Middle-aged male mice on a long-term diet high in fat and sugar have early divergent changes in body weight and fat mass associated with aging parameters.

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Abstract

The ability to respond to physical stress that disrupts normal physiological homeostasis at an older age embraces the concept of resilience to aging. A physical stressor could be used to induce physiological responses that are age-related, since resilience declines with increasing age. Increased caloric intake is a nutritional stress with a high prevalence of obesity in older people. In order to determine the effect of a high fat (HF) diet on resilience to aging, 18-month-old male C57BL/6J mice were fed a diet high in saturated fat (lard) and sucrose for ten months. Mice were designated as resistant or sensitive one month after starting the HF diet based on relative increase in body weight or fat mass, respectively. At the end of the 10-month study, sensitive mice showed increased cognitive impairment, decreased cardiac function and motor agility, and decreased survival compared to resistant mice. The degree of response aligned with resilience to the long-term adverse effects of the diet and aging in general. This observation suggests additional studies could be conducted to investigate the relationship between relative body weight and fat mass and healthy aging under different dietary conditions.

Key words. Resilience to aging, High fat diet, Physical stressor, C57BL/6J mice, Cognition, Cardiac function.

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Introduction

A decline in functionality with increasing age can be seen in changes in physical abilities [1], cognitive abilities [2], and overall morphological structures [3]. Basically, the ability to maintain normal function becomes impaired. However, not all people age at the same rate making some individuals more resilient to age-related changes compared to others [4]. The concept of physical resilience to aging builds on the heterogeneous response pattern to physical stress that disrupts normal physiological homeostasis and the rate of return to normalcy [5]. Therefore, a physical stressor could be used to induce physiological responses that are age-related for resilience measurement [6]. Resilience to aging then could be predicted by analyzing responses to a physical stressor that disrupts physiological functions correlated with the robustness of the response with aging endpoints.

One example of physical stress is nutritional stress. The prevalence of obesity in older people has dramatically increased in recent years. In the United States, more than 37 percent of men and women aged 60 years and over are obese, which puts the elderly at a much higher risk for developing disability and loss of function [7]. The potential of using a diet high in saturated fat to predict resilience to aging can be studied in mice using early metabolic changes such as increase in body fat. It is well established that certain inbred strains of mice, especially male C57BL/6J mice, are highly sensitive to diets high in fat and sugar [8]. Changes can occur relatively quickly so the possibility exists that the degree of response as measured by increases in body weight and fat mass could decipher which mice might be more resilient to the long-term adverse effects of these types of diets and aging in general.

In this study we show that middle-aged (18 months) C57BL/6J male mice fed a diet high in saturated fat (lard) and table sugar (sucrose) for ten months have early divergent changes in body weight and body fat mass that align with aging parameters of physiological function and survival.

Methods

Animals

C57BL/6J male mice were obtained from the United States National Institute on Aging aged rodent colony at 18 months of age. Mice were housed 3 to 5 per cage in an SPF facility at the University of Washington under a 12-hour light and 12-hour dark cycle with room temperature of 25℃±4 and reverse osmosis water in an automatic watering system. Mice were acclimated for two weeks. The high fat and sugar (HF) diet (Bioserv, 3282, paste, gamma irradiated) has been described [9]. Briefly, it consisted of lard, sucrose, casein, maltodextrin, vitamins and minerals with 36 percent fat, 36 percent carbohydrate, and 20 percent protein. The level of kilocalories per gram of food was 5.54. Body weight was measured weekly. Body fat mass was measured by quantitative magnetic resonance imaging (QMR) (EchoMRI) monthly with readouts in grams minus water content. Percent body fat mass was calculated by dividing fat mass in grams by body weight at the time of measurement. Food consumption was calculated in the first week of each month by weighing the food placed in each food holder, and three days later weighing the remaining amount, including any fines in the bedding. The diet was replaced weekly in each food holder over a ten-month period. The study was approved by the University of Washington IACUC.

Physiological performance tests

A spatial navigation learning task was used to assess learning impairment [10]. Mice were placed into a square box with seven blocked exits and one escape hole leading to a dark non-stressful cage. In each trial, mice were allowed to explore the cage for 120 seconds. Mice were tested continuously for four trials in one day and their escape times recorded.

Cardiac function was assessed by echocardiography. Echocardiography is a non-invasive procedure that allows the assessment of systolic and diastolic function in mice. A Seimans Acuson CV-70 system was used with standard imaging planes: M-mode, conventional and Tissue Doppler imaging, to measure cardiac function, e.g., LV mass index, LA dimension, end diastolic and systolic dimensions, LV fractional shortening. Ea/Aa (diastolic function) was measured by tissue Doppler imaging of the mitral annulus, and myocardial performance index, as described [11].

Rotarod performance is a measure of balance and coordination, and was assessed as the ability of mice to maintain balance on a rotating rod using a Rotamex 4/8 (Columbus Instruments, Inc.) with an accelerating rod protocol as described [12]. Up to four mice were placed on the rod within their individual lanes in the rotarod enclosure. The software recorded photobeam breaks as the animal's continuous participation in the task. Once an animal fell from the rotarod, there were no longer any beam breaks, and final time was recorded. Three successive runs were performed.

Grip strength, which is one of the measures used to assess frailty in older people, was determined using a Grip Strength Meter (Columbus Instruments, Inc.) by measuring the amount of force the mouse can apply in grasping a specially designed pull bar assembly.

Statistical analysis

Significance analysis was done by one- and two-way ANOVA. Mean values ± standard error of the mean (SEM) were presented in the figures. Statistical significance was established as $p \leq 0.05$. Correlation analysis and high/low responder separation were calculated using JMP pro software, version 14.

Results and Discussion

A rapid increase in body weight was associated with poor cognitive ability and motor coordination.

Mice fed the HF diet with body weight increases of 20 percent or more after the first month were considered sensitive (Fig. 1A). For assessing association with cognitive function, escape times in the spatial navigation task were quantified as the R^2 value of the learning curve [12]. Specifically, a linear trendline was drawn on escape times vs trials of each mouse and the R^2 value was recorded. Sensitive mice had poor learning ability (Fig. 1B) and spent less time on the rotarod (Fig. 1C) than resistant mice. There was no difference in grip strength between the two groups (data not shown).

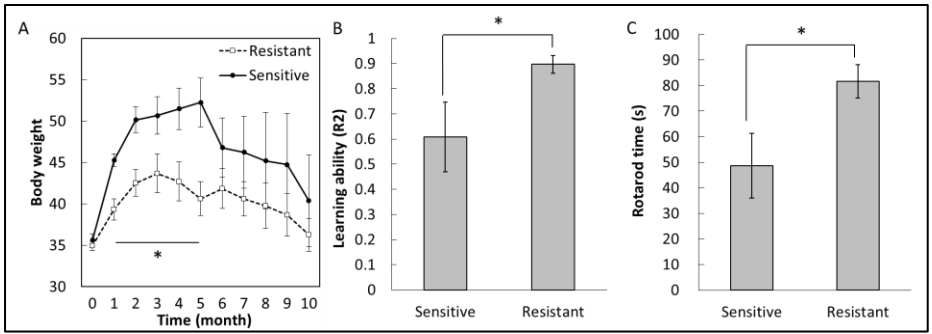

Figure 1. Early changes in body weight were associated with cognitive function and rotarod performance. A) Male mice with more than 20 percent increase in body weight in the first month on the HF diet were considered sensitive responders; **B)** and showed poor learning ability; and **C)** decreased rotarod time after 10 months on the diet. *p≤0.05**.** N=13-17 mice per cohort.

These observations suggest that a long-term diet consisting of high levels of saturated fat and table sugar are detrimental to cognitive function and motor coordination in middle-aged male mice, and provide a model system to study the mechanistic aspects as to why some mice are more resistant to developing these detrimental features, while others are very susceptible. There was no difference in amount of food ingested or caloric intake between the two groups (data not shown), so biological variation between individual mice is of interest to investigate.

A rapid increase in body fat mass was associated with poor cardiac performance and decreased survival.

Mice fed the HF diet could be separated into sensitive or resistant groups based on the relative increase in body fat mass after one month (Fig. 2A). The separation boundary was calculated as 32 percent by Jmp using regression tree, so that mice with more than 32 percent body fat were considered sensitive. These mice had lower survival (Fig. 2B) compared to resistant mice with less than 32 percent increase in fat mass. Sensitive mice had increased cardiac dysfunction after 5 months as indicated by an MPI of 0.56±0.06, while resistant mice had an MPI of 0.78±0.09 (Fig 2C).

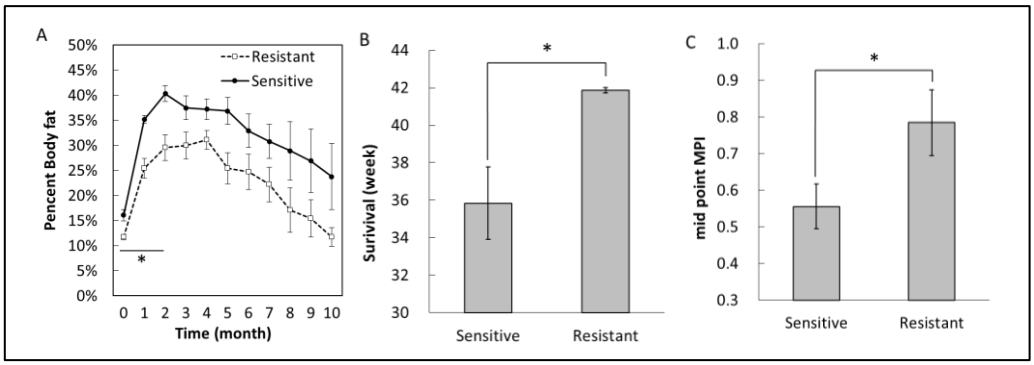

Figure 2. Early changes in body fat mass were associated with cardiac performance and survival. A) Mice fed the HF diet were categorized as resistant or sensitive by percent body fat measured after the first month. **B)** Mice with more 32 percent body fat were considered sensitive, and had decreased survival; and **C)** increased cardiac dysfunction as assessed by the myocardial performance index. *p≤0.05, N=13-17 mice per cohort.

The body fat mass data add additional evidence that a long-term HF diet in middle-aged male mice is stressful and enhances aging, at least in mice that are designated as sensitive. The association between sensitivity and poor cardiac performance is of special interest because it is well accepted that people ingesting diets high in fat and sugar are at increased risk for heart disease [13]. This increased risk

extends to overall survival as shown by an average survival of 36 weeks for HF diet sensitive mice. The fact that most of the HF diet resistant mice survived the entire study period suggests that cellular and molecular factors are involved in parameters associated with resilience to aging. Additional studies are currently being carried out on the differences in anatomic and cellular pathology seen in the two groups.

The HF diet used as a physical stressor provides observations on metabolic changes showing that middle-aged C57BL/6J male mice respond to this type of nutritional stress as expected. However, it is of interest to see that a diet containing large amounts of saturated fat and table sugar can segregate groups of mice based on how rapidly they gain weight and fat mass at the beginning of the feeding trial. One unexpected issue was the lack of concurrence between early body weight gain as a predictor of adverse aging effects and early body fat mass gain as a predictor. There was no significant correlation between the two measurements at this time point and no overlap was observed as neither was able to predict any aging parameters measured by the other. In addition, there were no differences in lean body mass between the two groups, so it is speculated that a rapid increase in body weight and fat mass in response to a diet high in fat and sugar align with different metabolic parameters associated with aging.

In conclusion, different increases in body weight and body fat mass in the first month of starting a diet with high amounts of saturated fat and sugar were seen in middle-aged C57BL/6J male mice and were associated with resilience to aging parameters after 10 months on the diet at 28 months of age. This diet and the inbred middle-aged C57BL/6J mouse strain will be useful to study physical resilience to aging and age-related diseases with relevance to clinical studies. Of particular interest will be the study of middle-aged female C57BL/6J mice to determine differences and similarities attributed to sex.

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