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Insulin Resistance and Obesity in a Mouse Model of Systemic Lupus Erythematosus

Michael J. Ryan, Gerald R. McLemore Jr, Steven T. Hendrix

Abstract**—**Accumulating data indicate that metabolic syndrome is an inflammatory condition. Systemic lupus erythematosus (SLE) is an autoimmune disorder associated with nephritis and cardiovascular disease. Evidence suggests that individuals with SLE are at risk for developing insulin resistance; however, this has not been directly examined. Using an established mouse strain with SLE (NZBWF1), we examined whether SLE is associated with increased body weight and fat deposition. Mean arterial pressure was significantly increased (140±4 versus 114±2 mm Hg; n≥5) in SLE mice by 36 weeks of age compared with control mice (NZW/LacJ). Body weight in SLE mice was higher at each age compared with controls by 12%, 22%, and 34% (n>30). Visceral adipose tissue weight was increased in SLE by 44%, 74%, and 117% at 8, 20, and 36 weeks, respectively (n≥12). Plasma leptin was increased in SLE mice (8.6±1.0 versus 24.7±2.2 ng/mL; n=5), and renal and adipose tissue exhibited macrophage infiltration. Fasted insulin was higher in SLE mice (0.6±0.1 versus 1.4±0.3 ng/mL; n≥10), but fasted glucose was not different (94±5 versus 80±9; n≥9). A glucose tolerance test caused a significantly greater and longer increase in blood glucose from mice with SLE compared with control mice. Food intake was not different between control and SLE mice. However, mice with SLE demonstrated lower levels of nighttime activity than controls. These data show that the NZBWF1 strain may be an important model to study the effects of obesity and insulin resistance on SLE-associated hypertension. **(*Hypertension*. 2006;48:988-993.)**

Key Words: SLE ■ autoimmune ■ inflammation ■ insulin ■ adipose ■ leptin ■ glucose

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Metabolic syndrome can be characterized by a group of metabolic risk factors that includes central obesity, insulin resistance, dyslipidemia, increased blood pressure, and endothelial dysfunction.[1] Individuals with metabolic syndrome are at a markedly increased risk for developing hypertension and renal disease. Recent evidence indicates that inflammatory cytokines are elevated in patients with metabolic syndrome,[2–4] thus suggesting a role for chronic inflammation as an underlying mechanism for progression of this disease.

Systemic lupus erythematosus (SLE) is a chronic inflammatory disorder that predominantly affects women during their reproductive years. The presence of autoantibodies (typically antinuclear antibodies) is used diagnostically, and although SLE can influence many organ systems, the skin, joints, and kidneys are typically affected. Like metabolic syndrome, a large percentage of individuals with SLE are hypertensive. Evidence suggests that individuals with SLE are also at increased risk for developing insulin resistance[5] and changes in body mass composition[6]; however, this has not been adequately examined.

The purpose of the present study was to test whether the progression of SLE is associated with changes in body composition, insulin sensitivity, and other characteristics of the metabolic syndrome. To examine this, we used a genetic mouse model of SLE (NZBWF1) that exhibits many features of human SLE, including a complex genetic origin, a bias for the female sex, immune complex glomerulonephritis, and the presence of antinuclear antibodies. We reported recently that these mice have hypertension and impaired endothelial-dependent relaxation.[7] The results of the present study show that NZBWF1 mice also have several other characteristics of the metabolic syndrome that may contribute to the hypertension, including central obesity, insulin resistance, and hyperleptinemia. These data show that the NZBWF1 strain may be an important model to study the effects of obesity and insulin resistance on SLE-associated hypertension.

Methods

Animals

Female NZBWF1 (SLE) and NZW/LacJ (control) obtained from Jackson Laboratories (Bar Harbor, ME) were maintained on a 12-hour light/dark cycle, normal chow, and water ad libitum. Mice were studied at 8 (7.8±0.7 weeks), 20 (21.2±0.3 weeks), and 36 (36.4±0.1 week) weeks of age. These ages correspond with the onset of sexual maturity, pre-SLE, and full SLE, respectively. At 36 weeks of age, female mice are fully capable of reproduction, and a decline in ovarian hormones would be expected to improve or delay the onset of SLE in NZBWF1 females. All of the studies were performed

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with the approval of the University of Mississippi Medical Center Institutional Animal Care and Use Committee and in accordance with National Institutes of Health guidelines.

Physical Characteristics

Body weight (in grams) was measured for all of the mice. A subgroup was euthanized and dissected for measurement of total visceral adipose (omental+ovarian/uterine+retroperitoneal) and to weigh individual adipose depots (ovarian/uterine, retroperitoneal, and thermogenic). Mice were acclimated for ≥5 days to being individually housed before daily food intake was measured (in grams) and normalized to grams per 24 hours. The effect of leptin on food intake was also measured in 36-week-old mice. Mouse leptin (R&D Systems) was administered via intraperitoneal injection (30 μg, twice daily) for 3 days. Intraperitoneal injection of vehicle (saline) was used as a control.

Blood Glucose, Insulin, and Leptin

Thirty-six-week-old NZBWF1 and NZW/LacJ mice were fasted for 6 hours before collecting blood samples with EDTA through retro-orbital punctures in lightly anesthetized animals (isoflurane). Fifteen microliters were used with the Accuchek Advantage glucometer (Roche) to measure blood glucose in milligrams per deciliter. Remaining blood samples were centrifuged, and plasma samples were stored at −80°C.

Fasted insulin (nanograms per milliliter) was measured using a Rat/Mouse Insulin ELISA kit (Linco Research). Plasma leptin levels (nanograms per milliliter) were measured using the Quantikine Mouse Leptin Immunoassay (R&D Systems).

Glucose tolerance was tested in 36-week-old NZBWF1 and NZW/LacJ mice. Carotid artery catheters were implanted as described previously.[8–10] The next day mice were fasted for 6 hours at which time a 15-μL sample of blood was analyzed for basal blood glucose levels. Each mouse was administered an intraperitoneal injection of isosmotic glucose (317 mmol/L). Fifteen-microliter blood samples were taken through the carotid catheter, and glucose was measured at 20, 40, 60, 120, and 180 minutes postinjection. This method has been reported previously.[11]

Mean Arterial Pressure

Mean arterial pressure (MAP) was measured with indwelling carotid artery catheters attached to an external force displacement transducer as described previously.[9,12] Mice were allowed 48 hours to recover before measurements were made. Surgeries were performed under isoflurane anesthesia, and bupivacaine (subcutaneous) was used postoperatively as an analgesic.

Animal Activity

Radiotelemeters (PAC10 or PAC20, DSI) were implanted in NZBWF1 and NZW/LacJ mice at 8, 20, and 36 weeks of age as described previously.[7] Mice recovered for ≥1 week before measuring activity continuously (24 hours per day) for 4 consecutive days. Measurements are a relative measure of locomotor activity (high counts means the animal was active) and cannot be used to calculate distance or type of activity.

Histology

Ovarian/uterine adipose or kidneys were fixed in 10% neutral buffered formalin and paraffin embedded for histological analysis. Sections were incubated with F4/80 rat anti-mouse antigen (1:10 in normal rabbit serum overnight, Serotec), biotinylated with rabbit anti-rat IgG, incubated with avidin-biotin complex (mouse avidin–biotin Vectastain, Vector), and visualized with diaminobenzidine substrate (Vector) to detect the presence of monocytes/macrophage. Slides were counterstained with Harris hematoxylin. At least 10 randomly selected fields were analyzed per slide from each animal, and data are presented as a percentage (total number of macrophage/total number of adipose cells counted). Frozen liver sections from 6 control and 6 SLE mice were incubated with Oil-Red-O to test for the presence of hepatic liver accumulation. Sections were counterstained with hematoxylin/eosin.

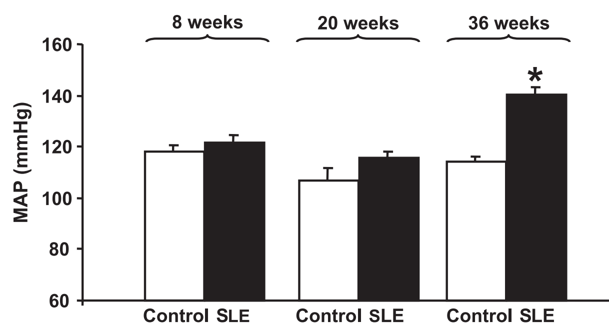

Figure 1. MAP is increased at 36 weeks of age in SLE mice compared with controls. Pressure was measured by indwelling carotid artery catheters in conscious freely moving mice. (*$P<0.05$, 1-way ANOVA).

Statistics

A 1-way ANOVA with Student–Newman–Keuls post hoc test for all of the pairwise comparisons was used to assess differences across different age groups and between control and SLE animals. A Student *t* test was used to assess statistical significance when comparing only control and SLE groups. Significance was accepted at $P<0.05$.

Results

As we have reported recently,[7] SLE mice are hypertensive compared with control animals by 36 weeks of age (Figure 1). Body weight in female SLE (NZBWF1) mice at 8, 20, and 36 weeks of age was significantly greater than control mice (NZW/LacJ) and by a progressively larger margin (12%, 21%, and 34%) at each age (Figure 2 and Table). Weights, indexed for body length (excluding tail), were significantly higher in SLE mice compared with controls at 36 weeks of age (0.36±0.00 versus 0.40±0.02 g/cm^2; $P<0.01$, Student *t* test). Visceral adipose tissue from SLE mice was significantly increased by 20 weeks of age with a progressively larger margin (73% to 117%; Table). Adipose tissue weight from individual depots, including ovarian/uterine, retroperitoneal, and thermogenic (brown), was also measured. Thermogenic adipose tissue was not different between control and

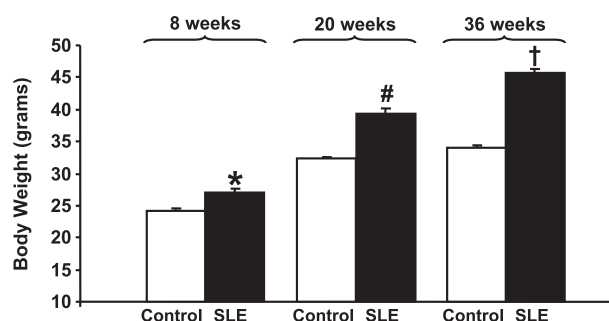

Figure 2. Body weight in control and SLE mice at 8, 20, and 36 weeks of age. *>8-week control mice, #greater than all groups except 36-week SLE, †greater than all groups ($P<0.01$, 1-way ANOVA). Control body weight was not different from 20 to 36 weeks of age.

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Body Weight, Food Intake, Activity, and Adipose Weight

Age, wk	Strain	Weight, g	Food Intake, g per 24 h	Activity, Counts per min	Visceral Fat, g	Uterine/Ovarian Fat, g	RP Fat, g	Brown Fat, g
8	NZW/LacJ	24.1±0.4	3.9±0.1	10.0±1.5	0.9±0.1	0.6±0.1	0.11±0.01	0.11±0.01
		n=33	n=5	n=5	n=10	n=4	n=4	n=4
	NZBWF1	27.1±0.5	3.9±0.1	7.5±0.9	1.3±0.1	1.0±0.1	0.17±0.01	0.13±0.01
		n=34*	n=5	n=4	n=12	n=5	n=5	n=5
20	NZW/LacJ	32.3±0.3	3.1±0.2	6.9±0.6	1.9±0.2	1.2±0.1	0.21±0.02	0.28±0.03
		n=55	n=5	n=5	n=15	n=4	n=4	n=4
	NZBWF1	39.3±0.8	3.0±0.2	5.7±0.5	3.3±0.2	3.8±0.3	0.48±0.06	0.27±0.04
		n=48*	n=5	n=5	n=13*	n=5*	n=5*	n=5
36	NZW/LacJ	34.1±0.4	3.2±0.1	8.5±0.7	2.3±0.2	2.6±0.2	0.47±0.08	0.17±0.02
		n=79	n=5	n=7	n=31	n=4	n=4	n=4
	NZBWF1	45.7±0.6	3.3±0.2	5.5±0.7	5.0±0.3	5.7±0.6	0.87±0.08	0.32±0.09
		n=97*	n=5	n=8*	n=46*	n=5*	n=5*	n=5

RP indicates retroperitoneal.
*$P<0.05$, 1-way ANOVA, vs age-matched NZW/LacJ.

SLE; however, reproductive and retroperitoneal adipose depots were significantly greater beginning at 20 weeks of age in SLE compared with controls (Table).

Food intake was not different between control and SLE mice at any age tested (Table). Data using radiotelemeters indicate that locomotor activity (during the night cycle) tended to be lower in SLE mice at 8 ($P=0.14$) and 20 ($P=0.11$) weeks of age. At 36 weeks, the age with the greatest disparity in weight and adipose, activity is significantly lower in SLE mice ($P<0.02$). Activity data in the Table represent the average nighttime activity over the course of the 4 days. Because the greatest differences in activity, body, and adipose weight were observed at 36 weeks, the remaining experiments were performed at this age.

Given the increased central adiposity, it was not surprising to find that plasma leptin levels in 36-week-old mice were significantly elevated compared with controls (Figure 3a). The elevated leptin and similar food intake led to the consideration of leptin resistance, at least centrally. To assess this, the effect of intraperitoneal leptin administration on food intake was tested in 36-week-old mice (Figure 3b). Leptin significantly reduced food intake to the same degree in both control and SLE mice, suggesting that the SLE mice are not resistant to its appetite-suppressing effects. In addition, the reduction in body weight caused by leptin injection was not different between control and SLE mice (−2.6±0.2 g versus −3.0±0.4 g; n=5; $P>0.33$ Student *t* test). Injection of vehicle (saline) did not affect food intake in either strain (data not shown).

The ovarian/uterine adipose tissue depot isolated from 36-week-old SLE mice had significant macrophage infiltration, an indication of inflammation, when compared with control mice (Figure 4). Similarly, sections of renal cortex from SLE mice exhibited macrophage infiltration, whereas those from control mice did not (Figure 5). Macrophage infiltration was not notable in the medulla (data not shown).

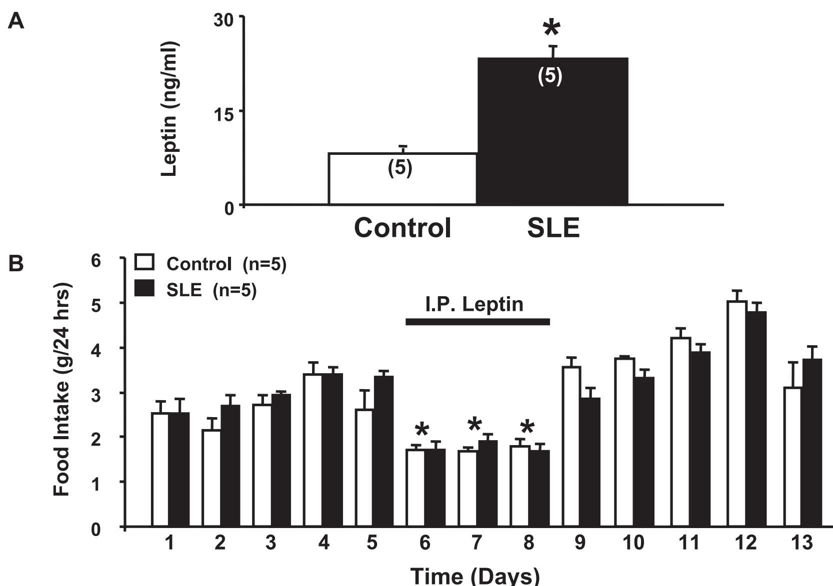

Figure 3. Plasma leptin levels are increased in 36-week SLE mice compared with controls. (A) $P<0.001$ compared with control (Student *t* test). Twice daily intraperitoneal injections of 30 μg of leptin in saline lead to a similar reduction in food intake in both control and SLE mice. (B) $P<0.05$ compared with basal food intake (1-way ANOVA).

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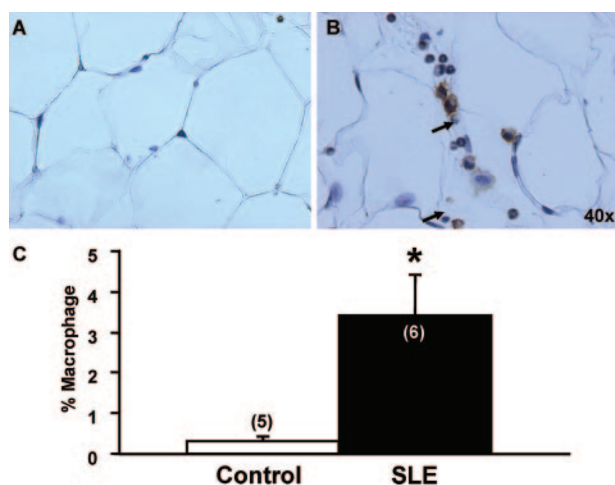

Figure 4. Visceral adipose tissue from 36-week-old SLE mice (B) had macrophage infiltration, whereas control adipose tissue did not (A). Percentage of macrophage (per total number of cells) was significantly increased in SLE mice compared with controls (C). $P<0.03$.

Frozen liver sections from SLE mice exhibited marked lipid accumulation in 5 of 6 mice studied compared with 0 of 6 controls (Figure 6).

Although fasted blood glucose levels were not different (Figure 7a), fasted insulin levels were significantly increased in SLE mice when compared with controls (Figure 7b). Moreover, when isosmotic glucose was administered after a 6-hour fast, SLE mice demonstrated a greater increase in blood glucose that took longer to return to basal levels (Figure 7c). These data indicate that the SLE mice are insulin resistant.

Discussion

The major new finding of this study is that an established model of SLE with hypertension has several characteristics of the metabolic syndrome. Therefore, the NZBWF1 strain may prove to be an important model for examining the connection between chronic inflammation and the metabolic syndrome. Specific findings include the following: (1) a hypertensive mouse model of SLE has increased body weight and central adiposity; (2) adipose tissue from SLE mice has monocyte/macrophage infiltrates as a marker of inflammation; (3) reduced physical activity may contribute to the increased body weight and adipose, but increased food intake does not; (4) SLE mice do not seem to be resistant to the satiating effects of leptin; and (5) the NZBWF1 model of SLE is insulin resistant.

Several risk factors comprise metabolic syndrome, including central obesity, insulin resistance, endothelial dysfunction, hypertension, inflammation, and dyslipidemia. The present data demonstrate that this model of SLE exhibits many of these features. For example, numerous other studies have reported an important role for inflammatory cytokines in the progression of SLE in NZBWF1 mice.[13–15] Recent evidence from our laboratory demonstrated that NZBWF1 mice are hypertensive, have impaired endothelial dependent relaxation, and have albuminuria by 36 weeks of age.[7] The pressure data from the present study measured by indwelling carotid catheters are consistent with the recently reported telemetry pressures. Although it is not yet clear, the impaired endothelial function[7] and elevated circulating leptin may be potential contributors to elevated blood pressure in this model. Although they were not measured in the present experiments, data from the literature indicate that these mice have dyslipoproteinemia.[16] The NZBWF1 strain has been widely used to examine the progression of SLE. These mice closely mimic human SLE, including the presence of antinuclear antibodies, glomerulonephritis, inflammatory cytokines, and a female predilection.[13,15,17,18,] Despite 4 decades of experimentation, this is the first study to report characteristics of the metabolic syndrome in this model. One potential limitation of this model is that the obesity and insulin resistance may be comorbid with SLE. Further examination will be required to establish a link between chronic inflammation in this model and the metabolic syndrome.

The relationship between SLE and changes in glucose handling or body composition has not been widely studied. In rare clinical cases, patients with SLE develop antibodies to the insulin receptor, which cause insulin resistance (type B insulin resistance).[19] Independent of these case studies, currently available data suggest that humans with SLE are at an increased risk to develop insulin resistance and obesity. For example, it has been reported that individuals with SLE have significantly increased body mass index at a 10-year follow-up compared with healthy controls.[20] In addition, women with SLE have an increased likelihood of developing insulin resistance[5] and changes in body fat distribution and composition.[6,20] The reason for increased body weight and adiposity in the NZBWF1 model is not yet clear; however, one possible mechanism seems to be a reduced physical activity level.

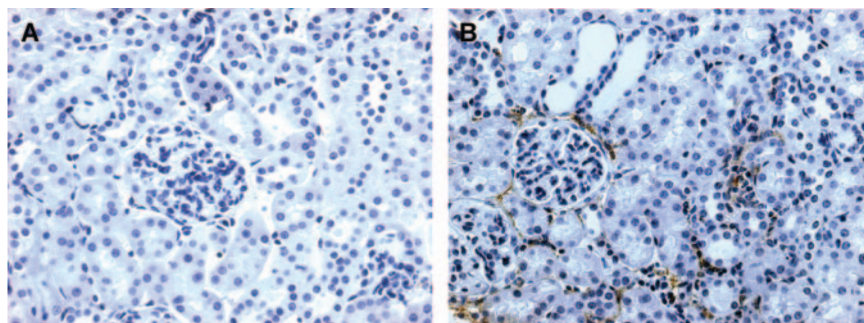

Figure 5. The renal cortex from 36-week-old SLE mice (B) exhibit macrophage infiltration, whereas the renal cortex from control mice did not.

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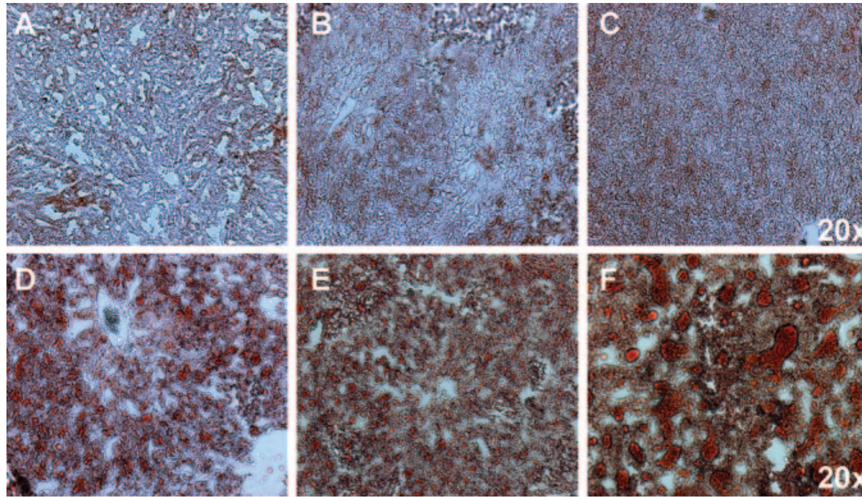

Figure 6. Liver sections from 3 control (A to C) and 3 SLE mice (D and E) stained with Oil-Red-O show increased hepatic lipid accumulation (red droplets) in SLE mice.

This new model of metabolic syndrome is potentially interesting, because previous genetic models typically have alterations in the leptin gene, its receptor, or its signaling pathways in the brain.[21–24] The NZBWF1 model of SLE is unique in that the cause of the metabolic syndrome is not clear. Although the present data suggest that the satiety actions of leptin in the brain are not different between control and SLE, the data do not rule out the possibility of selective leptin resistance.[25] In addition, it is equally plausible that the increased leptin may provide some benefit by maintaining normal blood glucose[26,27] and preventing even greater lipid accumulation in nonadipose tissues.[25] The findings of hepatic lipid accumulation are consistent with what has been observed in human metabolic syndrome.

The role of leptin and its contributions to obesity in rodent models has been studied extensively. Interestingly, leptin is important for promoting immune system function. Leptin (ob/ob) or leptin receptor (db/db) knockout mice are protected against the development of autoimmune diseases, including arthritis, inflammatory bowel disease, and multiple sclerosis.[28–30] There is also compelling evidence in humans that leptin may be important for the pathogenesis of autoimmune disease. For example, after a 7-day fasting period, 10 individuals with rheumatoid arthritis showed significant clinical improvement.[31] Another study demonstrated that women with SLE have significantly elevated plasma leptin when compared with age-, sex-, and body mass index–matched controls.[32] Therefore, it is possible that the elevated leptin in NZBWF1 may be a contributing factor to the chronic inflammation and progression of SLE in this model. Future studies will be required to determine the role of leptin in promoting autoimmunity and SLE hypertension.

Perspectives

Chronic inflammation is increasingly considered as a potential underlying mechanism for the progression of hypertension, insulin resistance, and the metabolic syndrome. SLE is a chronic autoimmune inflammatory disorder with a high incidence of hypertension. Evidence suggests that the incidence of insulin resistance, obesity, and lipodystrophy is higher in individuals with SLE; however, the mechanisms for this have not been investigated. The present study shows for the first time that a widely used mouse model of SLE has characteristics of the metabolic syndrome, including central adiposity, fatty liver, elevated leptin, and insulin resistance. Therefore, NZBWF1 may be an important model to examine the relationship between chronic inflammation during SLE and how this leads to insulin resistance and altered body composition.

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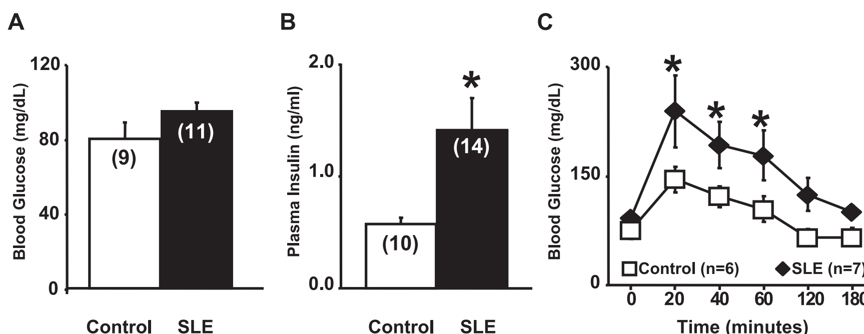

Figure 7. Fasted blood glucose was not different between 36-week-old control and SLE mice (A). Fasted plasma insulin was significantly increased in 36-week SLE mice vs controls (B). $P<0.05$. A glucose tolerance test showed that fasted glucose was significantly greater in 36-week SLE mice after an intraperitoneal injection of isosmotic glucose and took longer to return to basal levels (C). $P<0.05$ vs time-matched control (1-way ANOVA).

Disclosures

None.

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