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Nonneoplastic pathology in male Sprague-Dawley rats fed the American Institute of Nutrition–93M purified diet at ad libitum and dietary-restricted intakes ☆

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Abstract

This study evaluates the effects of age and chronic dietary restriction (DR) on nonneoplastic diseases in rats that were fed the American Institute of Nutrition (AIN)–93M purified diet. Male Sprague-Dawley (SD) rats were divided into an ad libitum (AL) group and a DR group that was fed the AIN-93M diet with intake reduced by 31%. Nonneoplastic disease profiles were developed to clarify whether the AIN-93M diet fulfills long-term nutritional requirements of rats. Subsets of rats were killed at 58 and 114 weeks of age, and histopathology was performed. At 58 weeks of age, the 2 main types of nonneoplastic diseases in AL rats were liver vacuolization and cardiomyopathy. Dietary restriction reduced the severity and incidence of both lesions. At 114 weeks of age, the most common lesions in AL rats were cardiomyopathy, nephropathy, liver vacuolization, and degeneration with renal failure and genitourinary infections causing the greatest mortality. Dietary restriction reduced the incidence and severity of these lesions. Nonneoplastic diseases accounted for 28.9% and 0.0% of total mortalities in the AL and DR groups, respectively; however, there was a higher incidence of unknown deaths in the DR rats (52.6%) compared to AL rats (28.9%), which may have limited the success of DR to improve survival. Although the AIN-93M diet supported chronic rat growth, alterations in some dietary component concentrations may be required to lower body weight in chronic rodent and human studies. Factors such as diet composition and digestibility may alter nonneoplastic diseases and mortality in rats and humans in a similar fashion.
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Keywords: Dietary; Restriction; Nonneoplastic; Rat; Pathology
Abbreviations: AIN, American Institute of Nutrition; AL, ad libitum; DR, Diet restriction; NIH, National Institutes of Health; SD, Sprague-Dawley.

1. Introduction

A need for nutritionally adequate purified diets to standardize research feeding regimens led to the development of the American Institute of Nutrition (AIN)–76 rodent

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diet [1,2]. However, several nutritional deficiencies were detected in subsequent studies with this diet [3,4]. For instance, deposition of amyloid protein in kidney, spleen, stomach, and liver in mice and hamsters was linked to the high levels of dietary casein protein and resulted in significant decreases in survival [3,4] in both sexes. In addition, the AIN-76 diet caused severe kidney calcification in female rats [5,6].

In an effort to improve rodent health and long-term survival, the AIN-93 purified diets were formulated to meet rodent requirements for growth and maintenance [2]. Nutritionists, toxicologists, and oncologists agreed that a new dietary formulation with lower protein, fat, and carbohydrate concentrations would be more suitable for chronic bioassays. Complete descriptions of AIN-93G (growth) and AIN-93M (maintenance) diet formulations, as well as comparisons to the AIN-76 diet, were reported previously [2]. Briefly, the carbohydrate source was modified to include dextrinized cornstarch; soy oil was added to improve essential fatty acid concentration ratios; protein was reduced in the maintenance formulation; and *tert*-butylhydroquinone was added as an antioxidant. Modifications to the vitamin and mineral concentrations were also made [2].

Many studies have reported the beneficial effect of DR on longevity and disease prevention and/or delay of disease pathologies among several species of experimental animals [7-13]. Recent reports showed that the efficacy of a drug to induce carcinogenesis in rats is reduced when food consumption was lowered by 40% [14,15]. Mortality associated with prescription drug use was significantly reduced by DR, and the relative toxicity of these compounds was decreased dramatically in aged animals [16,17]. Several DR studies related to aging and disease have been conducted using cereal (chow) diets such as the National Institutes of Health (NIH)–31 diet [18,19]. The results of a 114-week pathology evaluation in the Sprague-Dawley (SD) rats fed the NIH-31 diet showed that all levels of DR significantly reduced the incidence and severity of cardiomyopathy and nephropathy and increased longevity [18,19].

Several short-term studies have been conducted to determine pathologic end points related to the AIN-93G diet [6,20-22]. To our knowledge, no long-term study to determine the effects of DR and the AIN-93M diet on nonneoplastic diseases has been reported. The present study establishes nonneoplastic disease profiles for male SD rats fed the AIN-93M diet either AL or restricted by 31%. Nonneoplastic pathology end points were evaluated in the present study to determine whether the new AIN-93M dietary formulation provides adequate nutrition to promote improved long-term survival potential in rats. Further, these nonneoplastic disease profiles may help to clarify the findings that neoplastic diseases [23] accounted for less than 50% of the total early deaths in this study and that tumors are likely not responsible for the large number of unknown deaths. Purified diets such as the AIN-93M cannot be effectively used in future chronic nutritional, toxicological, and aging studies until accurate pathologic profiles are documented to justify their use. It is important to determine the effects of dietary composition and subsequent DR on disease processes and survival in rats as surrogate models of humans whose complex diet and intake levels contribute to disease. The results presented may lead to dietary modifications in human intake patterns that would potentially reduce the incidence and severity of age-related diseases and improve the quality of life. The use of purified diets such as the AIN-93M is significant, considering that they can be precisely formulated to conform to the needs of specific studies.

Our hypothesis is that changes in nonneoplastic diseases associated with intake level of the AIN-93M may explain why the survival curves for AL and DR rats in this study are not significantly different [24]. Further, we hypothesize that the nonneoplastic disease profiles for AL and DR rats fed the AIN-93M casein diet would be significantly different from those for similar rats fed the NIH-31 cereal diet in a previous study [19].

2. Methods and materials

2.1. Animal husbandry and feeding regimen

Animal procedures used in this study have been reported previously [17-19,23,24], and only a brief summary is provided here. The original founder stocks of SD rats (Charles River, caesarean-derived, SD strain) were obtained from Charles River Laboratory (Wilmington, Mass) in 1972, and the subsequent breeding colony stock has been bred and maintained in a specific pathogen-free environment at the National Center for Toxicological Research. The rat strain and all of the experimental procedures were approved by the Institutional Animal Care and Use Committee at the National Center for Toxicological Research. After weaning at 21 days of age, the male SD rats assigned to this study were singly housed in standard rat cages with metal lids and were maintained in clean, conventional animal rooms that were kept at 23°C. Rooms were scheduled for a 12-hour light/12-hour dark photoperiod cycle with lights on from 6 AM to 6 PM daily. All rats were fed at 10 AM daily, which was 4 hours after the onset of lights-on. Body weight was recorded weekly. Sentinel animals were sacrificed periodically, and tissues were sent to microbiology for bacterial screening. Rats that died spontaneously were necropsied and examined for any evidence of infectious disease. Room swabs, as well as food, water, and air samples were monitored for contamination.

The AIN-93M diet composition has been reported elsewhere [2]. This diet was fed AL to all animals from weaning until they were assigned to the experimental protocol at 6 weeks of age. At 6 weeks, 80 male rats continued to be fed the AIN-93M diet AL; 60 male rats were assigned to an intake regimen that was 31% of the AL level of intake (31% DR). A larger number of rats were assigned to