

Male and female middle-aged rabbit model fed a high-fat diet: Changes of weight, intake, and blood composition over time

Tomoyuki Kuroiwa¹, Hayman Lui¹, Ramona Reisdorf¹, Abigail Johnson¹, Koichi Nakagawa¹, Anne Gingery¹, Peter Amadio¹

Email of Presenting Author: kurorth@gmail.com

Disclosures: Tomoyuki Kuroiwa (N), Hayman Lui (N), Ramona Reisdorf (N), Abigail Johnson (N), Koichi Nakagawa (N), Anne Gingery (N), Peter Amadio (N)

INTRODUCTION: Regular consumption of a high-fat diet (HFD) is a major public health problem because it can lead to obesity, insulin resistance, and dyslipidemia, which are triggers for a number of diseases. In the orthopedic field, obesity and insulin resistance are also important factors known to have adverse effects on a wide variety of diseases, including osteoarthritis, tendonitis, carpal tunnel syndrome, ossification of the posterior longitudinal ligament, and osteoporosis. Thus, various HFD animal models have been proposed to evaluate the effects of HFD on the human organism. Rabbit models are widely used in studies because they easily mimic human pathology and can be studied with minimal staff, maintenance, and resources. However, previous studies did not report the extent to which rabbits actually consumed the HFD or what problems they encountered during the HFD feeding period. Furthermore, these studies were usually conducted on young male rabbits rather than mature male and female rabbits¹⁻⁴. Thus, the effect of HFD on aged female and male rabbits is still unclear. Nevertheless, given the worldwide increase in obesity and the fact that many of the aforementioned diseases affect both aged women and men, it is essential to study age-appropriate animal models of both sex. Therefore, we hypothesized that there would be sex differences in the effects of HFD on mature rabbits. To test the hypothesis, we fed HFD diets to male and female mature rabbits and evaluated their dietary intake trends and serological changes.

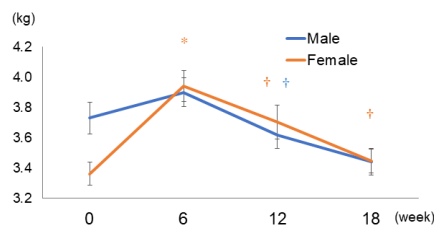
METHODS: This study was approved by The Mayo Clinic Institutional Animal Care and Use Committee. 48 retired breeder New Zealand White rabbits (24 female, 24 males) were used. Their average age was 2.6 (0.9–4.3) years. The HFD was made by adding 0.5% cholesterol and 4% peanut oil to the STD, as was previously reported. The rabbits were fed for 18 weeks and then sacrificed. The weight of the rabbits was measured every 6 weeks. Daily chow intake was defined as the amount given in the morning minus the amount left uneaten evaluated during the chow replacement. The blood samples of the rabbits were collected in the early morning after overnight fasting every 6 weeks. Insulin level was analyzed only in the blood collected at sacrifice. HOMA-IR was calculated with the glucose and insulin data. For weight and blood test results in the HFD group, one-way repeated measures ANOVA was used to compare the differences between time points, and the Tukey method was additionally performed as a post hoc analysis. The difference of intake between males and females every week was compared using Student's t test.

RESULTS: In males, body weight decreased significantly by HFD beginning at 12 weeks. Meanwhile, in females, body weight significantly increased after 6 weeks of HFD, followed by a significant decrease in body weight after 12 weeks (Figure 1). Overall, HFD intake trended downward during the study period. The intake in males was significantly smaller at weeks 1 through 6 and weeks 9 and 10 but significantly larger at weeks 14 and 15 than in females (Figure 2). Cholesterol was significantly increased by HFD in both males and females. Triglycerides were significantly increased by HFD in only males. Glucose showed a significant decrease in males from 12 weeks, while in females, it showed non-constant change (Figure 3). While, insulin level and HOMA-IR did not show a significant difference between STD and HFD groups in both males and females.

DISCUSSION: Unlike the previous study, no weight gain or increase in blood glucose was shown, despite feeding the same composition of HFD for a longer period. However, other HFD diet studies with young and old rats reported that glucose increase was not significant and weight gain occurred only temporarily in the aged rats, while the young rats had a significant increase in glucose and weight until the end of the experiment^{5,6}. That is consistent with the present findings. This further suggests that young animals may not be ideal models for elucidating the impacts of metabolic disruptions and their consequences. Focusing on male-female differences, we found three main differences in the study: (i) changes in triglyceride levels, (ii) changes in body weight from 0 to 6 weeks, and (iii) HFD intake (especially from 0 to 6 weeks). In a previous HFD mouse model study, males were shown to be more vulnerable to metabolic alterations⁷, and result (i) was consistent. On the other hand, in the above study, males were shown to be more vulnerable to HFD impact on body weight, which at first glance would seem to be inconsistent with the result (ii). However, this "impact on body weight" is based on standardized by caloric intake, and in light of result (iii) that male HFD intake itself was lower at 0-6 weeks than females, we can say that these are not conflicting results.

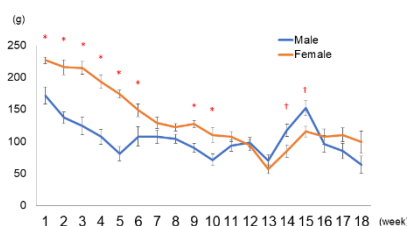
SIGNIFICANCE/CLINICAL RELEVANCE: This study showed that high-fat diets have different effects on rabbits of different ages and sex. This means that research should incorporate both sex and age-appropriate models. Further, it is important to assess the systemic biological responses to metabolic changes concurrently in experiments. We observed sex differences in the effects of HFD. This discovery may lead to the elucidation of sex differences in the development of many orthopedic diseases that are adversely affected by obesity and diabetes, and further studies will be conducted.

Figure 1. Body weight transition



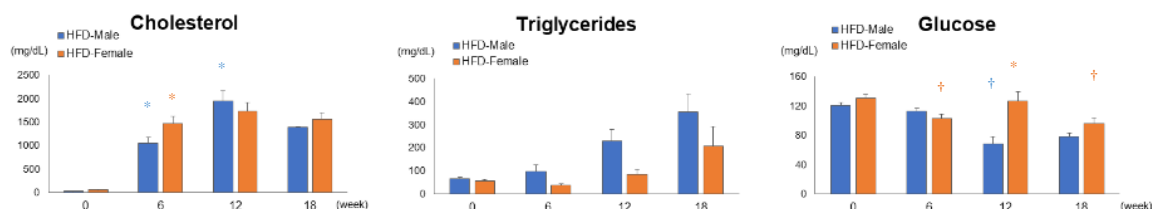
* and † indicates significant increase and decrease compared to previous, respectively.

Figure 2. Intake amount transition



* and † indicates amount was significantly higher and lower in the female group, respectively.

Figure 3. Blood test results



* and † indicates significant increase and decrease compared to previous, respectively.