

Mortality and Morbidity in Laboratory-maintained Rhesus Monkeys and Effects of Long-term Dietary Restriction

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Mortality and morbidity were examined in 117 laboratory-maintained rhesus monkeys studied over approximately 25 years (8 dietary-restricted [DR] and 109 ad libitum-fed [AL] monkeys). During the study, 49 AL monkeys and 3 DR monkeys died. Compared with the DR monkeys, the AL monkeys had a 2.6-fold increased risk of death. Hyperinsulinemia led to a 3.7-fold increased risk of death ($p < .05$); concordantly, the risk of death decreased by 7%, per unit increase in insulin sensitivity (M). There was significant organ pathology in the AL at death. The age at median survival in the AL was approximately 25 years compared with 32 years in the DR. The oldest monkey was a diabetic female (AL) that lived to be 40 years of age. These results suggest that dietary restriction leads to an increased average age of death in primates, associated with the prevention of hyperinsulinemia and the mitigation of age-related disease.

THE morbidity and mortality rates for many human diseases increases with age, leading investigators to examine the effects of aging on the onset and severity of human disease. However, successful gerontological intervention in age-related diseases has been complicated by the variation in the rate of aging processes across individuals as well as the variation in interaction of disease pathology with the aging process across individuals (1).

The rhesus monkey (*Macaca mulatta*) is an excellent model for the study of human aging, as it exhibits extraordinary similarity to humans. Behavioral and biomedical data have been documented on the rhesus monkey over at least the last eight decades, further increasing its value for the study of human aging.

However, in comparing the rhesus monkey model to studies of aging in humans, additional data on morbidity and causes of death, life expectancy, life span, survival information, and related characteristics of the rhesus is needed. Previous studies from various primate centers and colonies (2–5) have provided some of these data. For example, Smith (2) summarized mortality, fertility, and growth rates for approximately 450 captive rhesus monkeys compared with free-ranging (Cayo Santiago) primates and showed improved mortality and fertility in the captive monkeys. Gage and Dyke (4) examined the mortality statistics for 25 populations of the larger Old World monkeys (*Cercopithecinae*) with data analyzed for eight data sets and noted the importance of variation in environmental factors in comparing the mortality patterns in both wild and captive Old World primates. Tigges and colleagues (5) studied three groups of captive rhesus monkeys at the Yerkes

Regional Primate Research Center: wild-born, singly housed; wild or captive-born and socially housed; and captive-born and singly housed; mortality rates and maximum life span data were examined.

Ha and colleagues (6) carried out a demographic analysis of the Washington Regional Primate Research Center Pig-tailed macaque colony over nearly 30 years and examined growth, fertility, mortality, and survival. Rawlins and co-workers (7) summarized the demographic, reproductive, and anthropometric data from 1976 to 1983 on the free-ranging rhesus primate colony of Cayo Santiago, Puerto Rico.

Therefore, the current study is provided to further this primate information base, focusing on morbidity and mortality in laboratory-held rhesus monkeys. In addition, the lay population, clinicians, and gerontologists, in particular, have recently focused on which therapies or interventions might be used to achieve not only a longer life, but an increased quality of life. Clearly, in several rodent species studied over the last 60+ years, dietary restriction (generally referred to as a 30–40% decrease in caloric intake without malnutrition) has shown reproducible effects to prolong life span (8–10).

There are currently three groups studying dietary restriction in nonhuman primates: the National Institute on Aging (NIA), which began in 1987 (11,12), the University of Wisconsin at Madison (UW), which began in 1991 (13–15), and the current study at the University of Maryland Obesity and Aging Animal Resource Center (ODAAR), Baltimore (16–20). In addition, a shorter term (5-year) study of calorie restriction in nonhuman primates was conducted at Bowman Gray University (21).

Each of the groups has now compiled data addressing multiple effects of dietary restriction in nonhuman primates. Among these multiple physiological effects, dietary restriction leads to the prevention of obesity (16,19), decreased plasma insulin levels (12,13,15,16,21,22), increased insulin sensitivity (12,13,15,17), and decreased atherosclerosis (21,23), relative to ad libitum (AL)-fed primates. However, to date, there have been limited data regarding the effects of dietary restriction on mortality and morbidity.

The purpose of this study is to determine if there were differences in the survival (mortality) and morbidity (causes of death) of two groups of rhesus monkeys from the Obesity and Diabetes Research Center (ODRC) at the University of Maryland, Baltimore. The two groups of monkeys were defined based on their dietary regimen, which differed only in the amount of diet provided. The first group, referred to as the AL monkeys, was provided standard well-balanced primate chow, which was always available; that is, the amount consumed was not restricted. The second group, referred to as the dietary-restricted (DR) monkeys, was provided the identical well-balanced primate chow; however, the chow was provided in amounts adjusted to maintain a healthy adult body weight in a predetermined range (details of both feeding regimens are described below).

All the monkeys were metabolically well characterized and maintained under constant dietary and environmental conditions. The variables included were age, weight, fasting plasma insulin (IRI), fasting plasma glucose (FPG), glucose tolerance (glucose disappearance rate during an intravenous glucose tolerance test), acute insulin response during an intravenous glucose tolerance test (AIR), and insulin sensitivity (M; estimated during a euglycemic, hyperinsulinemic clamp). We sought to determine if there were differences in the survival of the two groups of monkeys based on AL versus DR conditions, and to identify the major morbidity and causes of death in each group, including associations with metabolic factors.

METHODS

Subjects

One hundred seventeen rhesus monkeys (*Macaca mulatta*) were studied (21 females and 96 males). One hundred nine of the monkeys (88 males and 21 females) were AL fed and 8 monkeys (all male) were DR (details of the protocol described below). All monkeys were part of a longitudinal study of aging, obesity, and spontaneous type 2 diabetes mellitus and were individually housed. Data were obtained from approximately January 1977 to July 1, 2001, a period of almost 25 years. The selection criteria for the subjects are described below. During the course of study, 52 monkeys died (49 of the AL-fed monkeys and 3 of the DR monkeys). These deaths were due to natural causes as detailed below.

All monkeys were maintained on monkey chow (Purina Mills, St. Louis, MO), composed of 70% carbohydrate, 17% protein, and 13% fat. In the case of 16 of the AL-fed monkeys, a nutritionally complete liquid diet, Ensure (Ross Laboratories, Columbus, OH), was occasionally used. For all

monkeys, the diet conditions described below were initiated as soon as the monkeys were released from quarantine.

The AL-fed monkeys were provided the diet in an amount that allowed for food to be readily available 24 hours/day and which allowed each monkey to determine its daily intake on an individual basis. Therefore, in the AL monkeys, food intake per monkey was entirely individually determined.

The DR monkeys were maintained on monkey chow (Purina Mills), which was calorically titrated on an individual monkey basis to maintain a goal weight of 10 to 11 kg, the weight of normal lean adult monkeys (19). This body weight is associated with a body fat ranging from approximately 17–24% (16,19,24). Each monkey was weighed a minimum of once weekly, and the individual daily chow allotment was adjusted up or down for each monkey depending on any change in its body weight. This caloric titration usually required an increase or decrease of one to two biscuits per day. The DR monkeys were fed in three equal amounts at approximately 8 AM, 1 PM, and 4 PM.

For both AL and DR monkeys, the primate husbandry in the ODRC was meticulously carried out, including counting of biscuits eaten per day in all monkeys and direct and discrete observation of all monkeys throughout the day. In addition, the monkeys were weighed weekly and all monkeys received a daily chewable multiple vitamin and fresh water ad libitum.

Selection Criteria and Methods

The monkeys in this study were obtained from the following sources: primate center breeding colonies (n = 41), commercial breeding colonies (n = 32), research laboratories (n = 42), and miscellaneous sources (n = 2). All monkeys were maintained on monkey chow prior to purchase and were research naïve. The prenatal conditions of the monkeys were not quantified or recorded.

Prior to purchase, each primate was screened by the clinical veterinarian for adequate health status and normal health parameters. This process included background information from the attending veterinarian at the originating facility and the medical record of the monkey. All monkeys were required to have a negative tuberculosis test, undergo a standard physical exam, have a normal chemistry/hematology profile (indicating normal electrolyte, liver, and kidney function), be research naïve, and have normal laboratory behavioral characteristics.

Monkeys were included in the study based on the following criteria: All monkeys had a recorded age and a known laboratory and medical history prior to acquisition into the colony. Fifty of the 52 monkeys that died were humanely euthanized due to a veterinary diagnosis indicating that death was imminent, including a diagnosis of a terminal condition or significant morbidity not amenable to treatment. Euthanasia was carried out using 100 mg/kg pentobarbital administered intravenously. Two of the 52 monkeys were found in the morning to have expired during the night. In all cases the necropsy was carried out immediately.

Data Analysis

Basic descriptive statistics regarding the two groups of monkeys were calculated. A Cox proportional hazards

regression model was used to estimate the difference in the mortality rate for the two dietary groups: AL versus DR monkeys. This model assumes that the hazard rates (i.e., the risk of death at a given age) for the two groups are proportional to each other. The survival times were left truncated; therefore, a monkey contributed to the survival estimate beginning at the age at which it entered the laboratory, the time that all monkeys were known to exist under the same environmental conditions. In addition, the model was tested for a possible effect of the source of acquisition on survival.

Secondary analyses compared a) the mortality rate for the DR monkeys with the AL-fed monkeys, and the AL-fed monkeys classified as follows: *normal* (fasting plasma glucose ≤ 126 mg/dl and fasting plasma insulin ≤ 70 μ U/ml), *hyperinsulinemic* (fasting plasma glucose ≤ 126 mg/dl and fasting plasma insulin > 70 μ U/ml), or *diabetic* (fasting plasma glucose > 126 mg/dl), and b) the mortality rate for the two groups of monkeys stratified by diet treatment (AL fed vs DR) after adjusting for the baseline metabolic characteristics: body weight, fasting plasma glucose, fasting plasma insulin, and peripheral insulin sensitivity.

The age at death, the major cause of death, and organ pathology present at death were determined for each monkey based on the individual necropsy reports. Associations between the causes of death and the age at death with gender and diabetic status of the monkeys were tested using two-sample *t*-tests and Fisher's exact test.

RESULTS

Table 1 presents the baseline characteristics of the monkeys in each dietary group. The AL-fed monkeys have been subgrouped as AL normal, AL hyperinsulinemic, and AL diabetic. The baseline metabolic characteristics of these AL monkeys (which ranged from young normal to obese hyperinsulinemic to type 2 diabetes) were as follows: Fasting plasma glucose concentrations ranged from 47–384 mg/dl and fasting plasma insulin concentrations ranged from 3–446 μ U/ml. Glucose disappearance rate during an intravenous glucose tolerance test ranged from 0.68–5.70 %/min and acute insulin response (baseline to 10 minutes) to intravenous glucose ranged from 1–1636 μ U/ml/min. Peripheral insulin sensitivity (M) ranged from 1.63–23.92 mg/kg FFM/min. Type 2 diabetes was defined as two or more observations of fasting plasma glucose concentration ≥ 126 mg/dl, consistent with the diagnostic criteria of the American Diabetes Association (25).

We found no statistically significant differences between the risk of death comparing the DR monkeys with the AL monkeys ($p = .1$), although we estimated that the free-feeding condition compared with DR feeding led to approximately 2.6-fold higher risk of death in the AL monkeys (95% Confidence Interval [CI]: 0.82–8.70). These results were not sensitive to adjustment for the source of acquisition (hazard ratio [HR]: 2.6; 95% CI: 0.77–8.85). Similar results were found when restricting the analysis to those monkeys that entered the study from 12 to 18 years of age (age range for DR monkeys): the estimated HR is 2.27 (95% CI: 0.65–7.94). Estimated median survival based on analysis of the AL-fed monkeys versus the DR monkeys was approximately 25 and 32 years for the AL and DR monkeys, respectively.

Table 1. Summary Characteristics of the Monkeys

Variable/Group	Mean	SD	N	Range
<i>Age at entry (y)</i>				
Dietary restricted	16.6	2.1	8	12–19
Ad libitum fed (normal)	12.6	5.7	64	4–28
Ad libitum fed (hyperinsulinemic)	12.8	5.4	22	7–26
Ad libitum fed (diabetic)	20.8	4.9	20	13–29
<i>Age at death/censoring (y)</i>				
Dietary restricted	26.9	3.6	8	21–30
Ad libitum fed (normal)	20.6	7.2	64	9–34
Ad libitum fed (hyperinsulinemic)	18.6	6.3	22	10–35
Ad libitum fed (diabetic)	25.5	6.0	20	16–40
<i>Body weight (kg)</i>				
Dietary restricted	10.3	0.8	8	9.5–11.6
Ad libitum fed (normal)	11.8	3.7	64	4.8–24.7
Ad libitum fed (hyperinsulinemic)	13.4	3.6	20	4.8–17.8
Ad libitum fed (diabetic)	9.5	4.5	20	4.5–22.2
<i>Fasting plasma glucose (mg/dl)</i>				
Dietary restricted	60	5	8	52–67
Ad libitum fed (normal)	64	8	62	47–92
Ad libitum fed (hyperinsulinemic)	67	6	22	50–77
Ad libitum fed (diabetic)	196	79	20	129–384
<i>Fasting plasma insulin (μU/ml)</i>				
Dietary restricted	14	6	8	5–23
Ad libitum fed (normal)	42	15	61	11–67
Ad libitum fed (hyperinsulinemic)	105	85	22	40–446
Ad libitum fed (diabetic)	63	82	19	3–239
<i>Glucose disappearance rate (%/min)</i>				
Dietary restricted	3.9	0.96	8	2.6–5.7
Ad libitum fed (normal)	3.6	0.90	51	2.0–5.5
Ad libitum fed (hyperinsulinemic)	3.1	0.85	18	2.1–5.1
Ad libitum fed (diabetic)	1.2	0.40	18	0.7–2.3
<i>Acute insulin response (μU/ml/min)</i>				
Dietary restricted	106	60	8	44–228
Ad libitum fed (normal)	131	89	45	27–470
Ad libitum fed (hyperinsulinemic)	378	388	15	73–1635
Ad libitum fed (diabetic)	27	54	15	1–160
<i>Peripheral insulin sensitivity (mg/kg FFM/min)</i>				
Dietary restricted	14.1	6.3	7	6.6–23.9
Ad libitum fed (normal)	12.3	3.7	48	4.6–19.5
Ad libitum fed (hyperinsulinemic)	7.6	3.3	16	1.6–13.9
Ad libitum fed (diabetic)	7.3	2.4	7	4.2–10.1

Note: All but "Age at death" were data collected on entry to the study.

We also compared the risk of mortality for three metabolic classifications of the AL-fed monkeys (normal, hyperinsulinemic, and diabetic) to the risk of mortality for the DR monkeys). Table 2 presents the estimated HRs and corresponding 95% CIs. The risk of death for a hyperinsulinemic monkey was 3.7 times higher than the risk of death for a DR monkey of the same age (95% CI: 1.02–13.62; $p < .05$). Figure 1 presents the estimated survival curves for the DR monkeys versus the normal, hyperinsulinemic, and diabetic groups of monkeys, with the age at median survival indicated for each metabolic group. When restricting the analysis to compare mortality for only AL normal monkeys and DR monkeys, the estimated HR is 2.33 (95% CI: 0.68–7.94).

Table 3 presents the estimated HRs and 95% CI comparing AL-fed versus DR monkeys after adjusting for body

Table 2. Estimated Hazard Ratios and 95% Confidence Intervals Comparing Four Classifications of Monkeys: Dietary-restricted, Ad Libitum Fed Normal, Ad Libitum Hyperinsulinemic, and Ad Libitum Diabetic Groups

Reference group	Comparison Group	N	Hazard Ratio (95% Confidence Interval)
Dietary restricted	AL Normal	58	2.56 (.76–8.57)
	AL Hyperinsulinemic	33	3.71 (1.02–13.62)
	AL Diabetic	18	2.37 (.65–8.68)
AL Normal	Dietary restricted	8	0.42 (.13–1.39)
	AL Hyperinsulinemic	33	1.58 (.79–3.15)
	AL Diabetic	18	2.65 (.78–9.04)

Note: AL = ad libitum.

weight, fasting plasma insulin, fasting plasma glucose, and peripheral insulin sensitivity (M). Results showed that the risk of death for an AL-fed monkey decreases by 7% per unit increase in insulin sensitivity (M) with a 95% CI of 0.80–1.07, although this value was not statistically significant.

In the course of the study, three of the DR monkeys died, and the oldest of these DR monkeys (age 30 years at death) had significant pathology present (case findings discussed further in Discussion). There were no significant differences between the average age at death of males to females ($p = .7$) and diabetics to nondiabetics ($p = .8$), and there were no differences in gender or diabetic status related to significant pathology at death.

Table 4 summarizes the proximal cause of death by organ system. The major (proximal) cause of death in this group of monkeys was cardiac related and included such pathology as endocarditis, aortic valve calcification, fibrosis, cardiac hypertrophy, myocardial infarction, acute cardiac arrest, and congestive heart failure. The next most-prevalent organ systems leading to death involved the gastrointestinal system and the kidney. Interestingly, based on age at death, the monkeys dying of gastrointestinal disease (mean \pm SD: 21 \pm 8 years of age at death) or renal disease (mean \pm SD: 22 \pm 6 years of age at death) were several years younger on average than the monkeys dying of cardiac-related disease (average age 25 \pm 8 years) or hepatic-related disease (26 \pm 5 years). The presence of type 2 diabetes mellitus in the monkeys was clearly a pathological factor, as the proportion of diabetic to nondiabetic monkeys in all categories (cardiac, respiratory, hepatic, renal, reproductive, and cerebrovascular) ranged from 25–100% by group. Only the gastrointestinal category (as the major cause of death) was without diabetic monkeys.

Table 5 summarizes the organ pathology identified at death by dietary treatment group. Considering severe and moderate pathology, the most prevalent organ pathology in all monkeys involved the respiratory system. In the AL-fed monkeys, the most frequent respiratory-related complications included: lung mites (*Pneumonyssus simicola*, ranging from mild to severe), pneumonia, and bronchiolitis. Other pathologic respiratory complications included emphysemic changes, pulmonary edema, pulmonary artery thrombosis, and pleural adhesions. Additional significant pathology was present in the gastrointestinal system (bowel obstruction,

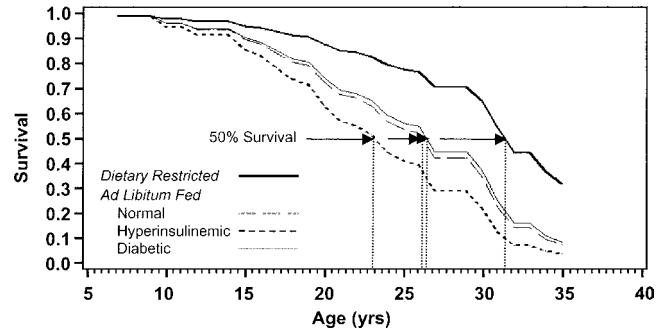


Figure 1. Estimated survival curves comparing the dietary-restricted monkeys

diverticulitis, and gastritis), cardiac (as noted previously), renal (glomerulosclerosis and glomerulonephritis), and liver (fatty infiltration, amyloidosis, hepatitis, and hepatic degeneration). The organ pathology of the three DR monkeys that died will be detailed in the Discussion.

DISCUSSION

The purpose of this study was to describe mortality in a well-established, metabolically well-characterized colony of laboratory-maintained rhesus monkeys. In addition, we examined the effects of long-term dietary restriction, if any, on these same parameters. Specifically, we addressed the questions of whether DR monkeys had improved quality of life, improved health status, and/or extension of the life span compared with the AL-fed, control monkeys.

Results showed the risk of death for an AL fed monkey was 2.6-fold higher than the risk of death for a DR monkey of the same age. Recognizing the limitations of the sample size in the DR group, we estimate that the power to significantly detect a 2.6-fold increase in risk of death in the AL monkeys compared with the DR monkeys is approximately 0.15. Although our study has low power to significantly detect a difference in the risk of death, the study does provide preliminary data that the calorie restriction regimen will improve survival in laboratory-held primates, as compared with AL feeding.

The median survival age in the AL-fed monkeys was 25 years of age. In contrast, the surviving DR monkeys as of July 1, 2001, have attained an average age of 30 \pm 2 years. What the cause(s) of death will be in the surviving DR monkeys is not yet known, and whether there will be a significant difference in the average age at death compared with the AL monkeys awaits further study.

Eight and one-half years after initiation of dietary restriction in the University of Wisconsin primate study, both the DR and control monkeys are in good health (15). Two deaths in each group (originally 15 monkeys each) have been noted: 1 control monkey death was due to herniation of the colon, and 1 DR monkey death was due to asymptomatic cardiomyopathy; in addition, 1 death in each group was anesthesia related (15). Support for increased longevity from dietary restriction feeding in primates compared with controls has been reported by a National Institute on Aging group showing trends consistent with decreased mortality

Table 3. Estimated Hazard Ratios and 95% Confidence Interval in the Two Dietary Groups After adjusting for Metabolic Characteristics

Group/Characteristic	Hazard Ratio	95% Confidence Interval
Dietary restricted	1.00	—
Ad libitum fed	4.63	.86–25.00
Body weight (kg)	.76	.63–.91
Fasting plasma insulin (μ U/ml)	1.03	1.00–1.06
Fasting plasma glucose (mg/dl)	1.07	.91–1.26
Insulin sensitivity (M) (mg/kg FFM/min)	.93	.80–1.07

Note: The hazard ratio for plasma glucose and insulin is shown per 10 unit change in these variables.

(26). This decreased mortality is due to a lower incidence of chronic disease, including cardiovascular disease, neoplasm, diabetes, endometriosis, and kidney failure in the DR versus AL-fed primates (27). Our present findings are consistent with these important studies.

The data were also examined in regard to the effect of different metabolic characteristics of the monkeys on survival. The risk of death for a hyperinsulinemic monkey was 3.7 times higher ($p < .05$) as compared with a DR monkey of the same age. Hyperinsulinemia has been implicated in the pathogenesis of a number of disorders, including obesity, glucose intolerance, and type 2 diabetes in primates (28–30) and dyslipidemia (31). Weyer and colleagues (32) studied 319 nondiabetic Pima Indians with normal glucose tolerance prospectively and found that fasting hyperinsulinemia has a primary role in the pathogenesis of type 2 diabetes, independent of insulin resistance. Additionally, Gwinup and Elias (33) have proposed systemic hyperinsulinemia as a major factor in the development of atherosclerosis, microangiopathy, nephropathy, and type 2 diabetes mellitus, a hypothesis consistent with the present findings.

Comparison of the AL-fed monkeys and the DR monkeys, after adjusting for baseline body weight, fasting plasma insulin, fasting plasma glucose, and peripheral insulin sensitivity (M), showed that the risk of death was decreased by 7% per unit increase in insulin sensitivity. There is good evidence in primates (34,35) and in humans (36,37) to support a close relationship between fasting plasma insulin and insulin sensitivity. Increased survival associated with improved insulin sensitivity is therefore consistent with the previous finding of increased survival and normoinsulinemia.

In addition, decreased peripheral insulin sensitivity (insulin resistance), such as hyperinsulinemia, is a risk factor in the development of obesity, glucose intolerance, dyslipidemia, type 2 diabetes mellitus, hypertension, and the metabolic syndrome in both primates (31) and humans (38–40). Increased mortality is also associated with these disorders. Dietary restriction has been shown to lead to improved glucose utilization in rodents (41) and in primates (12,14,15,17,21,42). Therefore, our data show preliminary evidence that the improved gluoregulation and insulin sensitivity associated with dietary restriction may be a factor underlying protection against age-related disease, decreased morbidity, and increased survival in primates.

Regarding major causes of death, in this study there were no significant differences between male and female

Table 4. Proximal (Major) Cause of Death by Organ System in All Monkeys

Organ System	Monkeys (N)	Age at Death (y) (Mean \pm SD)	Proportion With Diabetes (at Death)
Cardiac	11	25 \pm 8	7/11 (64%)
Respiratory	3	25 \pm 5	3/3 (100%)
Cardio/respiratory	4	30 \pm 5	1/4 (25%)
Gastrointestinal	6	21 \pm 8	0/6 (0%)
Hepatic	4	26 \pm 5	2/4 (50%)
Renal	6	22 \pm 6	4/6 (67%)
Reproductive	4	21 \pm 5	3/4 (75%)
Cerebrovascular	3	27 \pm 4	3/3 (100%)
Musculoskeletal	2	28 \pm 2	2/2 (100%)
TOTAL	43*	25 \pm 6	25/43 (58%)

Note: *Nine monkeys had multiple severe pathology, where the proximal cause of death was not determined.

monkeys and between nondiabetics and diabetics in regard to age at death. These findings are similar to those of Sievers and coworkers (43) who studied diabetic and nondiabetic Pima Indians and found that overall cause-specific death rates, when age- and sex-adjusted, were not significantly different between nondiabetic and diabetic subjects.

Regarding cardiac-related morbidity, the 11 monkeys that exhibited cardiac pathology as the (proximal) major cause of death had an average age at death of 25 \pm 8 years. None of the DR monkeys had cardiac pathology at death, although the three DR monkeys that died were 21, 23, and 30 years of age at death (average age, 25 \pm 5 years). These findings lend additional support to the importance of dietary restriction in maintaining normal plasma insulin levels and insulin sensitivity, and therefore, imparting metabolic protection to these monkeys regarding cardiac disease, atherosclerosis, and related pathology. Studies of type 2 diabetes mellitus in humans have shown that although hyperglycemia and hypertension contribute to the mortality of type 2 diabetes, atherosclerosis is the major cause of death. Ford and Stefano (44) found that approximately 50% of mortality in diabetic humans is related to coronary disease. Standl (45) estimated that coronary heart disease and stroke may account for greater than 75% of deaths in patients with type 2 diabetes.

Three of the eight DR monkeys died during the course of the study. In this limited sample to date, the organ pathology at death in these primates under the DR regimen was as follows. The first monkey died of acute gastrointestinal bloat at 21 years of age. At necropsy, the stomach was markedly dilated, filling almost the entire abdomen. Although there was no food in the stomach, there was approximately 20 ml of fluid in the intestinal tract, and no intestinal blockage or specific intestinal pathology was found. There were no significant lesions in the brain, kidney, liver, or spleen. Bronchiolitis, lung congestion, and pneumonia were also noted, associated with lung mite infestation. The condition of bloat in primates, known as gastric dilatation, was described in 1967 (46). Findings have included rupture of the stomach wall, bowel distension, and abdominal hernia (47), and this disorder has been described as one of sporadic occurrence with high mortality in apparently healthy monkeys (48). Etiology has been attributed to infection, anesthesia, changes in routine, and

Table 5. Incidence of Moderate and Severe Pathology in the Five Major Organ Systems in All Monkeys (Identified by Dietary Treatment Group) at Death

Organ System	Ad Libitum Fed	Dietary Restricted
Cardiovascular		
Severe	21	1
Moderate	0	0
Respiratory		
Severe	20	1
Moderate	12	1
Gastrointestinal		
Severe	20	2
Moderate	2	0
Hepatic		
Severe	16	1
Moderate	2	0
Renal		
Severe	6	0
Moderate	8	1

Note: Due to multiple pathology, particularly in the ad libitum-fed primates, monkeys may be represented in a disease category more than once.

excessive eating and drinking (47); our current monkey had received ketamine for an experiment the day prior to the morning when he was found dead.

The second DR monkey, which died at age 23 years, was diagnosed with systemic lupus erythematosus (SLE). In humans, this chronic inflammatory disease of connective tissue is classified as an autoimmune disorder that affects the skin, joints, kidneys, nervous system, and mucous membranes. In this primate, the clinical situation presented as inappetance, anemia, leukopenia, decreased activity, generalized muscular atrophy evident in the limbs, and decreased range of motion, particularly in the rear limbs.

Radiographs revealed severe discospondylosis in the thoracic, lumbar, sacral, and tail vertebrae as well as marked intravertebral bridging and narrowing of the disc spaces. A viral screen for herpes B virus, herpes simplex virus-1, measles, simian retrovirus, and simian immunodeficiency virus was negative. Additional diagnostic work-up showed an antinuclear antibody titer of 1:160 (reference values of a positive titer in young canines as $>1:20$ and in older canines as 1:60) and clinical signs that included proteinuria, anemia, and leukopenia. A clinical diagnosis of SLE was made. Spontaneous SLE has been rarely documented in macaques; an earlier case in an adult male rhesus (age not stated) was described by Anderson and Klein (49) with a clinical course similar to the present case. Diet-induced SLE in adult female cynomolgus macaques was produced by the feeding of alfalfa seeds (50,51). It has been noted (10) that dietary restriction has been shown to prevent the development of autoimmune disease in susceptible mice strains, including a lupus-like nephropathy. This response may be species specific, as a primate report (52) measuring the peripheral blood mononuclear cell response to various mitogens was decreased in DR rhesus monkeys versus control monkeys, indicating that perhaps the primate

immunological response was not improved by dietary restriction.

The third DR monkey died at age 30 with major gastrointestinal pathology as the proximal cause of death, specifically, colonic adenocarcinoma with metastases to the liver and mesentery. It is noteworthy that this DR monkey lived approximately 9 years longer than the average age of death of the AL-fed monkeys with gastrointestinal disease as the major cause of death.

Among the AL-fed monkeys, median survival was approximately 25 years of age. We would propose, therefore, that this biological threshold is a good indication of an "aged" rhesus monkey. At or after 25 years of age, and certainly by age 30 years and older, the rhesus monkey appears to reflect the characteristic senescent changes of the elderly human population. Currently, there are 15 monkeys in the ODRC colony that are older than 25 years; in this group there are 5 DR monkeys (average age 30 ± 2 years); next in age are 3 diabetic monkeys (average age $29 \pm .5$ years) and 1 old normal monkey (age 28.1 years), and 7 aged monkeys between 25 and 28 years of age. At this point, as the average age of the DR monkeys is about 7 primate years older than the median survival age in the AL-fed monkeys (25 years of age), we might speculate that dietary restriction, if applied to humans, might lead to an extension of median survival.

Although it is not yet known whether maximal life span will be extended by dietary restriction in primates, data relevant to AL feeding and longevity have resulted from the current study. Previously, Tigges and colleagues (5) documented the oldest known rhesus monkey (male), maintained at the Yerkes Regional Primate Research Center, Emory University. This monkey was 35 years old when he died, significantly older than the median survival age found in the present analysis. In the current study the oldest male rhesus (Monkey M-5) also lived to be 35 years old; in spite of life-long AL feeding and a history of obesity, he remained nondiabetic throughout life. His major pathology at time of death included cardiorespiratory pathology (enlarged heart with thickening of the heart valves and multifocal diffuse emphysemic changes in the lungs).

The oldest primate in the current study (Monkey L-9; a female rhesus), which was also maintained on ad libitum feeding, lived to the remarkable age of 40 years old. She had been diagnosed with type 2 diabetes mellitus at age 29 years and, therefore, was maintained on insulin therapy for approximately 11 years. Although this centenarian primate was diabetic, on insulin therapy, and had limited vision, she was otherwise in excellent health, physically active, and alert even at her advanced age. Approximately 6 hours before her death, her level of consciousness decreased, her color became dusky, and she began to deteriorate rapidly. Elective euthanasia was necessary. At necropsy, the proximal cause of death was cardiac related: endocarditis of the tricuspid valve and previous myocardial infarction. To our knowledge, she is the oldest rhesus monkey documented and provides evidence that the maximal life span of the rhesus monkey in the laboratory setting is 40 years of age.

Our findings in the rhesus monkey supplement the existing database from several other species, providing pre-

liminary data that the health-producing effects of dietary restriction led to an increase in average age of survival. In addition, our results suggest that dietary restriction decreased age-related morbidity in the nonhuman primate, associated with the prevention of hyperinsulinemia and the mitigation of several major age-related diseases.

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