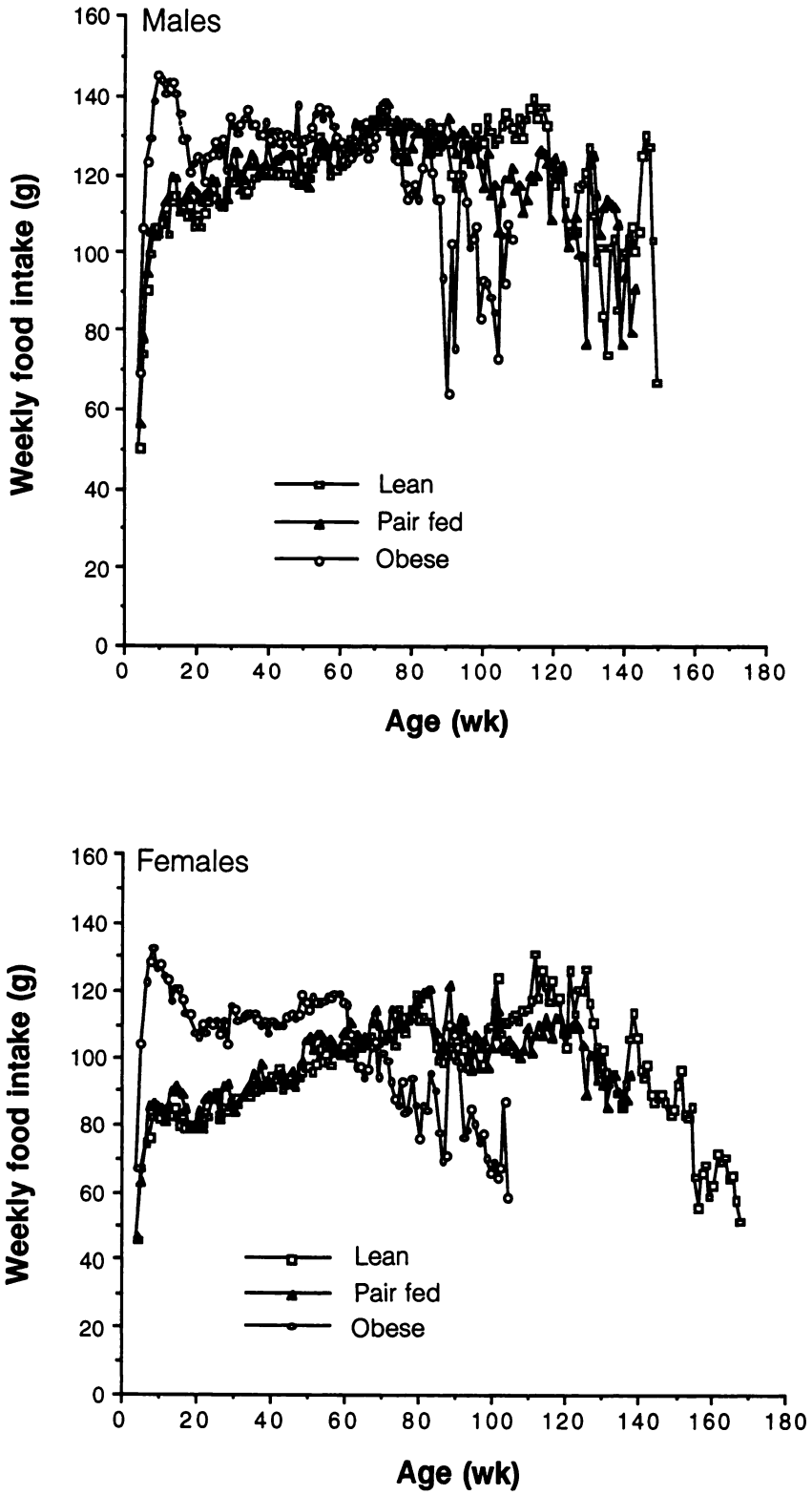

FIGURE 1. Average weekly food intake of male and female obese and lean Zucker rats. Data from rats within 2 wk of death are excluded. $n = 45$ for all groups except the male obese and lean groups ($n = 44$).

obese rats until 100 wk, at which point the body weights of the three groups converged.

Body weights of obese females (Figure 2) increased until 60 wks of age (580 g) and declined steadily thereafter with advancing age. Pair-fed obese rats reached their average maximum weight by 78 wk (550 g); it remained constant until 84 wk, after which it declined. Pair-fed obese rats reached the same body weight as ad libitum–fed obese rats at 68 wks of

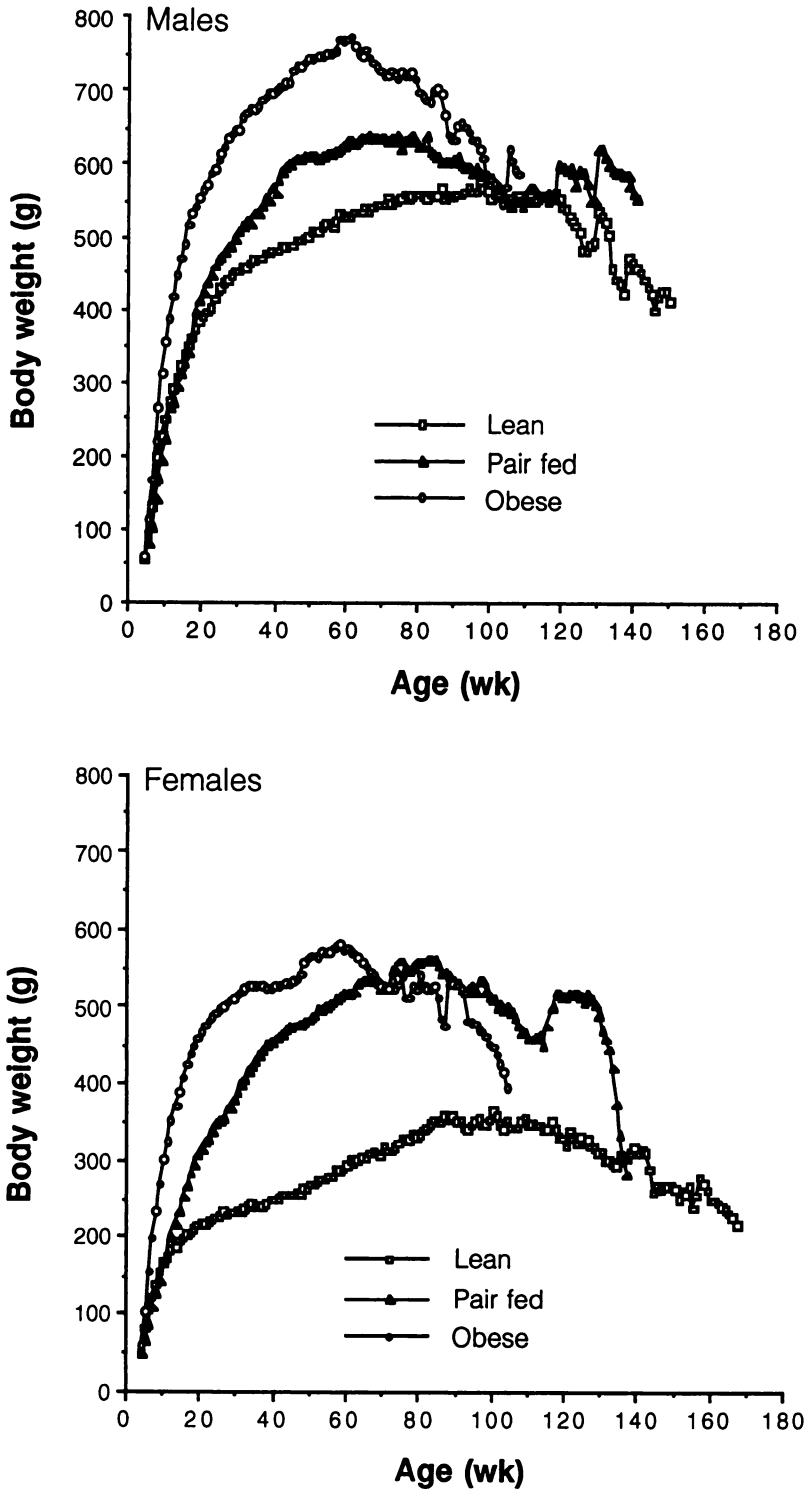

FIGURE 2. Average body weights of male and female obese and lean Zucker rats. Data from rats within 2 wk of death are excluded. n = 45 for all groups except the male obese and lean groups (n = 44).

age, a time at which pair-fed obese females were still increasing in body weight whereas ad libitum–fed obese females had begun their decline in body weight. Lean rats reached their average maximum weight by 88 wk of age (360 g); it remained constant to 110 wk, after which it declined gradually with advancing age. In contrast with pair-fed obese male rats who weighed less than ad libitum–fed obese rats for almost 2 y, pair-fed obese female rats reached the same body weight as ad libitum–fed obese females by 1.35 y, after which they exceeded the body weights of their ad libitum–fed controls.

Body composition

Body-composition data at death are given in **Table 1**. Several findings are notable when pair-fed rats are compared with ad libitum–fed obese rats: *1*) pair feeding did not significantly decrease either total or percentage carcass fat, *2*) pair feeding significantly decreased both protein and ash in both males and females and percentage protein only in females; the decrease in percentage protein was not statistically significant in males.

Survivorship

Survival curves of ad libitum–fed obese versus ad libitum–fed lean rats differed as did curves for pair-fed obese versus ad libitum–fed obese rats of both sexes ($P < 0.0001$, Kaplan-Meier test). In comparison with lean rats, ad libitum–fed obese rats had significantly lower survivorship by all measures (**Figure 3**). Pair feeding prolonged survival for both male and female obese rats. Curves for pair-fed obese males versus ad libitum–fed lean males were different ($P < 0.02$), but this comparison for females was not quite significant ($P = 0.06$). Survival curve analysis revealed that ad libitum–fed obese rats differed from both pair-fed obese and ad libitum–fed lean rats with respect to 50th and 10th percentile survivorship (**Table 2**). There were no significant differnces between the latter two groups.

Gompertz analysis revealed that the rate of acceleration of mortality (ie, slope) in male and female rats was higher in ad libitum–fed obese than in lean rats (Table 2). In obese female rats, pair feeding reduced the rate of acceleration of mortality compared with ad libitum–fed obese rats. In contrast, in males, pair feeding paradoxically increased the rate of acceleration of mortality in pair-fed obese compared with ad libitum–fed obese rats. However, because 50th percentile survivorship for pair-fed obese rats was greater than for ad libitum–fed obese rats, this increase may have been due to the death of a few male pair-fed obese rats at a relatively early age. MRDT was greater in lean and pair-fed obese female rats than in comparable male rats. However, for ad libitum–fed obese rats, mortality rate and MRDT were the same for males and females.

Gompertz analysis adequately describes the data for lean rats, but not for obese rats. This is reflected in the r^2 values (Table 2). For male and female lean rats, r^2 values exceed 0.93 (0.98 and 0.94, respectively). These values show that mortality rate as a function of age is highly correlated in lean rats. In contrast, for obese rats r^2 values ranged from 0.77 to 0.88. The Gompertz analysis suggests that there are at least two subpopulations in the four obese groups. There is a subpopulation of rats with a high mortality rate that dies at a younger age and a subpopulation of rats with a lower mortality rate that dies at an older age. Breaks in the curves occur at a younger age in obese females than in obese males. With pair feeding, breaks in the curves occur at an older age in both males and females.

Pathology

A spectrum of common pathologic conditions was observed at necropsy and subsequently confirmed by histologic examination of preserved tissues. As reported in numerous longevity studies, tumors increase with age. Pituitary adenomas are most common ($n = 46$). The large number of pancreatic adenomas (50% in ad libitum–fed males and females and pair-fed males, 38% in pair-fed females; **Table 3**) is consistent with the high degree of hyperinsulinemia and increased islet size (37). Other tumors identified included lymphoma ($n = 8$), mammary ($n = 6$), sarcoma ($n = 4$), and melanoma ($n = 2$) (**Table 4**). In no case was cause of death attributable to a tumor. Pathologies other than renal and cardiac ones included gastric ulcer ($n = 3$), pancreatitis ($n = 2$), and six occurrences of thrombi or hemorrhage to which death was attributable. The incidence of tumors was lower in lean males than in lean females (36.6% compared with 50.0%). A similar sex effect was seen in obese pair-fed males and females (17.8% compared with 35.6%). In ad libitum–fed obese males, incidence was 11.4% and no tumors (0%) were found in ad libitum–fed obese females.

In the case of renal pathology, the data reported are those instances in which death was attributable to renal failure consistent with ESRD (scores of 2–3 on a 0–3 point scale representing degeneration of glomeruli including protein-filled dilated tubules; **Figure 4**). The percentage of rats dying with ESRD was comparable for ad libitum–fed obese males (91.1%) and females (93.3%). Deaths attributable to ESRD decreased to 64.4% ($P < 0.05$) for pair-fed obese males and 51.1% ($P <$

TABLE 1
Carcass composition of Zucker rats[1]

	Male			Female		
	Lean ($n = 44$)	Pair-fed obese ($n = 45$)	Obese ($n = 44$)	Lean ($n = 45$)	Pair-fed obese ($n = 45$)	Obese ($n = 45$)
Carcass weight (g)	298.4 ± 14.6^{d}	406.8 ± 15.3^{b}	485.2 ± 12.9^{a}	188.9 ± 11.0^{e}	317.9 ± 15.9^{c,d}	342.3 ± 13.7^{c}
Total body water						
(g)	185.7 ± 7.7^{a}	154.2 ± 4.2^{b}	187.8 ± 4.8^{a}	121.7 ± 6.9^{c,d}	110.2 ± 3.5^{d}	129.1 ± 2.7^{c}
(%)	70.1 ± 0.6^{b}	67.1 ± 0.7^{c,d}	66.0 ± 0.4^{d}	72.2 ± 0.4^{a}	68.1 ± 0.5^{c}	67.9 ± 0.5^{c}
Fat						
(g)	34.2 ± 6.1^{e}	177.1 ± 11.0^{a,b}	200.7 ± 8.5^{a}	21.4 ± 4.2^{e}	155.9 ± 11.9^{b}	151.6 ± 10.3^{b}
(%)	9.8 ± 1.4^{e}	42.0 ± 1.3^{a,b}	40.9 ± 0.9^{b}	9.6 ± 1.4^{e}	45.4 ± 2.2^{a}	42.2 ± 1.4^{a,b}
Protein						
(g)	47.8 ± 2.8^{b}	48.2 ± 2.4^{b}	67.5 ± 2.5^{a}	21.0 ± 1.8^{e}	27.2 ± 1.8^{d}	36.5 ± 1.8^{c}
(%)	15.9 ± 0.4^{a}	12.1 ± 0.5^{b}	14.0 ± 0.4^{b}	10.6 ± 0.4^{d}	8.4 ± 0.3^{e}	10.6 ± 0.3^{d}
Ash						
(g)	30.8 ± 0.3^{a}	27.3 ± 0.2^{c}	29.0 ± 0.5^{b}	24.8 ± 0.3^{d,e}	24.2 ± 0.3^{e}	25.3 ± 0.2^{d}
(%)	11.0 ± 0.4^{b}	7.1 ± 0.3^{d,e}	6.1 ± 0.1^{e}	14.5 ± 0.6^{a}	8.7 ± 0.6^{c}	7.8 ± 0.3^{c,d}

[1] $\bar{x}$ ± SEM. Values within a row with different letter superscripts are significantly different, $P \leq 0.05$ (ANOVA with Neuman-Keuls post hoc analysis).

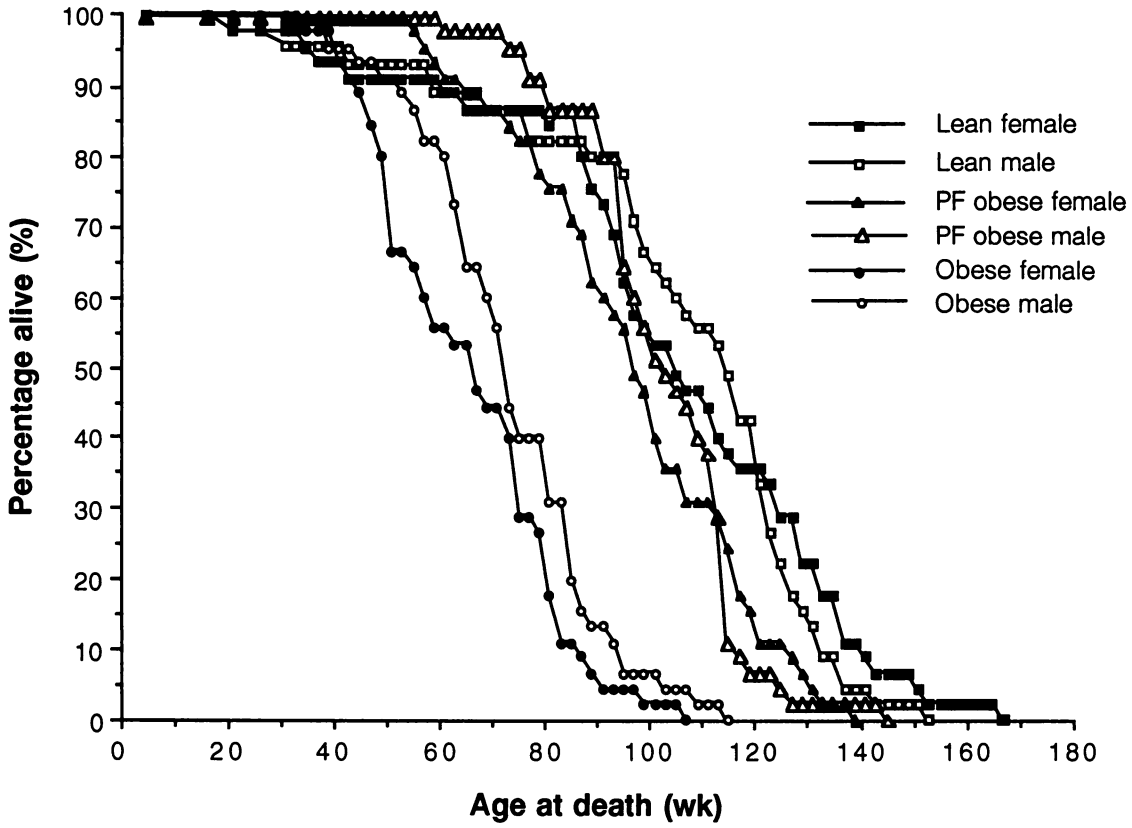

FIGURE 3. Survivorship of male and female obese and lean Zucker rats. Each survival curve is generated from 44 or 45 rats. PF, pair fed.

0.001) for such females. Although greatly decreased compared with ad libitum–fed obese rats, these percentages were still elevated compared with lean rats (males, 22.2%; females, 11.1%; $P < 0.05$). Severe renal disease occurred earlier in females than in males and its amelioration by prevention of hyperphagia was more effective in females than in males even though the incidence of ESRD was significantly lower in lean females than in lean males ($P < 0.05$). Significant cardiac pathology, defined as multifocal chronic cardiac necrosis and calcification (severity 2 and 3 on a 0–3 point scale) was present in ad libitum--fed obese rats, but the incidence was much lower in pair-fed rats (Figure 4).

DISCUSSION

For 60 y, studies of nutrition and aging have focused on observations that a lifetime of energy restriction without malnutrition extends life span in laboratory rodents. The original work of McCay and Crowell (38) has since been confirmed by many scientists. Dietary restriction appears to alter the aging process because it delays a variety of pathologic conditions or processes (for reviews *see* 31, 39–41). For example, in a series of studies in the 1960s, Berg and Simms (42) reported that rats restricted by 46% lived longer and had a lower incidence of degenerative diseases. At 800 d of age, food-restricted rats had

TABLE 2
Summary of longevity findings

Group by sex and genotype	Percentile		Maximum lifespan	Gompertz analysis		
	50th	10th		Slope	MRDT[1]	r^2
Males		*d*				
Obese (n = 44)	497	657	803	0.043	16.12	0.85
Obese, pair fed (n = 45)	732	839	1010	0.051	13.59	0.87
Lean (n = 44)	816	966	1067	0.031	22.35	0.98
Females						
Obese (n = 45)	475	620	744	0.044	15.75	0.77
Obese, pair fed (n = 45)	690	900	975	0.038	18.24	0.88
Lean (n = 45)	756	983	1163	0.026	26.65	0.94

[1] Mortality rate doubling time.

TABLE 3
Incidence and size of pancreatic islet adenoma and incidence of exocrine adenoma in Zucker rats[1]

Age, sex, genotype, and feeding group	Islet adenoma		Exocrine adenoma
	Occurrence	Size	Occurrence
		μm	
0–12 mo			
Male			
Obese, fed AL (n = 5)	4 [80][2]	51 (32–85)[3]	0
Obese, PF (n = 0)	—	—	—
Lean (n = 1)	1 [100]	33 (33)	0
Female			
Obese, fed AL (n = 15)	6 [40]	50 (32–100)	0
Obese, PF (n = 0)	—	—	—
Lean (n = 4)	4 [100]	25 (13–33)	0
12–18 mo			
Male			
Obese, fed AL (n = 72)	12 [54.5]	49 (11–92)	1
Obese, PF (n = 4)	1 [25]	35 (35)	0
Lean (n = 5)	2 [40]	56 (47–64)	0
Female			
Obese, fed AL (n = 17)	14 [82.3]	48 (30–84)	0
Obese, PF (n = 9)	9 [100]	35 (24–54)	0
Lean (n = 2)	2 [100]	36 (28–43)	0
18–24 mo			
Male			
Obese, fed AL (n = 15)	6 [40]	59 (31–99)	1
Obese, PF (n = 19)	10 [52.6]	171 (27–617)	2
Lean (n = 11)	2 [18.1]	49 (45–52)	2
Female			
Obese, fed AL (n = 12)	2 [16.6]	126 (50–201)	1
Obese, PF (n = 20)	4 [20]	238 (43–617)	1
Lean (n = 14)	0 [0]	50 (50)	1
24–30 mo			
Male			
Obese, fed AL (n = 2)	0 [0]	—	—
Obese, PF (n = 20)	6 [30]	151 (37–372)	2
Lean (n = 20)	1 [5.0]	72 (72)	6
Female			
Obese, fed AL (n = 1)	0 [0]	—	0
Obese, PF (n = 11)	4 [36.3]	233 (79–592)	0
Lean (n = 14)	2 [7.1]	36 (36)	1
30–39 mo			
Male			
Obese, fed AL (n = 0)	—	—	—
Obese, PF (n = 1)	1 [100]	37 (37)	0
Lean (n = 5)	2 [40]	372 (308–435)	1
Female			
Obese, fed AL (n = 0)	—	—	—
Obese, PF (n = 2)	0	—	—
Lean (n = 11)	3 [27.1]	NA	3

[1] AL, ad libitum; PF, pair fed; NA, not available.
[2] n; percentage occurrence in brackets.
[3] Range in parentheses.

a lower incidence of lesions such as glomerulonephritis, periarteritis, and myocardial degeneration.

Survivorship and the effects of pair feeding

As predicted, ad libitum–fed obese rats die at earlier ages than do their ad libitum–fed lean controls. MRDT of ad libitum–fed obese rats is significantly lower than for all male and female lean rats and female pair-fed obese rats, but not for male pair-fed rats. We also found a significant sex difference in this strain in that MRDT was greater in pair-fed obese and lean female rats than in their male counterparts. The data from this study agree with all previous reports that energy restriction extends life span in laboratory rodents. In this case, obese rats were restricted to the same intake as their lean controls, or in other words, their hyperphagia was prevented. Ad libitum–fed lean male Zucker rats have a longer 10th percentile survivorship (966 compared with 822 d, respectively) and maximum life span (1067 compared with 941 d, respectively) than do ad libitum–fed male F344 rats (32). This comparison suggests that the Zucker strain is a long-lived one, but that the presence of the *fa* mutation in the strain has a highly significant effect on longevity.

It is difficult to make direct comparisons of our data with those of others because the design of our study differs substantially from the often used practice of restricting energy intake by 40%. Because our study was designed to prevent hyperphagia, we restricted food intake of obese male rats from weaning at 4 wk until 48 wk of age and of obese females from 4 wk until 62 wk of age, after which time obese rats were no longer hyperphagic; their food intake was comparable with that of ad libitum–fed lean rats. Percentage food restriction varied and was greater when the rats were younger (17.7% for obese males and 28.7% for obese females 4–20 wk of age) and less as they became older (5.0% for males and 12.9% for females 40–60 wk of age). At no time did the degree of restriction approach 40%.

We compared our data on the Zucker strain with those of Yu et al (32), Iwasaki et al (33), Shimokawa et al (43), and Thurman et al (44) on the Fischer 344 strain and also with Keenan et al (27) on the Sprague-Dawley CD-COBS VAF strain (**Table 5**). These studies varied from one another and from ours with respect to dietary protein source and degree and duration of restriction. Yu et al (32) imposed a 40% food restriction from 6 to 78 wk of age in male F344 rats fed a casein-based, corn oil–containing diet. This degree of restriction increased 10th percentile survivorship by 43.1% and increased maximum life span by 37.1%. When soy was used as the protein source and a 40% restriction imposed (43), 10th percentile survivorship increased by 24.8%. Thurman et al (44) studied both male and female F344 rats. They began restriction at 14 wk, increasing the degree of restriction incrementally until by 16 wk rats were restricted by 40%. Forty percent restriction was continued until spontaneous death. These investigators used the NIH-31 open-formula diet, which contains fish meal, soy protein, and soybean oil. They reported a 25.8% increase in 10th percentile survivorship and a 36.7% increase in maximum life span for restricted males and an 11.2% increase and a 20.1% increase, respectively, for restricted females.

These data are in good agreement with our findings in obese male Zucker rats, although our degree of food restriction was only 12% from 4 to 48 wk. Even though both the degree and time of restriction were less than that used in these other studies, 10th percentile survivorship increased by similar degrees. The rats restricted in our study were genetically obese (body fat > 50%), whereas F344 rats have a much lower percentage body fat (22% in males and 31% in females at 24 mo) (25). Thus, we conclude that obese male Zucker rats are

TABLE 4
Tumor incidence, pituitary chromophobe adenoma and others, and other notable pathologies (renal and cardiac pathologies reported in Figure 4)[1]

Age, sex, genotype, and feeding group	Tumors			Rats affected	Nonrenal/noncardiac pathologies
	Total	Pituitary	Other		
0–12 mo					
Male					
Obese, fed AL (n = 5)	1	0	Lymphoma	1	None
Obese, PF (n = 0)	—	—	—	—	—
Lean (n = 1)	—		—	—	None
Female					
Obese, fed AL (n = 15)	0	—	—	—	Pancreatitis
Obese, PF (n = 0)	—	—	—	—	—
Lean (n = 4)	0	—	—	—	None
12–18 mo					
Male					
Obese, fed AL (n = 22)	0	—	—	—	None
Obese, PF (n = 4)	1	0	Osteosarcoma	1	None
Lean (n = 5)	0	—	—	—	None
Female					
Obese, fed AL (n = 17)	0	—	—	—	Cerebral hemorrhage
Obese, PF (n = 9)	3	3	—	3	Hepatic failure, intestinal obstruction, pylomyelitis
Lean (n = 2)	1	1	—	1	None
18–24 mo					
Male					
Obese, fed AL (n = 15)	3	3	—	3	None
Obese, PF (n = 19)	3	2	Prostate, gastric ulcer, thrombosis	2	None
Lean (n = 11)	4	4		4	None
Female					
Obese, fed AL (n = 12)	—	—	—	—	None
Obese, PF (n = 20)	6	6	0	6	None
Lean (n = 15)	8	3	Lymphoma, mammary, melanoma, unknown (2)	7	None
24–30 mo					
Male					
Obese, fed AL (n = 2)	1	1	0	1	None
Obese, PF (n = 20)	4	1	Sarcoma, skin, gastric ulcer, biliary, renal	4	None
Lean (n = 20)	9	2	Gastric, hepatic lymphoma (2), melanoma, renal, sarcoma	8	None
Female					
Obese, fed AL (n = 1)	0	—	—	—	None
Obese, PF (n = 11)	5	4	Sarcoma, lung hypertension	5	
Lean (n = 14)	9	4	Hepatic, gastric ulcer, mammary (4), pancreatitis	8	
30–39					
Male					
Obese, fed AL (n = 0)	—	—	—		None
Obese, PF (n = 2)	1	1	Gastric squamous hyperplasia	1	
Lean (n = 7)	4	4	Leukemia, lymphoma	5	Gastric ulcer, adrenal hemorrhage, brain focal hemorrhage
Female					
Obese, fed AL (n = 0)	—	—	0	—	None
Obese, PF (n = 5)	2	2	0	2	Thrombosis (2), hepatic congestion (2), necrosis, adrenal congestion and hemorrhage (2)
Lean (n = 11)	5	5	Adrenal, blood stem cell, mammary	5	Adrenal hemorrhage (2) Lymph, hepatic lymph (2) cardiac thrombosis
0–39 mo					
Male					
Obese, fed AL (n = 44)	5	4 [9.1][2]	1 [2.3][2]	5 [11.4][2]	
Obese, PF (n = 45)	10	4 [8.9]	6 [13.3]	8 [17.8]	
Lean (n = 44)	18	10 [22.7]	8 [18.2]	17 [38.6]	
Female					
Obese, fed AL (n = 45)	0	0 [0.0]	0 [0]	0 [0]	
Obese, PF (n = 45)	16	15 [33.3]	1 [2.2]	16 [35.6]	
Lean (n = 45)	26	13 [28.9]	13 [28.9]	23 [51.1]	

[1] AL, ad libitum; PF, pair fed.
[2] Percentage in brackets.

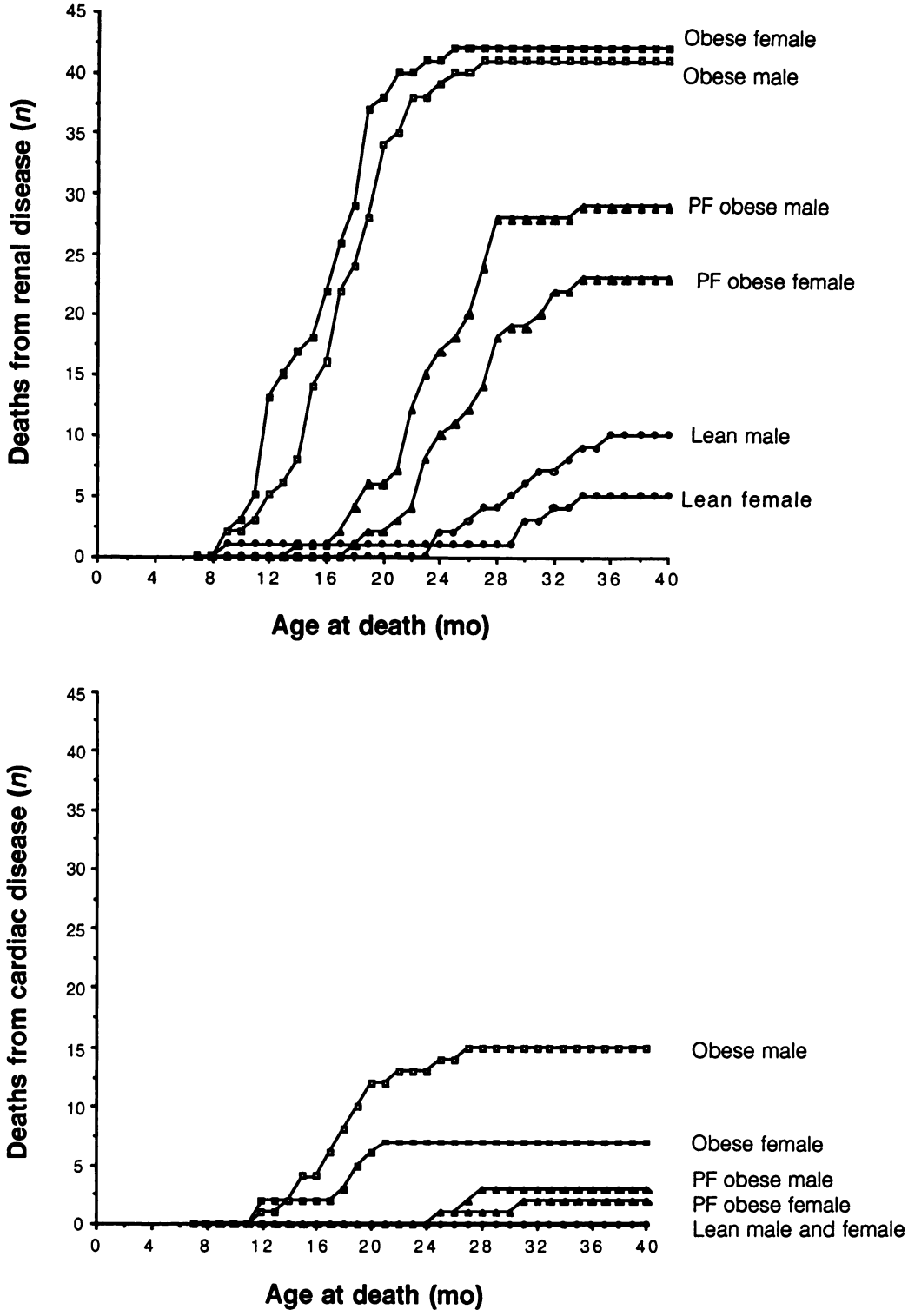

FIGURE 4. Cumulative renal and cardiac histopathology at necropsy as a function of age at death. n = 45 for all groups except the male obese and lean groups (n = 44). PF, pair fed.

more responsive to food restriction than are F344 male rats. This strain difference likely reflects an underlying disease process (chronic nephropathy) that occurs relatively early in genetically obese rats (*fa/fa*), and that is modified by prevention of hyperphagia. The effect of restriction on 10th percentile survivorship and maximum life span is greater when rats are fed casein-based diets compared with diets containing soy as a protein source because soy protein likely protects the animals against early development of renal disease, as suggested previously (33, 43, 45).

TABLE 5
Comparative data on survivorship and effects of energy restriction

Author, rat strain, sex, and feeding	Dietary protein source	Food restriction	Percentage restriction	Percentage body fat and age	10th percentile survivorship	Percentage increase in survival	Maximum lifespan	Percentage increase in lifespan	Percentage survivorship at 24 mo
		wk	*%*	*%*	*d*	*%*	*d*	*%*	*%*
Present study									
Zucker obese									
Male									
AL	Soy	0	0	41 (death)	657	—	803	—	4.4
R	Soy	4–48	12	42 (death)	839	27.7	1010	25.5	48.9
Female									
AL	Soy	0	0	42 (death)	616	—	744	—	2.2
R	Soy	4–62	18	45 (death)	914	20.0	975	8.0	35.6
Lean									
Male									
AL	Soy	0	0	—	966	—	1067	—	—
R	Soy	—	—	—	—	—	—	—	—
Female									
AL	Soy	0	0	—	1014	—	1163	—	—
R	Soy	—	—	—	—	—	—	—	—
Yu et al (32)									
F344									
Male									
AL	Casein	0	0	—	822	—	941	—	—
R	Casein	6–78	40	—	1177	43.1	1299	37.1	—
Iwasaki et al (33)									
F344									
Male									
AL	Casein	0	0	—	857	—	989	—	—
R	Casein	—	—	—	—	—	—	—	—
AL	Soy	0	0	—	937	—	1025	—	—
R	Soy	—	—	—	—	—	—	—	—
Shimokawa et al (43)									
F344									
Male									
AL	Casein	0	0	—	861	—	1078	—	—
R	Casein	6 until death	40	—	1197	39.0	1246	15.6	—
AL	Soy	—	—	—	—	—	—	—	—
R	Soy	6 until death	40	—	1169[1]	24.8	1295[1]	26.3[1]	
AL	Lactalbumin	0	0	—	889	—	1008	—	—
R	Lactalbumin	—	—	—	—	—	—	—	—
Thurman et al (44)									
F344									
Male									
AL	Soy and fishmeal	0	0	—	840	—	896	—	55[2]
R	Soy and fishmeal	14–25, 16 until death	10–25, 40	—	1057	25.8	1225	36.7	70[2]
F344									
Female									
AL	Soy and fishmeal	0	0	—	1001	—	1078		80[2]
R	Soy and fishmeal	14–15, 16 until death	40	—	1113	11.2	1295		90
Keenan et al (27)									
Sprague-Dawley CD-COBS VAF									
Male									
AL	Purina meal 5002 (fishmeal)	0	0	26.5 (24 mo)	—	—	—		7
R	Purina meal 5002 (fishmeal)	5–104	35	16.1 (24 mo)	—	—	—		44
Female									
AL	Purina meal 5002 (fishmeal)	0	0	35.0 (24 mo)	—	—	—		36
R	Purina meal 5002 (fishmeal)	5–104	35	11.0 (24 mo)	—	—	—		62

[1] Significantly different from values for soy feeding (AL) by Iwasaki et al (33), $P \leq 0.05$.
[2] Estimated from graph.

Thurman et al (44) reported that restricted female F344 rats have 10th percentile survivorship increased by 11.2%; restricted female Zucker rats (in this study) achieved a 20.0% increase. On the other hand, F344 females experienced a 20.0% increase in maximum life span compared with an 8.0% increase for restricted Zucker females. The fact that maximum life span is affected less for Zucker than for F344 females may reflect a greater susceptibility of the Zucker strain to degenerative renal disease, especially in females. Onset of renal disease occurred earlier in female than in male, obese Zucker rats (Figure 4). We have data that indicate a role for estrogen in accelerating the onset of renal disease in female obese Zucker rats (46). The mechanism of this effect is under active investigation in our laboratories.

Although Sprague-Dawley rats have historically been considered to be a relatively lean strain, the variant CD-COBS-VAF strain, introduced in 1988, is fatter than the F344 strain and is considered by some to be obese (1). Keenan et al (27–29) studied the effects of food restriction (35%) on survivorship at 24 mo of age in male and female CD-COBS-VAF rats fed Purina-certified rodent chow 5002. Restriction began at 5 wk of age (Table 5). In food-restricted males, survival at 24 mo increased from 7% to 74%. In food-restricted females, survival increased from 36% to 62%. In our study, percentage survival at 24 mo was also lower for obese ad libitum–fed than for obese pair-fed rats (males: 4.4% compared with 48.9%, respectively; females: 2.2% compared with 35.6%, respectively). Food restriction decreased body fat in Sprague-Dawley rats (36% in males, 69% in females) (27). In a second study of this strain (28, 29), energy restriction also significantly decreased body fat. In contrast, in our study, prevention of hyperphagia, which produces a lesser degree of food restriction, did not decrease percentage body fat, as reported previously for 33-wk-old obese Zucker rats (8). Thus, for obese Zucker rats, longevity was independent of body fat content. Note that this genetically obese strain decreases its food intake before death, as do most strains, but maintains its body fat content. Several authors have speculated on whether percentage body fat influences survivorship in laboratory rodents. There are studies to support the hypotheses that body fat both increases and decreases survivorship. Keenan (1) reviewed the reported data and concluded that the available data do not support a role for percentage body fat in determining survivorship. Our data on obese Zucker rats strengthen this conclusion. In this case, we are not referring to animals on a starvation regimen, for which the size of body fat stores clearly contributes to survival (PR Johnson and Zucker, unpublished observations, 1970).

Pathology: nephropathy

In the current study, > 90% of ad libitum–fed obese male and female Zucker rats died with severe nephropathy. Thus, genetic obesity is closely associated with early onset of degenerative renal disease, but the mechanism is not related to the body fat content per se because pair feeding slows renal disease progression but does not alter percentage body fat. It is more likely related to progression of the metabolic syndrome seen in obese rats, with hyperlipidemia being one factor that contributes (47). Although prevention of overeating decreased the occurrence of ESRD in obese males (64.4%) and females (51.1%), it continued to be a significant cause of death, but not premature death.

Kasiske et al (48) reported the appearance of renal disease as early as 9 wks of age in obese Zucker rats fed a chow diet. We have data from 20-wk-old lean and obese Zucker rats fed a soy protein diet ad libitum from weaning, which establishes that renal disease has an early onset in these rats. Urinary albumin excretion (UAE) was significantly elevated in both male and female obese rats in comparison with lean rats (49). Renal pathology includes mesangial matrix expansion and focal point glomerulosclerosis. Prevention of hyperphagia prevents these large increases in UAE and renal pathology by 20 wk of age. Our findings are consistent with those summarized by Yu (50). Histologic examination of the kidneys of F344 rats fed a 21%-casein diet ad libitum revealed severe nephropathy, whereas those that were energy restricted by 40% had minimal signs of nephropathy and a 50% extension of life span. Substitution of soy for casein as the protein source extended life span by 15% with only mild signs of nephropathy. Zucker and Seronde (personal communication, 1975) noted that at 240 d of age, progressive glomerulonephrosis was much more severe in Zucker obese than in lean rats (on a 0–4 point scale: 1.2 in obese compared with 0.1 in lean rats) fed a 24% casein diet. They also reported that the protein-creatinine ratio in 24-h urine samples exceeded 1.0 at an earlier age in obese (100 d) than in lean (950 d) rats. Kasiske et al (48) described more thoroughly the spontaneous focal glomerulosclerosis of Zucker obese rats and reported that treatment of hyperlipidemia somewhat ameliorated these glomerular changes (46). The occurrence of severe nephropathy in lean Zucker rats (*Fa/Fa*) was comparable with that in F344 rats (22.2% in males, 11.1% in females). The mechanism by which presence of the *fa* gene exacerbates the onset of renal disease remains unknown and is under active investigation in our laboratory.

Pathology: cardiomyopathy

The percentage of Zucker rats dying with cardiomyopathy (lesions graded 2–3) was higher in ad libitum–fed obese (males: 33.3%; females: 15.6%) than in pair-fed obese rats (males: 6.7%; females 4.4%) and was nonexistent in lean male and female Zucker rats. These data generally agree with the Zucker and Seronde study (personal communication, 1975), in which the incidence of elevated systolic blood pressure and myocardial necrosis and calcification was greater in obese than in lean rats. In contrast, whether fed ad libitum or restricted, F344 rats have a much higher incidence of cardiomyopathy at spontaneous death, ranging from 55% to 80%; this incidence is independent of the diet fed (casein, soy, or lactalbumin) (43).

Pathology: tumors

The incidence of tumors of all kinds is relatively low in this strain with the exception of pancreatic islet adenomas, which that occur at a rate of 50% in both male and female obese rats fed ad libitum. Pair feeding reduced the occurrence to 37.7% in females, but had no effect in males (49.9%). In no case was it possible to attribute death to the presence of a tumor.

Conclusions

Obesity is associated with increased morbidity and mortality and makes a major contribution to chronic diseases, such as NIDDM, cardiovascular disease, certain types of cancer, kidney disease, and hypertension in both women and men (51).

Controversy continues regarding the severity of obesity necessary to constitute a health risk in humans, but there is a consensus that morbid obesity is a health risk (51). Although obesity is associated with increased mortality, a significant number of obese individuals survive to an advanced age (52). This is likely because the effects of obesity on disease processes are not uniform and are influenced by factors such as genetic background and sex, a hypothesis well supported by the findings in this study on the Zucker strain. These data argue strongly for the beneficial effects of preventing overeating, with or without accompanying significant weight loss or a significant reduction in body fat, to reduce comorbid pathologies, particularly ESRD. Finally, focal glomerular sclerosis has been observed at autopsy in some obese individuals without overt renal disease (53). Thus, the Zucker strain offers a unique opportunity for investigation of early events leading to this pathology with the possibility of identifying potential interventions to slow or prevent its progression.

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