ABSTRACT. Background and aims: Daily nutrient intake and growth of male Sprague-Dawley (SD) rats fed compositionally different diets were monitored over 114 weeks to determine whether rats fed ad libitum (AL) or diet restricted (DR) followed normal growth parameters. A second objective was to evaluate the usefulness of the American Institutes of Nutrition’s AIN-93M (maintenance formulation) diet for aging and DR studies. Methods: Rats were fed NIH-31 cereal-based diet AL, or a vitamin-fortified modification of the NIH-31 diet at 10, 25, or 40% DR. Other SD rats were fed AIN-93M diet AL or 31% DR; daily nutrient intake and growth response were reported. Results: At all intervals up to 36 weeks of age, rats fed AL the NIH-31 diet consumed significantly \((p<0.001)\) more than rats fed AL the AIN-93M diet, and required more diet per unit of gain than AIN-93M AL rats. However, body weight (BW) gain in rats AL-fed the AIN-93M diet demonstrated that energy components were more efficiently metabolized than in those fed the NIH-31 diet. Whereas diet restriction decreased BW, the rate of maturation, i.e., the rate of reaching a mature BW, increased as intake level decreased. Growth response showed all growth curves were normal, but intake level effects on mature BW and maturation rate differed significantly \((p<0.001)\). Curves for rats AL- and DR-fed the AIN-93M diets were similar to those of rats AL- and DR-fed NIH-31 diet formulations, suggesting that diets adequately met growth requirements and supported normal growth parameters of male SD rats when fed AL or DR. Conclusions: A modification in the AIN-93M energy components to reduce total calories and an evaluation of other nutrient profiles could improve its usefulness as a maintenance and aging diet.

INTRODUCTION

Diet, a valuable tool in research planning, has a profound effect on experimental outcome, and it is important to understand the performance characteristics that it gives animals during protocol development. Laboratory diet formulations and selection criteria for cereal-based, purified, or chemically-defined diets can alter experimental outcomes influencing, for example, nutrient-compound metabolic interaction, disease onset, drug toxicity, genetic response, survival, and pathology (1-6) as well as growth response.

The need for nutritionally adequate purified diets allowing standardized research led to the formulation of the AIN-76 rodent diet (2). However, rodent pathology studies revealed that amyloid protein A deposition in kidney, spleen, stomach, and liver was associated with high casein diets and that replacing casein with soy protein delayed the progression of amyloid A-related pathology and increased survivability while decreasing end-stage chronic nephropathy among male Fischer 344 rats (5, 7-9). The mortality rate and incidence of malignancies were higher in mice fed the AIN-76 diet (casein protein) than in mice fed the NIH-07 non-purified diet containing soy, fish meal, and alfalfa protein when animals were exposed to 2-acetylaminofluorine (10). The AIN-76 diet was also linked to kidney calcification in female rats (11).

With the increased demand for long-term aging and toxicological studies, AIN-93G (growth) and AIN-93M (main-
tenance) purified diet formulations were developed as alternatives to the AIN-76 purified diet (4) and natural-ingredient chow diets typically used in these studies. The AIN-93M diet was found to successfully support a rodent aging study in which performance characteristics, for example, rodent survival, intake, and body weight (BW) curves of male Sprague-Dawley (SD) rats fed ad libitum (AL) and diet restricted (DR), were described (12). The DR component for the AIN-93M diet was included to evaluate whether caloric control would promote increases in survival similar to DR paradigms in which other rodent diets were given (13-17). Performance criteria were also evaluated in male SD rats fed the NIH-31 natural-ingredient chow diet AL and DR to determine the effects of different levels of diet restriction on aging and survival (1). The aims of the present investigation were: 1) to compare daily nutrient and energy intake levels when AIN-93M or NIH-31 diets were given AL and DR; 2) to further characterize the AIN-93M diet for use in aging studies and to evaluate a DR level comparable to that selected for the NIH-31 diet; and 3) to determine whether the criteria of normal parametric growth were met under the designated AL and DR paradigms when either diet type was fed.

METHODS

Animals

Husbandry procedures have been reported elsewhere (1, 12, 18). SD rats [Crl:CD®(SD)BR], obtained from the Charles River Laboratory, were bred in a specific pathogen-free (SPF) environment at the National Center for Toxicological Research (NCTR). Following weaning, the male rats assigned to this study were kept in clean, conventional animal rooms. Sentinel animals were sacrificed periodically and tissues were sent to the microbiology unit for bacterial screening. Rats that died spontaneously were necropsied and examined for evidence of infectious disease. Room swabs, and food, water, and air samples were monitored for contamination. Rats were housed singly in standard, polycarbonate rat cages with metal lids, maintained at 23°C. Rats, conditioned to a 12-hr cycle, showed no harmful peroxide concentrations in the activity, showed no harmful peroxide concentrations in the diet. Chemical analysis of peroxides, an indicator of fat degradation and potential microbial activity, showed no harmful peroxide concentrations in the diet. Contaminants were present in traces below the maximum acceptable limits established for NCTR (e.g., lead 1.5 mg/kg, arsenic 1.0 mg/kg, and cadmium 0.25 mg/kg). Selenium, which is considered a contaminant, but is required by the rat in low concentrations (0.15 mg/kg for growth), is present in the diet below potentially toxic levels. The acceptable NCTR limit established for selenium is 0.65 mg/kg. Daily intake was recorded so that nutrient consumption, on the basis of per kilogram BW, could be compared among the various AL and DR groups fed their respective diets at 6 and 24 months of age.

The energy density of the AIN-31M diet was approximately 3.8 kcal/g (physiological fuel value estimates). The digestible energy determined for the AIN-93M diet was 95%, based upon digestion coefficients of ingredient composition (20). The gross energy density of both NIH-31 diet formulations was 4.33 kcal/g (by bomb calorimetry); digestible energy of both NIH-31 diet formulations was estimated at 80% based upon digestibility studies employing Fischer 344 rats (1). Energy intake calculations were based upon approximate metabolizable energy (ME) values of 3.6 kcal/g for the AIN-31M diet, and 3.5 kcal/g for both NIH-31 diet formulations.

Six lots of both the NIH-31 and NIH-31+vitamins diets were chemically analyzed for nutrient concentrations (Table 1). All nutrient concentrations of either NIH-31 diet formulation were adequate or were in excess of rat requirements (21). Concentrations of vitamins A and E exceeded requirements 7.8- and 2.9-times, respectively, in the NIH-31 standard diet, and 11.3- and 4.3-times, respectively, in the NIH-31-vitamin formulation. Thiamine exceeded the requirement 10.6- and 14.2-times in the standard and vitamin-fortified formulations, respectively. The remaining water-soluble vitamins ranged from 1 to 8 times the requirement for the NIH-31 standard diet, and 1.5 to 10 times the requirement for the vitamin-fortified formulation. Although dietary mineral concentrations in the DR diet were not increased, all minerals were in excess of rat requirements. Chemical analysis of peroxides, an indicator of fat degradation and potential microbial activity, showed no harmful peroxide concentrations in the diet. Contaminants were present in traces below the maximum acceptable limits established for NCTR (e.g., lead 1.5 mg/kg, arsenic 1.0 mg/kg, and cadmium 0.25 mg/kg). Selenium, which is considered a contaminant, but is required by the rat in low concentrations (0.15 mg/kg for growth), is present in the diet below potentially toxic levels. The acceptable NCTR limit established for selenium is 0.65 mg/kg. Daily intake was recorded so that nutrient consumption, on the basis of per kilogram BW, could be compared among the various AL and DR groups fed their respective diets at 6 and 24 months of age.