The effects of ovariectomy and lifelong high-fat diet consumption on body weight, appetite, and lifespan in female rats

Authors

Takeshi Iwasa*, Toshiya Matsuzaki, Kiyohito Yano, and Minoru Irahara

Department of Obstetrics and Gynecology, Institute of Biomedical Sciences, Tokushima University Graduate School, 3-18-15 Kuramoto-Cho, Tokushima 770-8503, Japan

*** Corresponding author**

E-mail: iwasa.takeshi@tokushima-u.ac.jp (TI)

Key words

OVX, high-fat diet, longevity

Short title

The effects of ovariectomy and lifelong high-fat diet

Abstract

In females, ovarian hormones play pivotal roles in metabolic, appetite, and body weight regulation. In addition, it has been reported that ovarian hormones also affect longevity in some species. Recently, it was found that the consumption of a high-fat diet aggravates ovariectomy-associated metabolic dysregulation in female rodents. The aim of this study was to investigate the hypothesis that long-term high-fat diet consumption and ovariectomy interact to worsen body weight regulation and longevity in female rats. At 21 days of age, female rats were weaned and randomly divided into two groups, one of which was given the high-fat diet, and the other was supplied with standard chow. At 23 weeks of age, each group was further divided into ovariectomized and sham-operated groups, and then their body weight changes, food intake, and longevity were measured until 34 months of age. The sham – high-fat diet rats exhibited greater body weight changes and higher feed efficiency than the sham – standard chow rats. On the other hand, the ovariectomized – high-fat diet and ovariectomized - standard chow rats displayed similar body weight changes and feed efficiency. The sham – high-fat diet and ovariectomized – standard chow rats demonstrated similar body weight changes and feed efficiency, indicating that the impact of ovariectomy on the regulation of body weight and energy metabolism might be similar to that of high-fat diet. Contrary to our expectations, ovariectomy and high-fat diet consumption both had small favorable effects on longevity. As the high-fat diet used in the present study not only had a high fat content, but also had a high caloric content and a low carbohydrate content compared with the standard chow, it is possible that the effects of the high-fat diet on body weight and longevity were partially induced by its caloric/carbohydrate contents. These findings indicate that the alterations in body weight and energy metabolism induced by ovariectomy and high-fat diet might not directly affect the lifespan of female rats.

Introduction

It has been well established that obesity and overweight are major risk factors for several diseases, such as diabetes, cancer, and cardiovascular disease [Manson et al., 2004; Mokdad et al., 2001]. In females, ovarian hormones play a pivotal role in metabolic, appetite, and body weight (BW) regulation [Della et al., 2014; Hirschberg, 2012; Hong et al., 2009; Kaaja, 2008; You et al., 2004]. In humans, a deficiency of ovarian hormones in the postmenopausal period increases the risk of obesity and a range of metabolic disorders [Della et al., 2014; Kaaja, 2008; You et al., 2004]. Similarly, the short-term; i.e., for several weeks, abrogation of ovarian hormone production increases food intake (FI) and BW, and consequently induces obesity and associated comorbidities in experimental animals [Blaustein and Wade 1976; Butera 2012; Hirschberg 2012; Hong et al., 2009; Iwasa et al., 2016]. On the other hand, it remains unclear whether the long-term abrogation of ovarian hormone production also induces such unfavorable metabolic effects. Some studies have indicated that ovariectomy (OVX) induces increases in BW and fat deposition, even at 12 months after the surgery [Seidlova-Wutteke et al., 2012], whereas other studies have shown that it does not affect BW or fat mass at 8-10 months after surgery in female rodents [Gilbert and Ryan, 2014]. Similarly, although the effects of long-term OVX on longevity have been examined, the results of these studies varied markedly. It has been reported that ovarian hormones might have a positive effect on longevity in humans and experimental animals [Asdell et al., 1967; Benedusi et al., 2014; Parker et al., 2009]. Ovariectomy (OVX) is associated with a shortened lifespan in female rodents [Asdell et al., 1967; Benedusi et al., 2014], and women that undergo elective hysterectomy exhibit greater longevity than those who undergo hysterectomy combined with OVX [Parker et al., 2009]. On the other hand, OVX increases longevity in stroke-prone spontaneously hypertensive female rats [Stier et al., 2003]. Recently, Ludgero-Correia et al. reported that high-fat diet (HFD) consumption aggravates OVX-associated metabolic dysregulation in female mice [Ludgero-Correia et al., 2012]. OVX mice that were fed a HFD for 18 weeks displayed greater fat weight and larger adipocytes than OVX mice fed standard chow (SC). As the high-fat diet used in this study also had a high caloric content and a low carbohydrate content, it is possible that the effects of the high-fat diet on body composition were induced by its caloric/carbohydrate contents. On the other hand, the long-term effects of OVX and HFD consumption on BW, FI, and lifespan have not been examined in females.

Thus, the aim of this study was to investigate the hypothesis that long-term HFD consumption and OVX interact to worsen BW regulation and longevity in female rats.

Materials and Methods

Animals

All research involving vertebrates or cephalopods must have approval from the institutional animal care and use committee of the University of Tokushima and must be conducted according to the applicable national and international guidelines. Approval was received prior to the start of this research (No. 14060). Pregnant Sprague-Dawley rats (day 15 of pregnancy, BW: 350 – 380 g) were purchased (Charles River Japan, Inc., Tokyo, Japan) and housed individually under controlled lighting (12 h light, 12 h dark cycle) and temperature (24°C) conditions. All animal experiments were conducted in accordance with the ethical standards of the institutional animal care and use committee of the University of Tokushima. The day the pups were born was considered to be day 1, and only female newborn pups were used for the experiment. To remove litter-based effects, the litters were combined, and 10-12 pups were randomly assigned to each dam.

Experimental protocol

At 21 days of age (3 weeks (wk) of age), female rats were weaned and randomly divided into two groups, one of which was given the HFD (HFD-60; Oriental Yeast Co. Ltd., Tokyo, Japan; 506.2 kcal/100 g, 62.2% of the provided calories were derived from lard-based fat, 18.2% were from protein, and 19.6% were from carbohydrates) (n=14), and the other was supplied with SC (type MF; Oriental Yeast Co. Ltd., Tokyo, Japan; 359 kcal/100 g, 12.8% of the provided calories were derived from fat, 25.6% were from protein, and 61.6% were from carbohydrates) (n=15). These foods also contain adequate amounts of vitamins and minerals. At 23 wk of age, each group was further divided into OVX or sham-operated (sham) groups; i.e., the rats were divided into OVX-HFD (n=7), OVX-SC (n=7), sham-HFD (n=7), and sham-SC (n=8) groups. All surgical procedures were carried out under anesthesia with sodium pentobarbital. In the sham groups, the ovaries were just touched with forceps. Water containing ibuprofen (0.1 mg/ml) was provided during the three days after surgery to reduce the rats' postoperative pain. BW and food intake (FI) were weighed weekly throughout the experimental period (until 138 wk of age). Generally, two rats were housed in each cage, and they were differentiated by their ear hole markings. The food consumption of the rats in each cage was measured

once a week. When two rats were housed in one cage, the mean consumption value was used as the value for each rat. Housing the rats in pairs was considered to be appropriate for this study because it helped to avoid 1) the isolation stress that would have been induced by housing the rats individually and 2) discrepancies between calculated and real food consumption values due to group housing [Nilsson et al., 2001]. The health statuses of the rats were checked daily; i.e., we checked whether they were suffering from ill health or had died unexpectedly. The rats were euthanized via the intraperitoneal injection of sodium pentobarbital for humane reasons if their conditions worsened and it was considered that they were unlikely to survive for more than a week. Namely, when rats could hardly move and could not take any food and water by themselves, they were euthanized.

Statistical analysis

Statistical analyses were performed via mixed model ANOVA or two-way factorial ANOVA followed by the Tukey-Kramer post-hoc test or the Student's t test. All results are expressed as mean plus standard error of the mean (SEM) values. Cohen's d (small effect = 0.2, medium effect = 0.5, large effect = 0.8) and Eta squared ($\eta 2$) (small effect = 0.2, medium effect = 0.5, large effect = 0.8) are reported when analyses were undertaken by Student's t- test and ANOVA, respectively.

Results

The mean BW of the pups at 3 wk of age was 56.6 ± 0.27 g. As noted above, the rats were randomly divided into the HFD and SC groups. The mean BW of the HFD group was 56.7 ± 0.41 g, and that of the SC group was 56.5 ± 0.37 g. Mixed model ANOVA showed that the main effect of diet on BW was not significant during the period from 3 to 23 wk of age, whereas the interaction between diet and time had a significant effect on BW (diet: $F(1,27)=0.471$, $P=0.498$, $\eta 2=0.013$; time: $F(20,540)=990.1$, $P<0.01$, $\eta 2=10.15$; interaction: $F(20,540)=2.51$, $P<0.01$, $\eta 2=0.026$). It also demonstrated that the main effect of diet on the change in BW was not significant, whereas the interaction between diet and time had a significant effect on the BW change (mixed model ANOVA; diet: $F(1,27)=0.424$, $P=0.521$, $\eta 2=0.012$; time: $F(20,540)=993.4$, $P<0.01$, $\eta 2=10.8$; interaction: $F(20,540)=2.52$, $P<0.01$, $\eta 2=0.028$) (Figs. 1A and B). However, at 23 wk of age the BW and BW changes of the HFD group did not differ from those of the SC group (BW: Student's *t* test, P=0.13, Cohen's d=0.58; BW change: Student's *t* test, P=0.13,

Cohen's d=0.59) (Table 1). Mixed model ANOVA showed that the main effect of diet on FI was significant during the period from 3 to 23 wk of age, as was the effect of the interaction between diet and time on FI (diet: $F(1,27)=304.2$, $P<0.01$, $\eta 2=5.05$; time: $F(20,540)=64.4$, $P<0.01$, $\eta 2=1.35$; interaction: $F(20,540)=9.52$, $P<0.01$, $\eta 2=0.20$) (Fig. 2A). Cumulative FI during the period from 3 to 23 wk was significantly lower in the HFD group than in the SC group (Student's *t* test, $P<0.01$, Cohen's d=6.58) (Table 1). On the other hand, the main effect of diet on energy intake was not significant, whereas the interaction between diet and time had a significant effect on energy intake (diet: $F(1,27)=0.023$, $P=0.88$, $\eta 2=0.001$; time: $F(20,540)=59.1$, $P<0.01$, $\eta 2=1.33$; interaction: $F(20,540)=5.82$, $P<0.01$, $\eta 2=0.13$) (Fig. 2B). Cumulative energy intake during the period from 3 to 23 wk did not differ between the HFD and SC groups (Student's *t* test, $P=0.58$, Cohen's d=0.21) (Table 1).

Mixed model ANOVA showed that the main effect of diet/surgery on BW was significant during the period from 23 to 55 wk of age, as was the effect of the interaction between diet/surgery and time on BW (diet/surgery: $F(3,25)=16.9$, $P<0.01$, $\eta 2=0.71$; time: $F(32,800)=283.2$, $P<0.01$, $\eta 2=0.93$; interaction: $F(96,800)=11.0$, $P<0.01$, $\eta 2=0.15$). It also demonstrated that the main effect of diet/surgery on BW change was significant, as was the interaction between diet/surgery and time on BW change (diet/surgery: $F(3,25)=38.1$, $P<0.01$, $\eta 2=1.42$; time: $F(32,800)=25.5$, $P<0.01$, $\eta 2=2.79$; interaction: $F(96,800)=5.06$, $P<0.01$, $\eta 2=0.40$) (Figs. 3A and B). Surgery-induced BW reductions were observed in the OVX groups, but not the sham groups, at 24 wk. Although, as noted above, the rats' ovaries were touched with forceps during the surgery in the sham group, it is possible that the surgical stress experienced by the sham groups was weaker than that experienced by the OVX groups. Two-way factorial ANOVA detected main effects of surgery ($F(1,28)=23.6$, $P<0.01$, $\eta 2=13.7$) and diet ($F(1,28)=17.2$, $P<0.01$, $\eta 2=10.0$) on the total BW gain from 23 to 55 wk of age, but no significant interaction between these factors was observed, indicating that they acted independently (Table 2). The sham-HFD group displayed significantly greater BW changes than the sham-SC group (Table 2), whereas the BW changes seen in the OVX-HFD and OVX-SC groups did not differ. The OVX-SC group exhibited significantly greater BW changes than the sham-SC group. Similarly, the OVX-HFD group displayed significantly greater BW changes than the sham-HFD group. Mixed model ANOVA showed that the main effect of diet/surgery on FI was significant during the period from

23 to 55 wk of age, as was the effect of the interaction between diet/surgery and time on FI (diet/surgery: $F(3,25)=38.8$, $P<0.01$, $\eta 2=2.55$; time: $F(32,800)=25.5$, $P<0.01$, $\eta 2=0.45$; interaction: $F(96,800)=5.06$ $P<0.01$, $\eta 2=0.27$). It also demonstrated that the main effect of diet/surgery on energy intake was significant, as was the effect of the interaction between diet/surgery and time (diet/surgery: $F(3,25)=9.26$, $P<0.01$, $\eta 2=0.60$; time: $F(32,800)=26.9$, $P<0.01$, $\eta 2=0.49$; interaction: $F(96,800)=5.15$, $P<0.01$, $\eta 2=0.28$) (Figs. 4A and B). The cumulative energy intake of the sham-HFD and sham-SC groups did not differ during the period from 23 to 55 wk of age (Table 2). Similarly, the cumulative energy intake of the OVX-HFD and OVX-SC groups did not differ. The cumulative energy intake of the OVX-SC group was significantly greater than that of the sham-SC group, and the cumulative energy intake of the OVX-HFD group was significantly greater than that of the sham-HFD group. The feed efficiency of the sham-HFD group was significantly greater than that of the sham-SC group during the period from 23 to 55 wk of age (Table 2). On the other hand, feed efficiency did not differ between the OVX-HFD and OVX-SC groups. The feed efficiency of the OVX-SC group was significantly greater than that of the sham-SC group, and the feed efficiency of the OVX-HFD group was significantly greater than that of the sham-HFD group. As rats had started to die at 56 wk of age, BW and FI could not be compared among the groups after this age.

Kaplan-Meier analysis and the log-rank test showed that lifespan was affected by HFD consumption and/or OVX ($P=0.04$) (Fig. 5). Specifically, the lifespan of the OVX-SC group was significantly longer than that of the sham-SC group. There were no differences in lifespan among the OVX-SC, OVX-HFD, and sham-SC groups. Although the causes of death were not pathologically confirmed in this study, some rats developed breast or abdominal tumors during the observation period (5, 3, 1, and 2 in the sham-SC, sham-HFD, OVX-ND, and OVX-HFD groups, respectively).

Discussion

In this study, we have shown that long-term HFD consumption affected BW changes and feed efficiency in ovarian intact (sham) female rats, whereas it did not influence such changes in OVX rats. Sham rats that were fed a HFD displayed greater BW changes and higher feed efficiency than sham rats fed SC. On the other hand, OVX rats that were fed a HFD and OVX rats that were fed SC demonstrated similar BW changes and feed

efficiency. It has been reported that HFD consumption over a short period induced excessive BW gain and obesity in male rodents. For example, HFD consumption for 25 days resulted in greater BW gain and an increase in the amount of visceral fat in male rats [Xu et al., 2016]. Similarly, HFD consumption for around 10 weeks induced excessive BW gain in male mice [Yura et al., 2005]. As estrogen acts to prevent excessive BW gain and obesity by suppressing appetite and/or increasing energy metabolism in females [Hirschberg, 2012], it could be assumed that these activities of estrogen attenuate the effects of HFD consumption in younger sham rats. It can also be speculated that the reductions in ovarian function caused by aging cannot completely abrogate the effects of HFD consumption on BW and energy metabolism, resulting in greater BW changes in sham rats at around 50 weeks of age. Another possibility is that the different diets might have influenced ovarian function; reproductive longevity; and/or adrenal function, which is often altered by OVX. However, because we did not check the rats' estrous cycles, these hypotheses could not be confirmed in the present study. On the other hand, feed consumption was decreased by the consumption of the HFD in both the sham and OVX groups in the current study (data not shown). It has been reported that chronic HFD consumption increases the levels of leptin, an anorexigenic factor, and decreases the levels of ghrelin, an orexigenic factor. Therefore, these changes might reduce cumulative food consumption in HFD-supplied groups [Handjieva-Darlenska and Boyadjieva, 2009]. As the sham rats fed the HFD and the OVX rats fed SC exhibited similar BW changes and feed efficiency values, OVX might have similar effects on the regulation of BW and energy metabolism to HFD consumption. It appears that the two OVX groups exhibited similar increases in BW in the early days after surgery and then maintained similar shallow BW growth slopes for the rest of the study period. In contrast, the females fed the HFD demonstrated a steeper BW growth slope, and this was maintained throughout their lives, indicating that the mechanisms by which OVX affects BW and energy metabolism might differ from those of the HFD. As we only evaluated BW changes and FI, but did not measure other physiological or nutritional factors, we cannot provide any precise mechanisms by which HFD and OVX affect the regulation of BW and energy metabolism in the present study. Further experiments are needed to clarify these mechanisms.

It has been shown that HFD consumption exacerbates obesity and increases fat mass and adipocyte size in OVX female mice, indicating that HFD consumption and OVX

interact to worsen lipid metabolism and BW regulation [Ludgero-Correia et al., 2012]. Similarly, it has been reported that OVX shortens the lifespan of female mice, suggesting that ovarian hormones play pivotal roles in lifelong health status [Xu et al., 2016]. In addition, even senescent ovaries and germ cell-depleted ovaries have positive effects on health and longevity [Shoupe et al., 2007]. Thus, we speculate that long-term HFD consumption and OVX also interact to reduce longevity in females. Contrary to our expectations, OVX and HFD consumption had weak but favorable effects on longevity in female rats in the current study. We speculate that there are two possible explanations for the discrepancy between the results of the present and past studies. One possible reason is that the interactive effects of OVX and HFD consumption on BW and energy metabolism seen in female mice are not observed in female rats. As mentioned above, the BW changes and feed efficiency of the OVX rats fed the HFD did not differ from those of the OVX rats fed SC. Therefore, the combination of OVX and HFD consumption might not be enough to affect the longevity of female rats. Another possible reason is that the effects of gonadal hormones on longevity might differ between female rats and female mice. On the contrary, germline removal increases lifespan in many species, such as *C. elegans* and *D. melanogaster* [Hsin and Kenyon, 1999; Flatt et al., 2008]. Similarly, there is some evidence that the increases in longevity that result from chronic food restriction are secondary effects of the inhibition of the reproductive system/the secretion of gonadal steroids. As there is not enough energy for both reproductive and longevity processes, many species make trade-offs. In other words, females enjoy a high level of reproductive success at the expense of a long life. Reproductive processes, e.g., hormone production, gamete production, fetal development, and lactation, often preclude longevity processes, e.g., immune functions and tissue maintenance. In addition, gonadal steroid exposure increases the chances of many kinds of cancer. Thus, the OVX rats in the present study might have been able to expend greater amounts of energy on longevity and could also have been at reduced risk of cancer. Furthermore, it could also be speculated that estrogen or other sex hormones might have adverse, as well as favorable, effects on the health status of female rats, and these effects might be modulated by OVX. However, because the levels of estradiol and other sex hormones were not measured, these hypotheses could not be confirmed in the present study. Recently, it has been reported that the upregulation of the basal inflammatory states of metabolic organs (e.g., adipose tissue, the aorta, and the liver), which can be induced by reductions in estrogen-mediated anti-inflammatory

activity and a lack of gonadal control of energy metabolism in female mice [Benedusi et al., 2014]. Therefore, it is also possible that changes in immune status and energy metabolism might have contributed to the differences in longevity between the OVX and sham groups in this study. We could not confirm the reasons why OVX had different effects on longevity in mice and rats. Although, as noted above, reduced estrogen levels can lead to the upregulation of the basal inflammatory state, it has also been reported that a physiological level of estrogen stimulates inflammatory cytokine responses to immune stress in female rodents [Geary et al., 2004]. Therefore, we speculate that OVX might blunt stress responses in female rats, resulting in longer longevity compared with sham rats. Further studies, including evaluations of serum and tissue inflammatory cytokine levels, insulin sensitivity, and blood lipid profiles, are needed to clarify these issues.

The current study had some limitations. As the HFD used in the present study not only had a high fat content, but also had a high caloric content and a low carbohydrate content compared with the SC, it is possible that the effects of the HFD on BW and longevity were induced by its caloric/carbohydrate contents. In addition, when two rats were housed in one cage the mean consumption value was used as the value for each rat. Thus, the sample size for FI was half of that for BW, and so it is possible that the variance in FI was underestimated.

In summary, we have shown that HFD consumption and OVX cause similar increases in BW and feed efficiency in female rats. On the other hand, neither long-term HFD consumption nor OVX affected the longevity of female rats, indicating that the alterations in BW and energy metabolism induced by OVX and HFD consumption might not directly affect the lifespan of female rats.

Conflict of interests

All authors declare that there are no conflicts of interest.

References

Asdell, S.A., Doornenbal, H., Joshi, S.R., Sperling, G.A., 1967. The effects of sex steroid hormones upon longevity in rats. J. Reprod. Fertil. 14, 113-120.

Benedusi, V., Martini, E., Kallikourdis, M., Villa, A., Meda, C., Maggi, A., 2014. Ovariectomy shortens the life span of female mice. Oncotarget 6, 10801-10811.

Blaustein, J.D., Wade, G.N., 1976. Ovarian influences on the meal patterns of female rats.

Physiol. Behav. 17, 201-208.

Butera, P.C., 2010. Estradiol and the control of food intake. Physiol. Behav. 99, 175-180.

Della Torre, S., Benedusi, V., Fontana, R., Maggi, A., 2014. Energy metabolism and fertility-a balance preserved for female health. Nat. Rev. Endocrinol. 10, 13-23.

Flatt, T., Min, KJ, D'Alterio, C., Villa-Cuesta, E. Cumbers, J., Lehmann, R., Jones, D.L., Tatar, M., 2008. Drosophila germ-line modulation of insulin signaling and lifespan. Proc. Natl. Acad. Sci. USA. 105, 6368-6373.

Geary, N., Asarian, L., Sheahan, J., Langhans, W., 2004. Estradiol-mediated increases in the anorexia induced by intraperitoneal injection of bacterial lipopolysaccharide in female rats. Physiol. Behav. 82, 251-261.

Gilbert, E.L., Ryan, M.J., 2014. Impact of early life ovariectomy on blood pressure and body composition in a female mouse model of systemic lupus erythematosus. Am. J. Physiol. Regul. Integr. Comp. Physiol. 307, R990-997.

Handjieva-Darlenska, T., Boyadjieva, N., 2009. The effect of high-fat diet on plasma ghrelin and leptin levels in rats. J. Physiol. Biochem. 65, 157-164.

Hirschberg, A.L., 2012. Sex hormones, appetite and eating behavior in women. Maturitas 71, 248-256.

Hong, J., Stubbins, R.E., Smith, R.R., Harvey, A.E., Nunez, N.P., 2009. Differential susceptibility to obesity between male, female and ovariectomized female mice. Nutr. J. 8, 11.

Hsin, H., Kenyon, C., 1999. Signals from the reproductive system regulate the lifespan of C. elegans. Nature 399, 362-366.

Iwasa, T., Matsuzaki, T., Tungalagsuvd, A., Munkhzaya, M., Yiliyasi, M., Kato, T., Kuwahara, A., Irahara, M., 2016. Effects of chronic testosterone administration on body weight and food intake differ among pre-pubertal, gonadal-intact, and ovariectomized female rats. Behav. Brain. Res. 309, 35-43.

Kaaja, R.J., 2008. Metabolic syndrome and the menopause. Menopause. Int. 14, 21-25.

Ludgero-Correia, A. Jr, Aguila, M.B., Mandarim-de-Lacerda, C.A., Faria, T.S., 2012. Effects of high-fat diet on plasma lipids, adiposity, and inflammatory markers in ovariectomized C57BL/6 mice. Nutrition 28, 316-323.

Manson, J.E., Skerrett, P.J., Greeenland, P., Vanltallie, T.B., 2004. The escalating pandemics of obesity and sedentary lifestyle. A call to action for clinicians. Arc. Intern. Med. 164, 249-258.

Mokdad, A.H., Bowman, B.A., Ford, E.S., Vinicor, F., Marks, J.S., Koplan, J.P., 2001. The continuing epidemics of obesity and diabetes in the United States. J. Am. Med. Assoc. 286, 1195-1200.

Nilsson, C., Larsson, B.M., Jennische, E., Eriksson, E., Bjorntorp, P., York, D.A., Holmang, A., 2001. Maternal endotoxemia results in obesity and insulin resistance in adult male offspring. Endocrinology 142, 2622-2630.

Parker, W.H., Broder, M.S., Chang, E., Feskanich, D., Farquhar, C., Liu, Z., Shoupe, D., Berek, J.S., Hankinson, S., Manson, J.E., 2009. Ovarian conservation at the time of hysterectomy and long-term health outcomes in the nurses' health study. Obstet. Gynecol. 113, 1027-1037.

Seidlova-Wuttke, D., Nguyen, B.T., Wuttke, W., 2012. Long-term effects of ovariectomy on osteoporosis and obesity in estrogen-receptor-β-deleted mice. Comp. Med. 62, 8-13.

Shoupe, D., Parker, W.H., Broder, M.S., Liu, Z., Farquhar, C., Berec, J.S., 2007. Elective oophorectomy for benign gynecological disorders. Menopause 14, 580-585.

Stier, C.T. Jr, Chander, P.N., Rosenfeld, L., Powers, C.A., 2003. Estrogen promotes microvascular pathology in female stroke-prone spontaneously hypertensive rats. Am. J. Physiol. Endocrinol. Metab. 285, E232-239.

Xu, P., Dai, S., Wang, J., Zhang, J., Liu, J., Wang, F., Zhai, Y., 2016. Preventive obesity agent montmorillonite adsorbs dietary lipids and enhances lipid excretion from the digestive tract. Sci. Rep. 6, 19659.

You, T., Ryan, A.S., Nicklas, B.J., 2004. The metabolic syndrome in obese postmenopausal women: relationship to body composition, visceral fat, and inflammation. J. Clin. Endocrinol. Metab. 89, 5517-5522.

Yura, S., Itoh, H., Sagawa, N., Yamamoto, H., Masuzaki, H., Nakao, K., Kawamura, M., Takemura, M., Kakui, K., Ogawa, Y., Fujii, S., 2005. Role of premature leptin surge in obesity resulting from intrauterine undernutrition. Cell. Metab. 1, 371-378.

Figure legends

Fig. 1.

Body weight (BW) (A) and BW changes (B) seen in high-fat diet (HFD)- or standard chow (SC)-fed rats between 3 and 23 wk of age. BW changes are expressed as percentages of BW at 3 wk of age. Data are expressed as mean and SEM values.

Fig. 2.

Daily food (FI) (A) and energy intake (B) in high-fat diet (HFD)- or standard chow (SC)-fed rats between 3 and 23 wk of age. Data are expressed as mean and SEM values.

Fig. 3.

Body weight (BW) (A) and BW changes (B) seen in ovariectomized (OVX) or sham-operated rats fed a high-fat diet (HFD) or standard chow (SC) between 23 and 55 wk of age. BW changes are expressed as percentages of BW at 23 wk of age. Data are expressed as mean and SEM values.

Fig. 4.

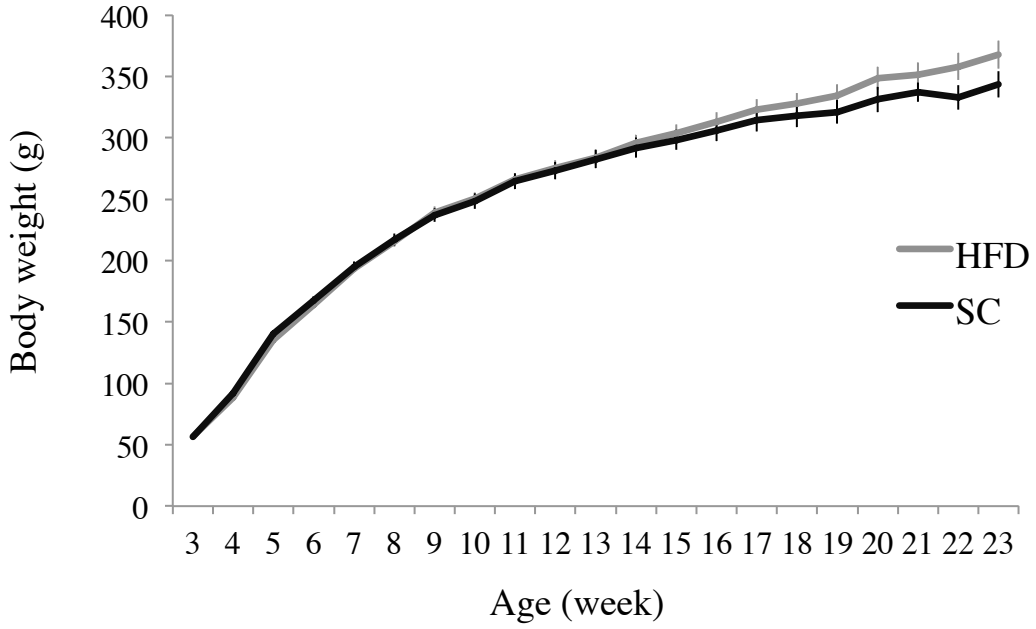
Daily food (FI) (A) and energy intake (B) of ovariectomized (OVX) or sham-operated rats fed a high-fat diet (HFD) or standard chow (SC) between 23 and 55 wk of age. Data are expressed as mean and SEM values.

Fig. 5.

Survival rates (%) of ovariectomized (OVX) and sham-operated rats fed a high-fat diet (HFD) or standard chow (SC) during the experimental period.

Fig. 1

A


Body weight (g)
400
350
300
250
200
150
100
50
0
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Age (week)
HFD
SC

B

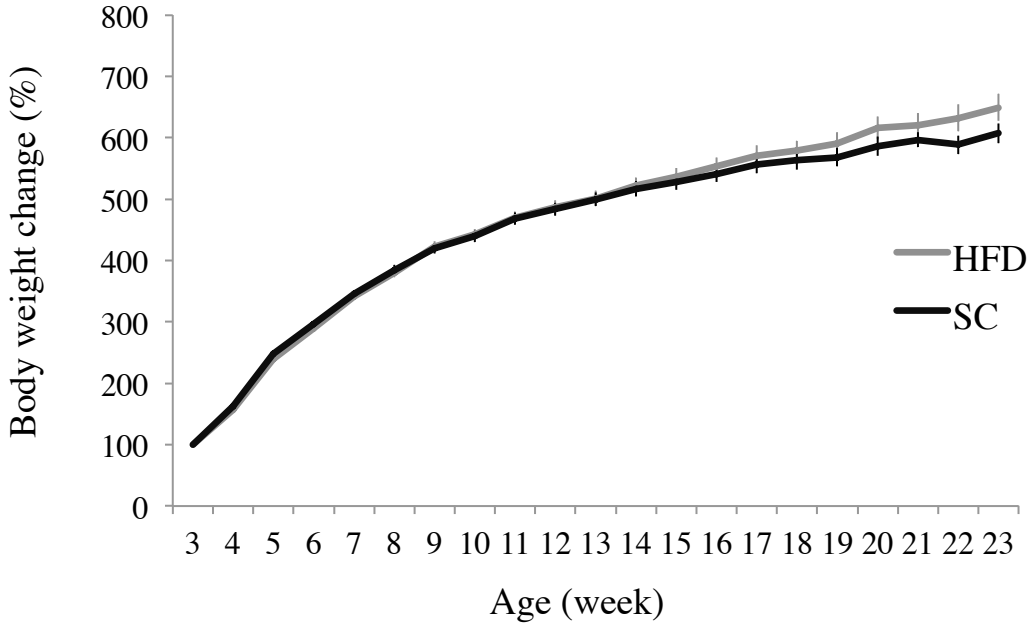
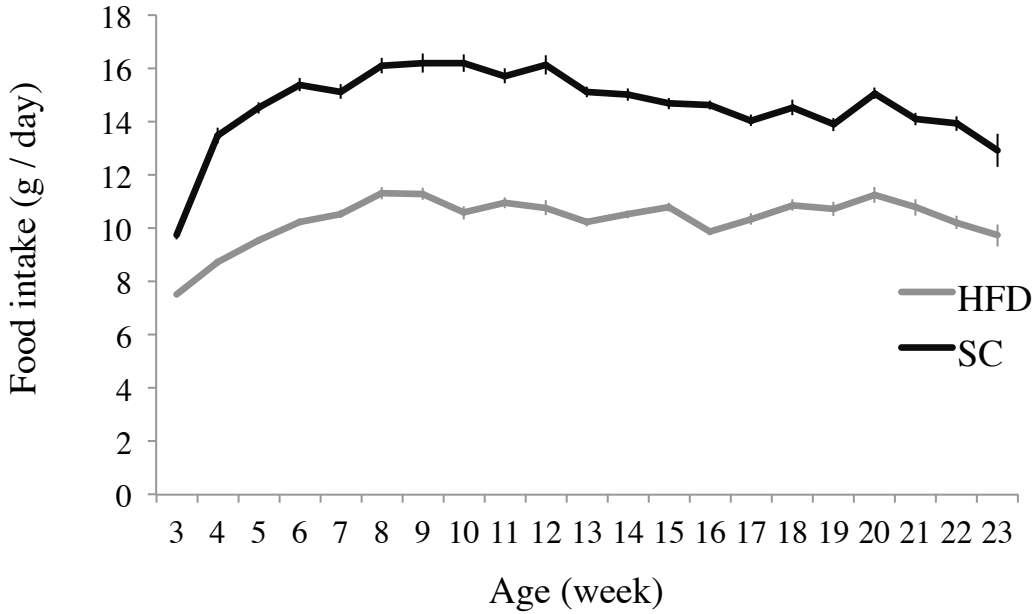

Body weight change (%)
800
700
600
500
400
300
200
100
0
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Age (week)
HFD
SC

Fig. 2

A


Food intake (g / day)
0
2
4
6
8
10
12
14
16
18
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Age (week)
HFD
SC

B

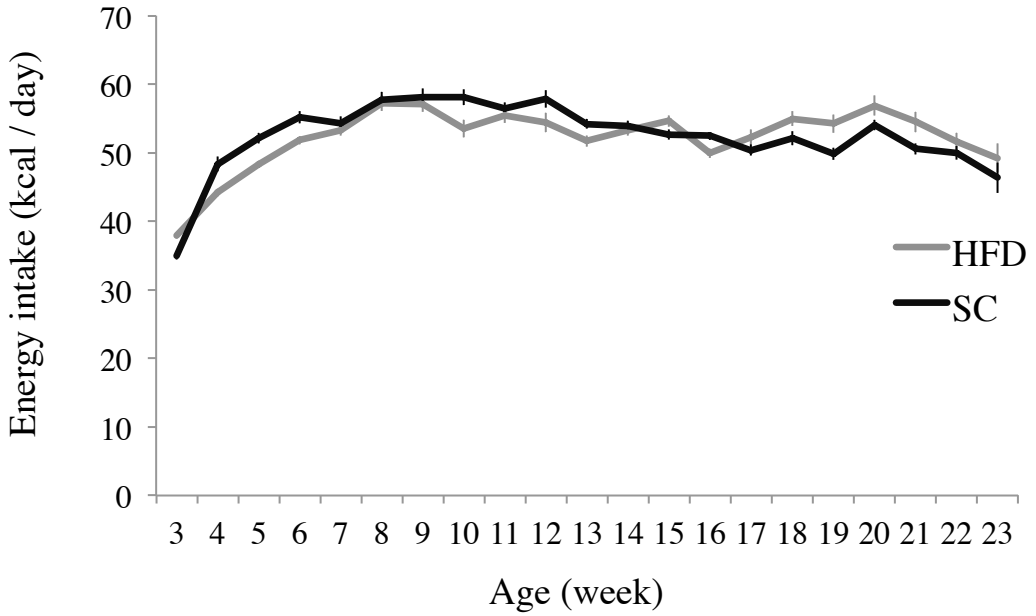
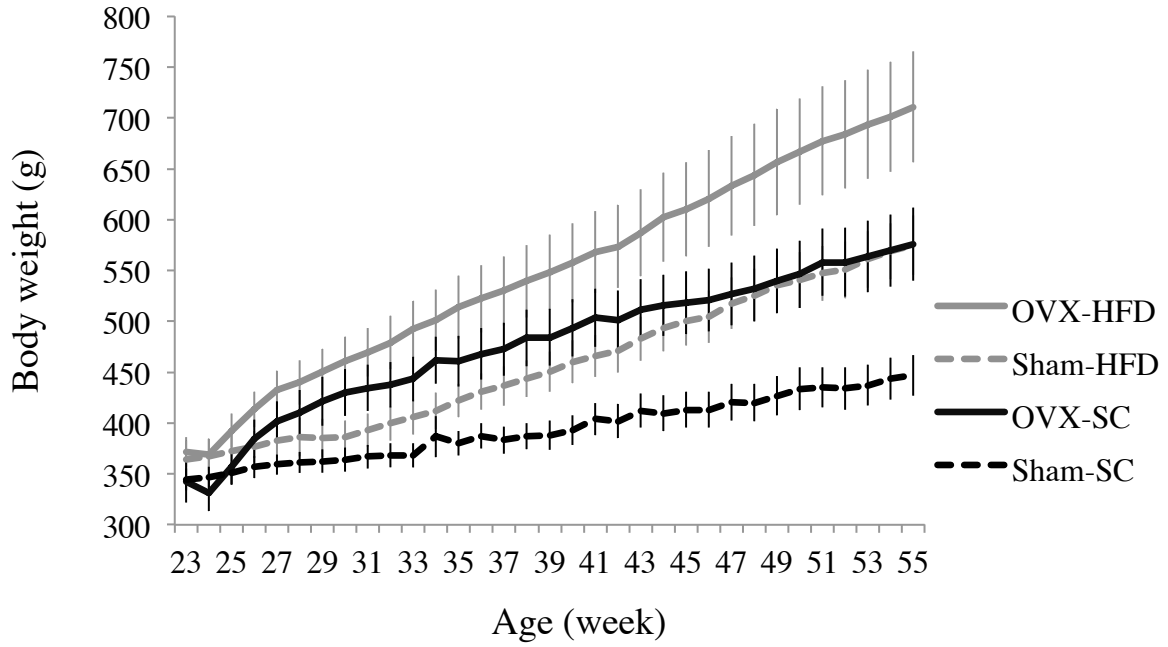

Energy intake (kcal / day)
0
10
20
30
40
50
60
70
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Age (week)
HFD
SC

Fig. 3

A


800
750
700
650
600
550
500
450
400
350
300
Body weight (g)
23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55
Age (week)
OVX-HFD
Sham-HFD
OVX-SC
Sham-SC

B

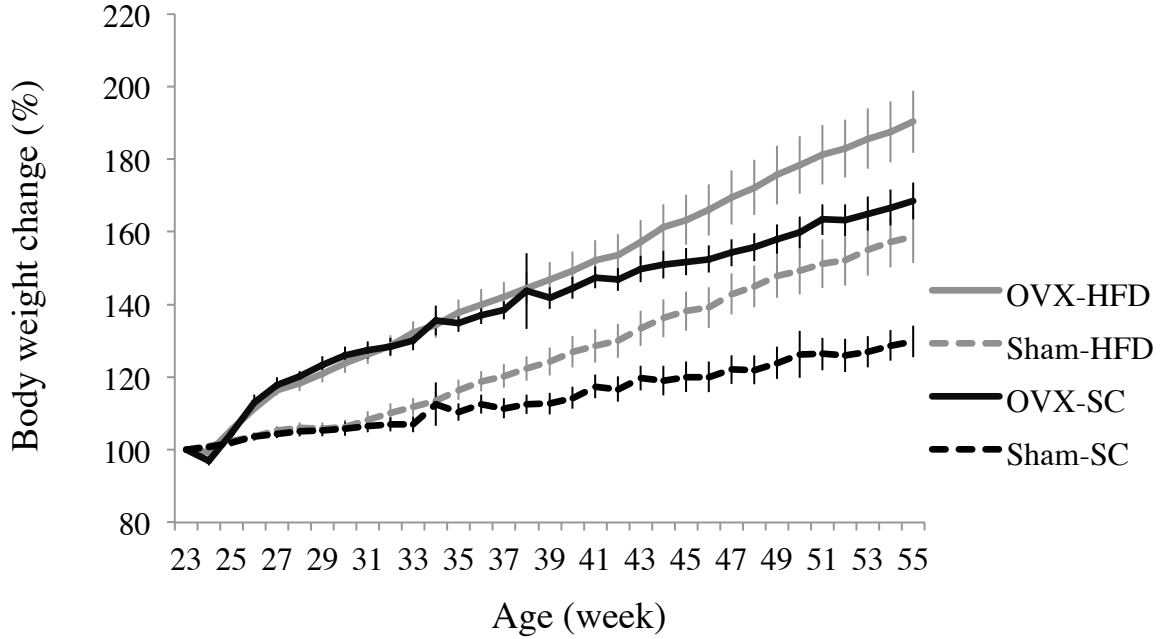

220
200
180
160
140
120
100
80
Body weight change (%)
23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55
Age (week)
OVX-HFD
Sham-HFD
OVX-SC
Sham-SC

Fig. 4

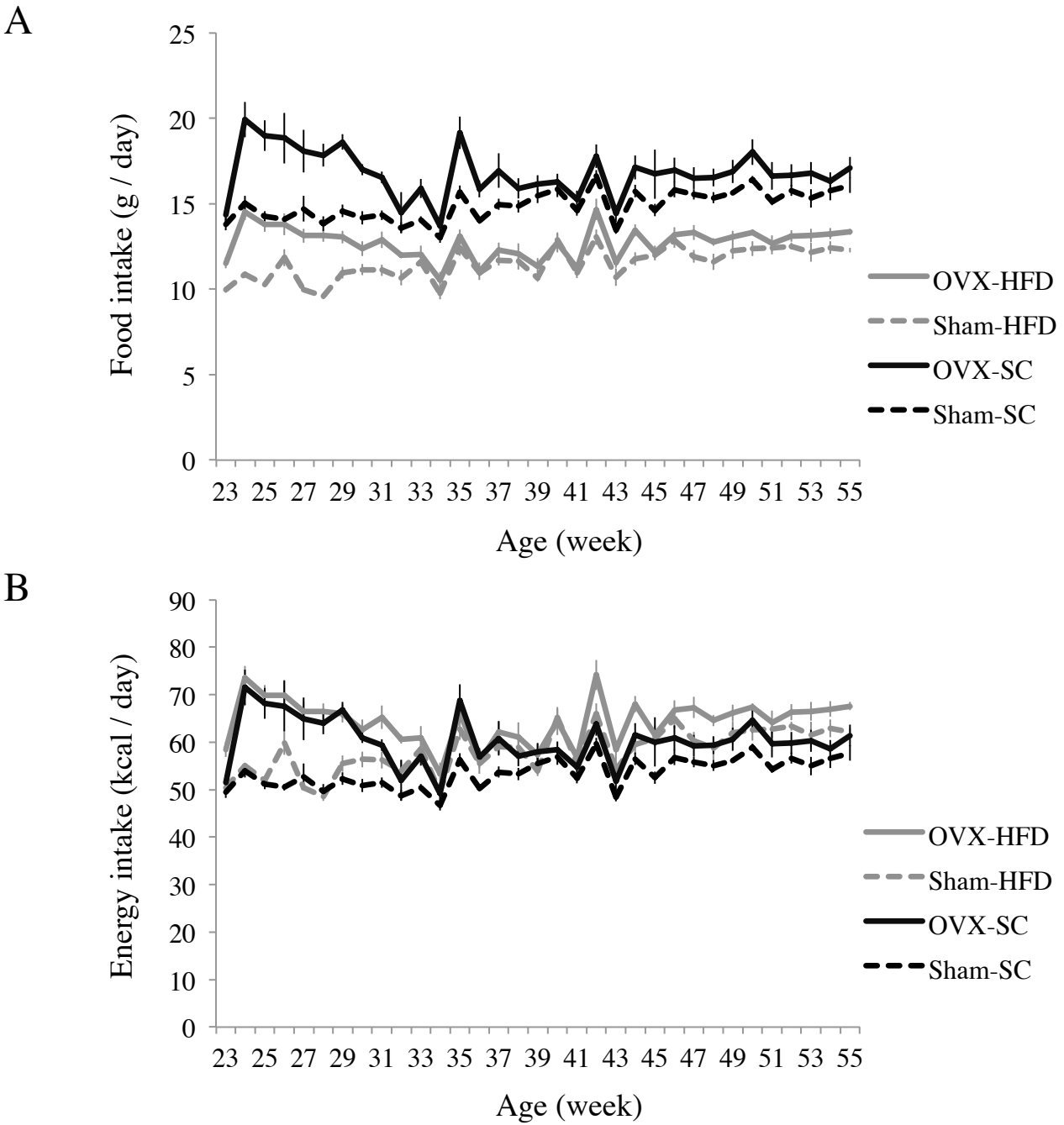

A
Food intake (g / day)
25
20
15
10
5
0
23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55
Age (week)
OVX-HFD
Sham-HFD
OVX-SC
Sham-SC
B
Energy intake (kcal / day)
90
80
70
60
50
40
30
20
10
0
23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55
Age (week)
OVX-HFD
Sham-HFD
OVX-SC
Sham-SC

Fig. 5

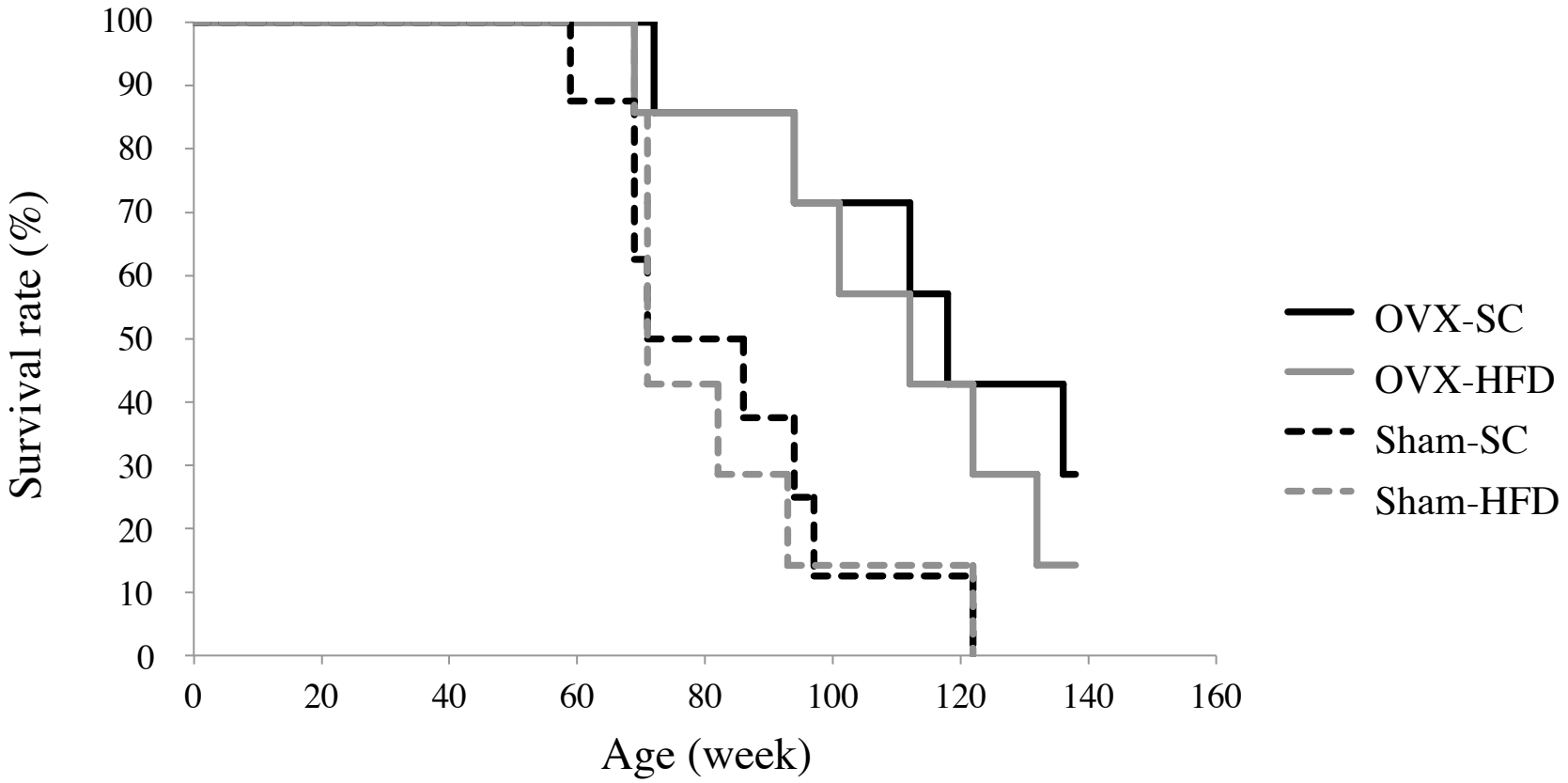

Survival rate (%)
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
140
160
Age (week)
OVX-SC
OVX-HFD
Sham-SC
Sham-HFD

Table 1 Biometric data of each group at 23 wk of age

	SC	HFD
Body weight (g)	343.4 ± 10.6	367.8 ± 11.4
Body weight change (%)	607.3 ± 16.0	649.5 ± 21.8
Cumulative food intake during 3–23 wk (g)	2144.7 ± 31.3	1505.8 ± 16.3**
Cumulative energy intake during 3–23 wk (kcal)	7699.4 ± 112.5	7619.6 ± 82.5
Feed efficacy	3.73 ± 0.14	4.10 ± 0.17

** $P < 0.01$ using student's *t* test. HFD; high-fat diet, SC; standard chow. Feed efficacy; bodyweight gain / energy intake. Data are expressed as mean and SEM.

Table 2 Biometric data of each group at 55wk of age

	Sham-SC	Sham-HFD	OVX-SC	OVX-HFD
Body weight (g)	446.9 ± 19.9	576.6 ± 29.1	575.9 ± 35.9	710.9 ± 54.8[a]
Body weight change (%)	129.8 ± 4.4	158.8 ± 7.4[a]	168.5 ± 5.1[a]	190.3 ± 8.5[a,b]
Total body weight gain (g)	102.4 ± 15.9	210.4 ± 22.3[a]	233.7 ± 20.4[a]	339.5 ± 43.0[a,b]
Cumulative food intake during 23–55 wk (g)	3445.2 ± 59.3	2655.2 ± 52.5[a]	3879.7 ± 141.0[a,b]	2937.4 ± 71.8[a,c]
Cumulative energy intake during 23–55 wk (kcal)	12368.3 ± 212.8	13435.3 ± 265.8	13928.0 ± 506.3[a]	14863.0 ± 363.4[a,b]
Feed efficacy during 23-55wk	0.81 ± 0.13	1.56 ± 0.15[a]	1.67 ± 0.10[a]	2.28 ± 0.26[a,b]

Where indicated, $P < 0.05$ using two-way ANOVA followed by Tukey-Kramer post-hoc test. [a]; compared with Sham-chow group, [b]; compared with Sham-HFD group, [c]; compared with OVX-chow group. HFD; high-fat diet, SC; standard chow, OVX; ovariectomized rats. Feed efficacy; 100 × bodyweight gain / energy intake. Data are expressed as mean and SEM.