Impact of early life ovariectomy on blood pressure and body composition in a female mouse model of systemic lupus erythematosus

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Running Title: Ovariectomy, Pressure, Body Composition, and SLE

Word Count: No. of Figures: 8

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Abstract

Due to the preponderance of women affected by the chronic autoimmune disease systemic lupus erythematosus (SLE), estrogen is thought to contribute to SLE disease progression. This is supported by evidence from experimental animal models of SLE showing that removal of estrogen in young female mice delays autoantibody production and renal injury and lengthens survival. Blood pressure and changes in body composition are important cardiovascular risk factors that can be regulated by estrogens. Because cardiovascular disease is the leading cause of death in patients with SLE, we used an established female mouse model of SLE (NZBWF1) to test whether early life removal of estrogen impacts the development of hypertension and changes in body composition commonly associated with SLE. Eight-week-old female SLE and control mice (NZW/LacJ) underwent either a sham or ovariectomy (OVX) operation. Body weight, body composition (fat mass, lean mass), and renal injury (albuminuria) were monitored until mice reached 34 weeks of age at which time mean arterial pressure was assessed in conscious animals by carotid catheter. Early life removal of the ovaries delayed the onset of autoantibody production and albuminuria while causing an increase in body weight and fat mass. Blood pressure in the adult was not altered by early life removal of the ovaries. These data suggest that estrogens may have a permissive role for the development of SLE while helping to maintain normal body weight and composition that would be associated with reduced cardiovascular risk.

Keywords: lupus, estrogen, hypertension, immune, inflammation
Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder that predominantly affects women of reproductive age (28, 45). It is characterized by a loss of immune tolerance leading to the production of autoantibodies that promote the formation of immune complexes resulting in tissue injury and inflammation. Although multiple organ systems can be affected in women with SLE, the leading cause of mortality is cardiovascular disease (2). This increased cardiovascular risk may be attributed, in part, to the prevalent hypertension (1, 13, 33, 38, 42) and changes in body composition (i.e. increased fat mass) (23, 24, 41) that are commonly associated with SLE in women. Because of the partiality of SLE towards women, estrogen is commonly implicated as an important contributor to the development of lupus disease. However, the impact of estrogens on blood pressure and body composition during SLE is not clearly understood.

Estrogens are known immunomodulators that can promote humoral (antibody mediated) immunity (21, 32), and therefore, contribute to SLE disease activity. In addition, the loss of estrogen or its receptors early in the life of experimental mouse models of SLE provide convincing evidence that estrogens have an important role in the development and progression of the disease (5, 35, 43). Surprisingly, the contribution of estrogens to SLE disease progression in humans remains unclear, and understanding their role in the cardiovascular risk is complicated by the large body of literature pointing to cardio protective actions of estrogens in women (22, 47), as well as their relatively safe use in women with SLE (3, 7, 8, 20, 26, 31, 40). Using an established experimental model of
SLE (female NZBWF1 mice), we recently reported that loss of estradiol in adulthood exacerbates the hypertension and renal injury (12) which, when considering that loss of estrogens early in life delays disease onset, suggests that there are distinct temporal effects of estrogens on SLE disease progression and its consequences. The major goal of the present study was to extend the findings of our recent work (12) by testing whether early life removal of estrogens in female NZBWF1 mice delays the onset of SLE and attenuates the hypertension in adulthood. Because loss of estrogens can have a profound impact on body composition, another cardiovascular risk factor, the second goal of the study was to assess the impact of early life ovariectomy on body weight and body composition.

Methods and Materials

Animals. Female NZBWF1 (SLE) and NZW/LacJ (control, Ctrl) (Jackson Laboratories, Bar Harbor, ME) mice were obtained at 3-5 weeks of age. Mice were maintained on a 12 hour light/dark cycle in temperature controlled rooms with access to chow and water ad libitum. All studies were performed with the approval of the University of Mississippi Medical Center Institutional Animal Care and Use Committee and in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Ovariectomy. When SLE and control mice were 8 weeks old, an ovariectomy (OVX) was performed through a dorsal midline incision as previously described (12). The OVX was performed at this age in order to mimic the early life removal of estrogens or its
actions previously described by others (5, 35, 43). In order to confirm the efficacy of OVX, the uterus was collected and weighed at the conclusion of the study.

**Blood Pressure Measurements in Adulthood.** At the conclusion of the study (34 weeks of age), mean arterial pressure (MAP in mmHg) was recorded via indwelling carotid artery catheters in conscious mice as previously described by our lab (37).

**Autoantibody Production.** Plasma anti-dsDNA antibodies, a hallmark of SLE disease, were measured by commercial ELISA (Alpha Diagnostic International, San Antonio, TX) as previously described (46).

**Albuminuria.** Urinary albumin was monitored weekly by dipstick analysis (Albustix, Tarrytown, NY) of overnight urine samples. Animals were considered to be positive for albuminuria at $\geq 100\text{mg/dL}$ as previously described (46). Urinary albumin was confirmed by ELISA (Alpha Diagnostic International, San Antonio, TX) in urine samples collected at the end of the study as previously published by our laboratory (46).

**Body weight and composition.** Changes in body composition of the mice were monitored by utilizing magnetic resonance imaging (EchoMRI-900TM, Echo Medical System, Houston, TX) (9). MRI measurements were performed in conscious mice placed in a thin-walled plastic cylinder with a cylindrical plastic insert added to limit movement of the mice. Mice were briefly submitted to a low-intensity electromagnetic field in order to measure total body fat mass, lean mass, free water, and total water.
Each mouse was weighed prior to placement in the Echo MRI. The Echo MRI analysis was run two times per mouse at each time point. Values were then averaged and normalized to the body weight of the mouse.

Insulin. Insulin was assessed by commercial ELISA (EMD Millipore, St. Charles, Missouri, Catalog # EZMRI-13K) in plasma samples collected from fasted mice (6 hour fast) as previously described (37).

Food Intake. Food intake was assessed by individually housing mice in shoebox cages and weighing the food each day for 8 days as previously described (37).

Protocol 1 (Impact of early life ovariectomy on SLE progression and blood pressure in the adult). SLE and Ctrl mice underwent either an OVX or sham operation at 8 weeks of age. Urine was collected every week and assessed for the presence of albumin as previously described (36). When mice reached 34 weeks of age, MAP was measured in conscious animals, and tissues were collected at the time of sacrifice.

Protocol 2 (Impact of early life on body weight and composition during SLE). The body weight of each mouse was measured weekly beginning at 8 weeks of age and continuing to the end of the study at 34 weeks of age. Body composition by EchoMRI was monitored every 3 weeks beginning at 9 weeks of age. When mice reached 20 weeks of age, body composition was analyzed every other week until the conclusion of the study at 34 weeks.
Statistical Analysis. Data are presented as mean±SEM. Statistical analyses were performed using Graph Pad Prism 6 software. A two-factor ANOVA was used to test for the main effects of group and treatment and their interaction among the uterine weight, mean arterial pressure, urine albumin excretion, food intake, and plasma insulin data. When ANOVA indicated significance, Tukey’s post hoc test was utilized. A two-factor ANOVA with repeated measures with factors treatment and time was utilized to test for statistically significant differences in the anti-dsDNA antibody data, followed by Tukey’s post hoc analysis. A two-factor ANOVA with repeated measures with factors group and time was utilized to test for statistically significant differences among the body weight, fat mass, and lean mass data, followed by Tukey’s post hoc analysis to determine individual differences. p<0.05 was considered statistically significant.

Results

Uterine Weight. In order to confirm the efficacy of the OVX operation, uterine weight was measured at the time of tissue collection. Uterine weight was significantly reduced in both control and SLE mice subjected to OVX in comparison to sham animals (Figure 1; Ctrl sham: 0.10±0.015 g, Ctrl OVX: 0.049±0.004 g, SLE sham: 0.10±0.011 g, SLE OVX: 0.049±0.009 g, p<0.05 sham vs. OVX).

Anti-dsDNA Antibodies. Circulating anti-dsDNA (IgG) autoantibodies were measured in plasma samples collected throughout the study. Figure 2 shows the increasing levels of autoantibodies over time in SLE sham and SLE OVX animals. The production of
autoantibodies is delayed in the OVX animals, significantly diverging at 28 weeks of age. When compared to sham control animals, the autoantibodies were significantly higher beginning at 24 weeks of age (Ctrl Sham: 24±4 kU/ml; SLE Sham: 206±46, p<0.05). Autoantibodies were similar between control and SLE mice at 8 weeks of age (in kU/ml, Ctrl Sham: 17±4; Ctrl OVX: 15±3; SLE Sham: 25±13; SLE OVX: 28±12) and remained low in the control sham group even at 34 weeks (61±12 kU/ml). OVX in control mice did not alter autoantibody levels in comparison to control shams.

Mean Arterial Pressure. We recently reported that OVX during adulthood (at 30 weeks of age) exacerbates the hypertension associated with SLE (12). In the present study we tested whether blood pressure in adulthood during SLE is impacted by OVX in young animals (8 weeks of age). Consistent with our previous results, MAP was significantly higher in SLE shams compared with control shams (Figure 3; Ctrl Sham: 119±4 mmHg, SLE Sham: 138±5 mmHg; p< 0.05). When OVX was performed in young control and SLE mice, MAP was not altered in the adult (Ctrl OVX:125±2 mmHg, SLE OVX: 138±3 mmHg).

Albuminuria. Over the course of the study, 42% of SLE Sham mice developed albuminuria (Figure 4A) whereas only 20% of the SLE OVX mice developed albuminuria. No control mice developed albuminuria. Urinary albumin, measured by ELISA in samples collected at 34 weeks of age, was significantly increased in SLE sham mice compared to controls (Figure 4B; 3.57±1.9 mg/day v. 0.03±0.006 mg/day Ctrl sham, p<0.05). OVX in young female SLE mice significantly reduced urinary
albumin (0.8±0.3 mg/day, p<0.05 vs. SLE sham). OVX in controls did not alter urinary albumin (0.06±0.05 mg/day).

**Body Weight.** At the beginning of the study, body weight was similar between groups (Figure 5A; Ctrl Sham: 23.41±0.36 g; Ctrl OVX: 24.54±0.65 g; SLE Sham: 25.18±0.50 g; SLE OVX: 28.72±1.1 g). At 9 weeks of age, body weight was significantly greater in OVX SLE mice compared to control shams and remained significantly elevated to the conclusion of the study (9 weeks: 32.46±0.83 g vs. 25.17±0.41 g control sham, p<0.05; 34 weeks: 46.10±1.6 g vs. 34.03±0.43 g control sham, p<0.05). Body weight was significantly elevated after OVX in SLE mice compared to SLE shams beginning at 14 weeks of age and continuing to the conclusion of the study (14 weeks: 38.24±1.4 g vs. 33.62±1.2 g SLE sham, p<0.05; 34 weeks: 46.10±1.6 g vs. 37.51±1.4 g SLE sham, p<0.05). OVX in control mice did not significantly alter body weight in comparison to control shams. % increase in body weight from baseline was also significantly greater in ovariectomized SLE mice compared to their intact counterparts (Figure 5B).

**Body Composition.** Fat mass as a percentage of body weight was similar between groups at 9 and 12 weeks (Figure 6A; 9 weeks: Ctrl Sham: 17.3±0.5 %; Ctrl OVX: 15.0±0.8 %; SLE Sham: 15.3±0.7 %; SLE OVX: 16.6±0.7 %). Removal of the ovaries accelerated the total body gain in fat mass in the mice with SLE but not in control animals. From 18 to 26 weeks, fat mass was significantly higher in OVX SLE mice when compared to all other groups. Fat mass in OVX SLE mice remained significantly increased compared to SLE shams to the conclusion of the study at 34 weeks (31.6±1.7
% vs. 20.4±2.2 % SLE sham, p<0.05). OVX did not alter fat mass in controls in comparison to control shams.

Lean mass as a percentage of body weight was similar between all groups at 9 and 12 weeks (Figure 6B; 9 weeks: Ctrl Sham: 75.0±0.5 %; Ctrl OVX: 78.2±0.8 %; SLE Sham: 77.0±0.7; SLE OVX: 76.0±1.0 %). Ovariectomy caused a reduction in lean mass in SLE mice beginning at 15 weeks of age that paralleled the increase in total fat mass. Lean mass was significantly reduced in OVX SLE mice compared to SLE shams (64.3±1.2 % vs. 69.8±1.5 % SLE sham, p<0.05) and remained so until the conclusion of the study at 34 weeks (60.6±1.6 % vs. 68.3±2.3 % SLE sham, p<0.05). Lean mass was significantly lower in OVX SLE mice compared to control shams from 18 weeks to 30 weeks. OVX in control mice did not alter lean mass in comparison to control shams.

Food Intake. OVX in young mice did not alter food intake at 29 and 30 weeks of age (Figure 7; Ctrl Sham: 3.2±0.2 g/day, n=4; Ctrl OVX: 3.4±0.1 g/day, n=3; SLE Sham: 3.1±0.3 g/day, n=9; SLE OVX: 3.2±0.1 g/day, n=5).

Plasma Insulin. Plasma insulin was significantly increased in female SLE mice compared with controls (Figure 8; 1.13±0.3 ng/ml, n=14 vs. 0.61±0.05 ng/ml, n=9; p<0.05). OVX in young female SLE mice did not alter plasma insulin in comparison to intact female SLE mice (1.38±0.3 ng/ml, n=7 vs. 1.13±0.3 ng/ml, n=14). Plasma insulin
was not different between ovariectomized controls and intact control mice (0.65±0.05
ng/ml, n=6 vs. 0.61±0.05 ng/ml, n=9).

Discussion

The role of estrogens in human SLE is surprisingly unclear. In experimental animal
models of SLE, evidence convincingly shows that the absence of estrogens, or its
receptors, early in life delays the onset and progression of SLE. However, recent data
from our laboratory show that loss of estrogens in adulthood does not affect antibody
production and exacerbates both the renal injury and hypertension associated with SLE.
Taken together, these data suggest that there may be distinct temporal roles for
estrogens during SLE. The major goal of the present study was to explore further this
temporal role by examining the long term impact of estrogens on blood pressure and
renal injury during SLE. In addition, we sought to determine whether loss of estrogens
contributes to body composition changes that would be consistent with increasing
cardiovascular risk. The major new findings of this study are as follows: (1) Blood
pressure was not different between intact and ovariectomized SLE mice during
adulthood after early life ovariectomy. (2) SLE mice experienced a greater weight gain
than control mice after early life ovariectomy. (3) The increased body weight caused by
early life ovariectomy was associated with an increase in total body fat mass with a
corresponding decrease in lean mass.

Impact of early life ovariectomy on SLE. Because of the preponderance of females
affected by SLE, estrogen is commonly perceived as a contributor to disease
Some of the strongest evidence to support this comes from studies using experimental animal models of SLE, including female NZBWF1 mice. For example, estrogen receptor alpha knockout NZBWF1 mice (ER-α KO) (5) not only survived longer but also had reduced levels of pathogenic inflammatory anti-dsDNA autoantibodies, a hallmark of SLE disease, and reduced renal injury. This important work complemented an early study in which female NZBWF1 mice ovariectomized at 2 weeks of age were given supplemental estradiol and experienced a worsened disease course with increased renal injury and mortality (35). Consistent with these previous studies, we report here that early life ovariectomy delays both the development of autoantibodies and the onset of albuminuria, thus providing verification of the role estrogen that plays in early life as a contributor to SLE disease development.

In humans, SLE typically impacts women beginning in the third or fourth decade of life, and the role of estrogens in human SLE is not as clear. For example, oral contraceptive use, ovulation induction therapy, and hormone therapy treatment traditionally invoke fear of lupus flare. However, there is growing evidence that oral contraceptive use and hormone therapy are safe in women with SLE and do not significantly exacerbate disease activity (3, 8, 20, 40) suggesting a more complex role for estrogens than perhaps previously assumed. Moreover, multiple studies report no additional risk of SLE flare and support the utility of hormone therapy in SLE (4, 7, 26, 31, 39), especially to alleviate menopausal vasomotor symptoms (7). Given the uncertainty surrounding the role of estrogen in human SLE, the published work on the protective effect of early life removal of estrogen during SLE in mice (supported by our current manuscript), and
our recent study showing that removal of estrogens in adult mice does not attenuate
SLE disease, our work supports the concept that there distinct temporal actions of
estrogen during SLE. Importantly, female NZBWF1 mice represent a widely utilized and
established experimental model with which to carefully examine these actions.

**Impact of early life ovariectomy on blood pressure in adulthood during SLE.** In contrast
to the increased blood pressure that results from removing estrogens in the adult SLE
mouse (12), blood pressure was not different between adult SLE OVX and SLE sham
mice when the procedure was performed early in life. One interpretation of this data
could be that early life exposure to estrogens does not have an important physiological
role in the pathogenesis of hypertension, especially given that blood pressure is
significantly elevated over control animals. A second interpretation is that the removal
of estrogens by ovariectomy early in life was protective against the development of
hypertension much in the same way that early life removal of estrogens delays the
onset of autoantibodies and albuminuria. This could be reasoned from data in rodents
showing that long term removal of the ovaries is expected to exacerbate hypertension
(15, 16, 18), whereas there is no such effect in the mice with SLE. In addition, it is
important to note that the hypertension in the adult was not increased even in the face
of the increased body weight and increased fat mass. While we favor the latter
interpretation, confirmation will require future experiments to assess blood pressure
longitudinally from the time of the ovariectomy until adulthood. Without longitudinal
assessment of blood pressure, the question of whether the increase in blood pressure
precedes the development of albuminuria arises. However, based on our previously
published work showing that blood pressure and albuminuria are not necessarily associated (12, 29) we are confident this is not the case.

Studies assessing the effect of hormone therapy on cardiovascular disease and risk factors such as hypertension in women with SLE are limited. Hochman et al. examined the effects of hormone therapy and risk of cardiovascular disease in postmenopausal women with SLE (17). This study suggested that hormone therapy does not predispose to coronary artery disease in postmenopausal women with SLE. Although the prevalence of hypertension among women with lupus was high in this study, the effect of hormone therapy on blood pressure was not reported. In the Lupus in MInorities NAture versus nurture (LUMINA) study in a multiethnic US cohort showed that use of hormone therapy by postmenopausal women with SLE was not associated with vascular arterial events (11). Therefore, while experiments have not been designed to test directly the impact of estrogen on blood pressure in SLE these studies suggest that women with SLE were able to utilize hormone therapy without enhanced risk of adverse cardiovascular events.

Estrogens and body composition during SLE. Obesity and increased fat mass are known cardiovascular risk factors and are also associated with increased blood pressure. Body composition changes are typical of women with SLE, and SLE disease itself has independent effects on body composition (24). A comparative study of women with SLE, women with rheumatoid arthritis (RA), and female controls found that abnormal body composition was more prevalent in the inflammatory autoimmune
conditions of SLE and RA (41). In a longitudinal study, 28 premenopausal women with SLE were tracked for three years to assess alterations in body composition (23). Both body mass index (BMI) and fat mass increased over the three year follow-up period (23). A study of childhood-onset SLE reported that SLE disease was associated with increased fat mass and decreased lean mass compared to age-matched healthy controls (27). Similarly, females with juvenile onset SLE had overall decreased lean mass and a higher percentage of fat than age and weight-matched controls (34).

We previously demonstrated that the female NZBWF1 mouse model mirrors the body composition changes observed in human SLE with increased adipose depots and central adiposity (37). In the present study, removal of estrogen significantly increased body weight with ovariectomized SLE mice gaining more weight both in terms of absolute number of grams and percent increase in body weight. In addition, fat mass increased in ovariectomized female SLE mice, and lean mass decreased over the course of the study, a result that is consistent with the effect of ovariectomy in young C57BL6/J mice (44). While the ovariectomized SLE mice exhibited significant changes in body weight and fat mass, the weight gain in ovariectomized control mice was not as pronounced. The reason for this moderate weight gain relative to the study in C57BL6/J mice (44) is not clear; however, it may relate to the genetic background of the mice or differences in the diets utilized.

The increased body weight and fat mass, observed in the ovariectomized SLE mice, most likely does not result from hyperphagia as food intake was similar between all
groups. Importantly, this finding is consistent with evidence from estrogen receptor-α and aromatase deficient mice in which no hyperphagia was observed (14, 19). Therefore, the mechanisms involved in the increased body weight and fat mass in the SLE OVX may be due to reductions in spontaneous activity, or from reductions in metabolism following estrogen deficiency. In women under the age of 40, similar alterations have been reported after bilateral oophorectomy including higher mean percent body fat and 30I (44). Another small study of young women ages 18 to 30 demonstrated that young women with primary amenorrhea had large amounts of fat tissue in a predominantly android pattern (25). Hormonal status affects body composition in women with SLE as well. For example, Kipen et al. demonstrated that increases in fat mass were significantly associated with loss of ovarian hormones (24). Although it was not examined in women with SLE, the effect of hormone therapy on body composition was investigated in the estrogen plus progestin (E+P) randomized controlled clinical trial of the Women’s Health Initiative (WHI) (6). Women who received E+P treatment for three years lost less lean soft tissue mass and had reduced redistribution of fat to a central location. Clearly estrogen affects body composition and may reduce fat tissue mass while preserving the lean tissue mass. Body composition changes may be one component that contributes to the overall cardiovascular disease risk in SLE beyond classic risk factors which alone do not completely account for the higher cardiovascular disease risk in SLE (10).

Conclusions and Perspectives. Much of the work conducted using experimental animal models of SLE, including the present study, shows that early life removal of estrogens,
or the absence of estrogen receptors, delays the onset of SLE. These studies clearly demonstrate the impact that estrogens have on the development of SLE. However, the onset of SLE in humans typically occurs in adulthood reaching a peak between ages 29-36 (28) and the role of estrogens in human SLE is far less certain. Interestingly, removal of estrogens in adult mice with SLE does not afford any protection and actually exacerbates the hypertension and renal injury associated with SLE (12). Therefore, early in life estrogens may have an important permissive role for the development of SLE, while their role in adulthood remains unclear and may even be cardioprotective.

The purpose of this study was to build upon our recent work and advance our understanding of the different temporal effects of estrogens on cardiovascular risk factors. In light of the fact that cardiovascular disease is the leading cause of death in patients with SLE, it may be prudent to carefully consider the time course of estrogen removal in the design of future studies in experimental animal models of lupus.

Sources of Funding

E.L. Gilbert is the recipient of an American Heart Association Greater Southeast Affiliate Predoctoral Fellowship (12PRE12050150). This work was supported by HL085907, AHA12GRNT12060203, and a UMMC Intramural Research Support grant to M.J. Ryan, and HL051971 to UMMC Physiology.

Conflict of Interest/Disclosures

None.
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Figure Legends

Figure 1. Uterine weight in control and SLE mice subjected to either an ovariectomy (OVX) or sham procedure. A two-factor ANOVA was used to test for the main effects of group and treatment and their interaction. Tukey’s post hoc analysis was utilized when ANOVA indicated significance. * p<0.05 Sham vs. OVX

Figure 2. Plasma anti-dsDNA antibodies in SLE mice subjected to either an ovariectomy (OVX) or sham procedure. A two way ANOVA with repeated measure was utilized to test for treatment or time interactions. Tukey’s post hoc test was utilized when ANOVA indicated significance. The * denotes the results of Tukey’s post hoc analysis comparing between the effect of treatment at the specific time point indicated. * p<0.05 SLE Sham vs. SLE OVX at the corresponding week.

Figure 3. Mean arterial pressure (MAP) in control and SLE mice subjected to either an ovariectomy (OVX) or sham procedure. A two-factor ANOVA was used to test for the main effects of group and treatment and their interaction. Tukey’s post hoc analysis was utilized to assess individual differences. * p_{group}<0.05 Ctrl vs. SLE

Figure 4. A, Weekly percentage of SLE mice with positive urinary albumin as measured by dipstick assay. Control mice did not develop albuminuria and therefore are not included in the graph. B, Urine albumin excretion in control and SLE mice at 34 weeks of age as measured by ELISA in mice subjected to either an ovariectomy (OVX) or sham procedure (n≥7). A two-factor ANOVA was used to test for the main effects of
group and treatment and their interaction. Tukey’s post hoc analysis was utilized to assess individual differences. * p<0.05 Ctrl vs. SLE  # p<0.05 SLE OVX vs. SLE Sham

**Figure 5.** A. Body weight (BW) in control and SLE mice subjected to either an ovariectomy (OVX) or sham procedure. A two way ANOVA with repeated measure was utilized with factors group and time included. Tukey’s post hoc test was utilized when ANOVA indicated significance. * p<0.05 SLE OVX vs. Ctrl across the weeks denoted by the bar; # p<0.05 SLE OVX vs. SLE Sham across the weeks denoted by the bar. B. Body weight data expressed as % increase. A two way ANOVA with repeated measure was utilized with factors group and time included. Tukey’s post hoc test was utilized when ANOVA indicated significance. * p<0.05 SLE OVX vs. Ctrl across the weeks denoted by the bar; # p<0.05 SLE OVX vs. SLE Sham across the weeks denoted by the bar.

**Figure 6.** Body composition in control and SLE mice subjected to either an ovariectomy (OVX) or sham procedure. A, Fat mass as a percentage of body weight (BW). A two way ANOVA with repeated measure was utilized with factors group and time included. Tukey’s post hoc test was utilized when ANOVA indicated significance. * p<0.05 SLE OVX vs. Ctrl Sham; # p<0.05 SLE OVX vs. SLE Sham. B, Lean mass as a percentage of BW. A two way ANOVA with repeated measure was utilized with factors group and time included. Tukey’s post hoc test was utilized when ANOVA indicated significance. * p<0.05 SLE OVX vs. Ctrl Sham; # p<0.05 SLE OVX vs. SLE Sham.
**Figure 7.** Food Intake in control (Ctrl) and SLE mice subjected to either an ovariectomy (OVX) or sham procedure. A two-factor ANOVA was used to test for the main effects of group and treatment and their interaction.

**Figure 8.** Plasma Insulin in control (Ctrl) and SLE mice subjected to either an ovariectomy (OVX) or sham procedure. A two-factor ANOVA was used to test for the main effects of group and treatment and their interaction. with the p value corresponding to the factor group. *p_{\text{group}} < 0.05 \text{ Ctrl vs. SLE}
Figure 1

![Bar chart showing uterine weight (g) for various groups.](chart)

- Ctrl Sham: (n=7)
- Ctrl OVX: (n=6) with * indicating significance
- SLE Sham: (n=13) with * indicating significance
- SLE OVX: (n=8)
Figure 3

![Bar chart showing MAP (mmHg) for different groups.](image)
Figure 4b

Bar graph showing the albumin excretion rate (mg/day) for different groups:

- Ctrl Sham
- Ctrl OVX (n=7)
- SLE Sham (n=9)
- SLE OVX (n=10)

Key:
- * indicates a significant difference
- # indicates another significant difference

The graph compares the albumin excretion rates across these groups, with the y-axis ranging from 0.0 to 6.0 mg/day.
Figure 5b

The graph depicts the body weight (% increase) over age (week) for different groups: SLE Sham, SLE OVX, Ctrl Sham, and Ctrl OVX. The x-axis represents age in weeks, ranging from 8 to 34. The y-axis represents body weight (% increase), ranging from 0 to 100. Significant differences are indicated by asterisks (* and #).
Figure 7

Food Intake (g/24 hrs)

- Ctrl Sham: (n=4)
- Ctrl OVX: (n=3)
- SLE Sham: (n=9)
- SLE OVX: (n=5)
Figure 8

![Graph showing plasma insulin levels for different groups.](image)

- **Ctrl Sham**: (n=9)
- **Ctrl OVX**: (n=6)
- **SLE Sham**: (n=14)
- **SLE OVX**: (n=7)

The data shows a significant difference between the SLE Sham and SLE OVX groups, indicated by an asterisk (*).