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Wang, Hui, Xue, Y, Wang, B, Zhao, J, Yan, Xu, Huang, Yan, Du, M and Zhu, M-J (2014) Maternal obesity exacerbates insulitis and type 1 diabetes in non-obese diabetic mice. Reproduction, 148 (1). 73 - 79. ISSN 1470-1626

Published in final edited form as:
Reproduction. 2014 July ; 148(1): 73–79. doi:10.1530/REP-13-0614.

Maternal obesity exacerbates insulitis and type I diabetes in non-obese diabetic (NOD) mice

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Abstract

Accompanying the dramatic increase in maternal obesity, the incidence of type I diabetes (T1D) in children is also rapidly increasing. The objective of this study was to explore impacts of maternal obesity on the incidence of T1D in offspring using non-obese diabetic (NOD) mice, a common model for TID. Four-week-old female NOD mice were fed either a control diet (10% energy from fat, CON) or a high fat diet (60% energy from fat, HFD) for 8 weeks before mating. Mice were maintained in their respective diets during pregnancy and lactation. All offspring mice were fed the control diet to 16 weeks. Female offspring (16-wk-old) born to obese dams showed more severe islet lymphocyte infiltration (major manifestation of insulitis) ($P < 0.01$), concomitant with elevated nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) p65 signaling ($P < 0.01$) and tumor necrosis factor (TNF) α protein level ($P < 0.05$) in the pancreas. In addition, maternal obesity resulted in impaired ($P < 0.05$) glucose tolerance and lower ($P < 0.05$) serum insulin levels in offspring. In conclusion, maternal obesity resulted in exacerbated insulitis and inflammation in the pancreas of NOD offspring mice, providing a possible explanation for the increased incidence of T1D in children.

Keywords

Maternal obesity; offspring; inflammation; type 1 diabetes; pancreas; insulitis

Introduction

Obesity is rapidly increasing worldwide, with the current obesity rate in USA more than 35%. Accordingly, more than 30% of women aged 20–39 (child bearing age) are obese (Ogden *et al.* 2012). Meanwhile, according to the statistics from American Autoimmune

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Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Related Diseases Association and National Institutes of Health, up to 23.5 million Americans suffer from autoimmune disease and the prevalence is rising. Type I diabetes is one of the major autoimmune diseases occurring in patients at young age, with about 30,000 diagnosed with Type 1 diabetes (T1D) annually (NIH autoimmune diseases statistics). The prevalence rate in developed countries is increasing at around 3% per year (Gale 2002; Sgouroudis and Piccirillo 2009).

Maternal obesity is considered as a risk factor for a number of adult diseases in offspring, especially cardiovascular disease and type 2 diabetes, so called “developmental programming” (Barker *et al.* 2002; Nathanielsz *et al.* 2007; Li *et al.* 2011; Portha *et al.* 2011; Poston 2012; Reynolds and Caton 2012). Recent epidemiological evidence indicates that maternal nutrition is correlated with the risk of childhood T1D (D'Angeli *et al.* 2010). T1D is an autoimmune disease caused by gradual pancreatic lymphocyte infiltration and the destruction of insulin-producing β cells in the islets.

The etiology of T1D is complicated and multifactorial, including both genetic and environmental risk factors. Epidemiological studies indicate that maternal intrauterine environment and early postnatal nutrition are among environmental factors contributing to the onset of T1D in children. Moreover, maternal over-nutrition or under-nutrition predisposes offspring to metabolic disease syndromes by altering the development of less vital organs such as the pancreas (Hales and Barker 2001; Harding 2001). Therefore, intrauterine environment, birth weight and postnatal early life nutrition play a role in β cell autoimmunity and are associated with a risk for T1D (Dahlquist *et al.* 1996; Kimpimaki *et al.* 2002; Oge *et al.* 2007). Indeed, the pancreatic β cell function is altered in the fetuses of obese sheep (Zhang *et al.* 2011). Maternal low protein diet, calorie restriction or neonatal low protein exposure are associated with delayed T1D onset or decreased apoptosis of β cells (Boujendar *et al.* 2002; Oge *et al.* 2007; Chamson-Reig *et al.* 2009). Though there is recent epidemiological evidence supporting the association between maternal obesity and T1D in offspring (D'Angeli *et al.* 2010), the possible causal relationship between maternal obesity and increased risk of T1D in offspring has not been tested. Most available studies on maternal obesity and offspring metabolic diseases focus on Type 2 diabetes (Barker *et al.* 2002; Yan *et al.* 2010). Non-obese diabetic (NOD) mice are susceptible to the spontaneous development of T1D, with histological results showing peri-insulitis at 3 to 4 weeks of age and severe insulitis by 10 weeks of age (Andre *et al.* 1996). Using NOD mice as an experimental model, the aim of this study is to examine the effects of maternal obesity on the onset of T1D in offspring.

Materials and methods

Animals and diet treatments

Forty three-week-old female NOD/ShiLtJ mice were obtained from the Jackson Laboratory (Bar Harbor, ME). Mice were housed in a temperature-controlled room with a 12 h light: 12 h darkness cycle and were given food and water at *ad libitum*. At 4 weeks of age, mice were randomly separated into two groups (n = 20 per group) and fed a control diet (CON, 19.2% protein, 67.3% carbohydrate and 4.3% fat with 10% energy from fat; D12450B, Research diet, New Brunswick, NJ) or a high fat diet (HFD, 26.2% protein, 26.3% carbohydrate and

34.9% fat with 60% of energy from fat; D12492, Research diet) *ad libitum*, respectively for 8 weeks to induce obesity. These two diets mimick western "healthy" and "obesogenic" diets respectively, and have been widely used for diet-induced obesity studies (Zhang *et al.* 1994). After inducing obesity, these female mice were mated with CON fed male mice of a similar age. At birth, mice with abnormally large (above 8) or small litter sizes (less than 4) were excluded. The size of remaining litters (n = 10 per treatment) were balanced to 6 pups per litter by reducing large and increasing small litters. Maternal mice were maintained on their respective CON or HFD diet during gestation and lactation. After weaning, all offspring were fed with CON diet so that the only difference between treatments was the maternal effect. The body weight and feed intake of both maternal and offspring mice were measured weekly. Because female NOD mice are known to be more susceptible to the development of T1D than male mice (Wicker *et al.* 1995), female offspring mice were chosen for further analyses, to avoid the possible confounding effects of sex. All animal procedures were approved by the Institutional Animal Use and Care Committee of University of Wyoming.

Tissue collection

On the day of necropsy, after overnight fasting, one female mouse with average body weight from each litter was selected. These mice were anesthetized intraperitoneally with tribromoethanol (250 mg/kg body weight). Blood samples were collected under anesthesia, and serum was used for insulin analysis. Mice were sacrificed by cervical dislocation, the pancreas was removed, and a small portion of the pancreas from each mouse was fixed in 4% (w/v) paraformaldehyde dissolved in phosphate buffer (0.12 M; pH 7.4), processed and embedded into paraffin per standard methods. The remaining pancreatic tissue was frozen in liquid nitrogen, and stored at −80 °C for further biochemical analyses. In addition, the gonadal fat was collected and weighed, because gonadal fat weight is highly correlated with adiposity in mice (Rogers and Webb 1980).

Insulitis scoring

The paraffin embedded pancreatic tissues were sectioned at 5 μm thickness. Sections were collected at 50 μm interval, subjected to hematoxylin-eosin staining (HE) for evaluation of insulitis (lymphocyte infiltration) using the following scale: 0, no infiltration; 1, minimal focal infiltration; 2 peri-islet infiltration (<25%); 3, intra-islet infiltration (25<50%); 4, extensive infiltration (>50%). The mean insulitis score of each offspring was calculated by dividing the sum of the insulitis scores by the number of islets examined. At least 20 islets per animal were analyzed by two independent examiners in a blind manner.

Intraperitoneal glucose tolerance test

Following 8-week treatment maternal NOD mice with CON or HFD diet (n = 20 per dietary group), or female offspring mice at age of 16 week (one mouse per litter of average body weight) were subjected to intraperitoneal (i.p.) glucose tolerance tests after overnight fasting with free access to water. Glucose was injected i.p. into mice at 2 g/kg body weight. Glucose concentrations of each mouse were measured with a glucometer (Bayer's Contour, Tarrytown, NY) at 0, 15, 30, 60, 90 and 120 min after injection using blood collected from

the tail vein. The glucose level of mice within dietary treatment at each time point post injection was averaged and graphed against time post injection. The total area under the glucose disposal curve (AUC) was calculated for each animal with GraphPad Prism 5.0 software without subtracting the baseline, and means computed. Glucose levels at each time point were analyzed by Repeated Measures ANOVA, and AUC was analyzed by ANOVA.

Insulin analysis

After overnight fasting, blood serum samples collected at necropsy day were used for insulin analysis. Serum insulin concentration was determined in duplicate in a single assay by commercial ELISA kit (Linco Research Inc., St. Charles, MO, USA) according to the manufacturer's instructions. Mean intra-assay and inter-assay CV for insulin was < 10%.

Immunoblotting analyses

Immunoblotting analysis was conducted as previously described (Zhu *et al.* 2010; Yan *et al.* 2011). Antibodies against phospho-NF-κB p65 (Ser536), NF-κB p65, phospho-Akt (Ser473), and Akt were purchased from Cell Signaling Technology (Beverly, MA). Tumor necrosis factor (TNF) α antibody was purchased from Invitrogen (Grand Island, NY), while β-actin antibody was purchased from Sigma (St. Louis, MO). Density of bands was normalized with reference to the β-actin content.

Statistical Analysis

Data were analyzed as a complete randomized design using GLM (General Linear Model of Statistical Analysis System, SAS, 2000). For GTT, glucose levels at different time points were analyzed as repeated measures ANOVA using SAS Proc Mixed. Each maternal mouse and the subsequent litter was considered as an experimental unit. Means ± standard errors of mean (SEM) are reported. The differences between group means were analyzed by ANOVA. Statistical significance is considered as $P < 0.05$.

Results

High fat diet leading to obese phenotype in NOD mice

During 8 weeks of dietary treatment, the overall feed intake was similar between CON and HFD fed maternal NOD mice (Fig. 1A). Starting the first week of feeding, maternal NOD mice fed with HFD gained more body weight than those fed the CON diet (Fig. 1B, $P < 0.01$). After 8 weeks of dietary treatment, HFD fed mice developed obesity with more gonadal fat (Fig. 1C & D). The fasting baseline blood glucose level were higher (Fig. 1E, $P < 0.05$) in NOD mice fed with HFD than those in CON fed mice at 4 weeks of dietary treatments. Although the fasting baseline blood glucose was similar, the HFD fed mice had impaired glucose tolerance compared to those fed CON diet after 8 weeks of dietary treatments (Fig. 1F, $P < 0.01$).

Body weight of female NOD offspring

Offspring born to CON and HFD fed dam had similar food intake and body weight (Fig. 2AB). The ratios of heart ($P < 0.05$) weight to offspring body weight were higher in female offspring exposed to maternal obesity (Fig. 2C).

Offspring from HFD dams developed more severe insulitis

Fig. 3A illustrates the histological scoring system that was used to evaluate effects of maternal obesity on pancreatic insulitis of female offspring. As shown in Fig. 3B & C, the female offspring exposed to maternal HFD showed more severe ($P < 0.05$) insulitis than those from CON dams.

Pancreatic inflammatory signaling pathways in female offspring

Consistent with more severe lymphocyte infiltration, the major mediator of inflammatory NF-κB signaling, phos-p65, was elevated ($P< 0.01$) in pancreatic tissue of offspring from HFD fed dams (Fig. 4A), suggesting an exaggerated pancreatic inflammatory response. Accordingly, higher TNFα protein level was also detected in pancreatic tissue of offspring born to HFD fed dams (Fig. 4B, $P < 0.05$).

Insulin signaling in female offspring

Although the intraperitoneal glucose tolerance test at 16 weeks of age revealed relatively minor differences, the area under curve (AUC) was greater (Fig. 5A, $P < 0.05$) in offspring from HFD fed dams, suggesting impaired glucose tolerance. This could be related to the trend of reduction of phos-Akt in pancreas tissue of offspring from HFD fed dam (Fig. 5B, $P < 0.10$). Conversely, offspring born from HFD fed dams had lower ($P < 0.05$) serum insulin level compared to those from CON fed dam (Fig. 5C), indicating decreased insulin production which could also explain the impaired glucose tolerance.

Discussion

While limited studies have shown that maternal obesity impairs fetal pancreatic β-cell development and postnatal function (Han *et al.* 2005; Ford *et al.* 2009; Zhang *et al.* 2011), the relationship between maternal obesity and the onset of T1D in offspring remains unclear. To clarify this relationship, we induced obesity in NOD mice to test whether maternal obesity predisposes offspring to T1D. NOD mice are prone to develop TID and are the most frequently used model for studying this disease. Wild-type mice were not used here because their incidence of TID is too low. In addition to obesity, NOD maternal mice had an elevated fasting blood glucose level after 4 weeks of HFD treatment and impaired glucose tolerance after 8 weeks. Together, these maternal mice were obese, with elevated blood glucose and impaired glucose tolerance, similar to obese human mothers.

Maternal obesity accelerates the development of T1D in offspring mice. We observed severe lymphocyte infiltration in the pancreatic islets of offspring exposed to maternal obesity, showing the increased degree of insulitis. Consistent with lymphocyte infiltration data, pancreatic tissue in offspring from HFD fed dam had augmented TNF-α content, and heightened inflammatory NF-κB signaling as indicated by elevated p65 activation. These

data are in line with previous reports, where maternal obesity elevates cytokines and inflammatory signaling in other tissues of fetuses and offspring (White *et al.* 2008; Bilbo and Tsang 2010; Leibowitz *et al.* 2012). The observed inflammatory responses in offspring pancreatic tissue from obese dams might be a contributing factor to their exaggerated insulitis (Kurylowicz and Nauman 2008; Thomas *et al.* 2012). Cytokines (IL1β, TNF-α and IFN-γ) can induce β cell stress leading to apoptosis in human and rodent T1D models by increasing the expression of pro-apoptotic proteins (Rabinovitch 1998; Rabinovitch and Suarez-Pinzon 1998; Pavlovic *et al.* 2000; Petrovsky *et al.* 2002). Our data are in line with a previous report, where maternal obesity resulted in pancreatic dysfunction in offspring and was associated with the development of T2D. However, in that study, the possible link of maternal obesity to T1D was not studied or discussed (Han *et al.* 2005). Consistent with our observations, maternal calorie restriction and low maternal nutrition has been shown to delay onset of T1D (Oge *et al.* 2007; Chamson-Reig *et al.* 2009).

We also detected glucose intolerance in maternal obesity offspring, which coincided with lower insulin levels. Akt activation contributes to insulin sensitivity in peripheral organs and also could suppress stress induced β cells death (Leibiger *et al.* 2008). We observed a tendency of decreased Akt phosphorylation in pancreas of maternal obesity offspring. This down-regulated Akt signaling in pancreas might further accelerate pancreatic cell apoptosis.

In summary, maternal obesity resulted in exaggerated insulitis, impaired glucose tolerance and reduced baseline insulin in female NOD offspring by 16 weeks of age. The mechanism for accelerated onset of T1D due to maternal obesity might be associated with augmented inflammation in the offspring pancreas. Up to now, the impact of maternal obesity on offspring metabolic diseases has been focused on T2D. To our knowledge, this is the first report showing that maternal obesity predisposes offspring to T1D, which is an important addition to our understanding regarding the long-term programming effects of maternal obesity on offspring health. Considering that more than 30% pregnant women are obese, the current finding has important clinical implications.

Acknowledgments

The authors gratefully acknowledged Dr. Joseph R. Powers in the School of Food School at Washington State University for his critical review on the manuscript, and Mr. David Hanna in the Department of Animal Science at University of Wyoming for taking care of mice and facilitate experiments.

Funding

Funding was supported by NIH R15HD073864 and NIH INBRE P20RR016474.

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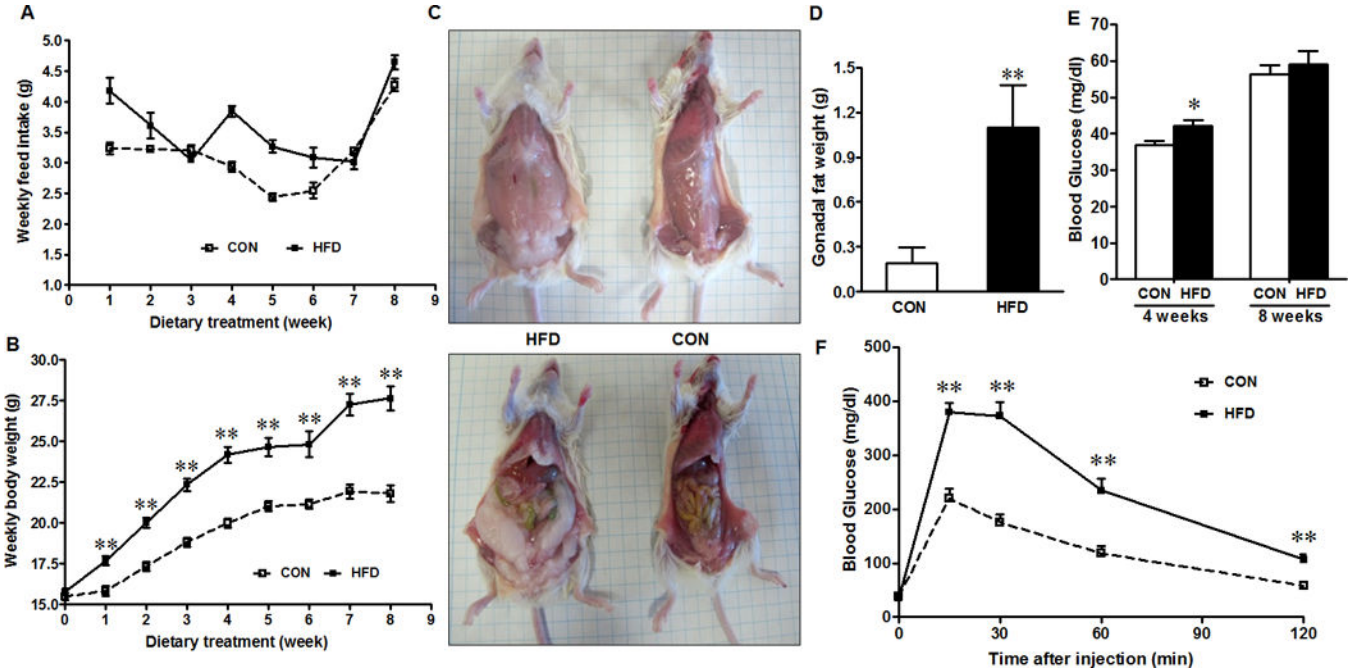

Figure 1.

High fat diet (HFD) induced obese phenotypes in maternal NOD mice. A: Weekly feed intake; B: Weekly body weight; C: Representative images of fat tissue in maternal NOD mice of CON and HFD treatments at necropsy; D: Gonadal fat weight at necropsy; E: Base line blood glucose concentration of maternal NOD mice following 4 or 8 weeks of CON or HFD diet; F: Intraperitoneal glucose tolerance test of maternal NOD mice following 8 weeks of CON or HFD treatment. Glucose was injected i.p. at 2 g/kg body weight. Blood glucose concentrations were measured at 0, 15, 30, 60, 90, and 120 min post glucose injection. (Mean ± SEM; **: $P < 0.01$,*: $P < 0.05$; N = 20).

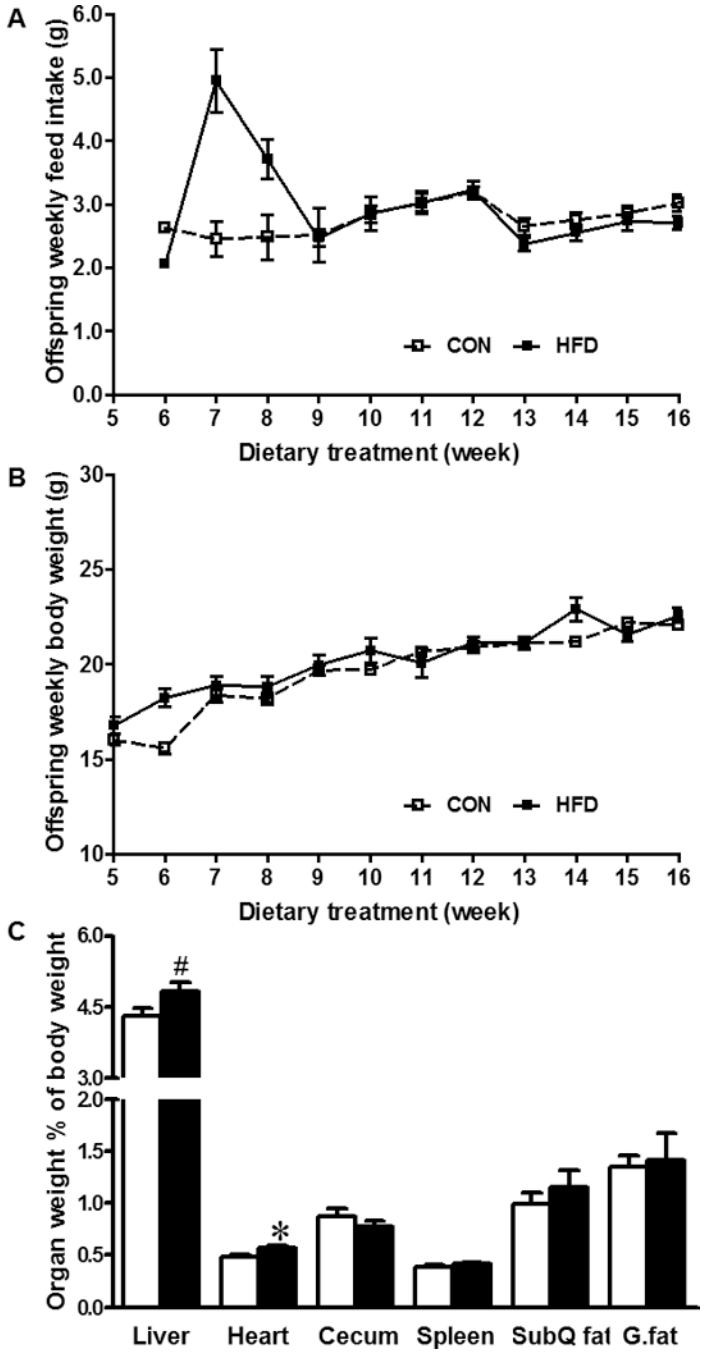

Figure 2.

Effects of maternal obesity on female offspring (16-week-old) feed intake, and body, organ and tissue weights. A: Offspring weekly feed intake; B: Offspring weekly body weight; C: Organ weights normalized as a percentage of total body weight. (Mean ± SEM; *: $P < 0.05$; #: $P < 0.10$; N = 10).

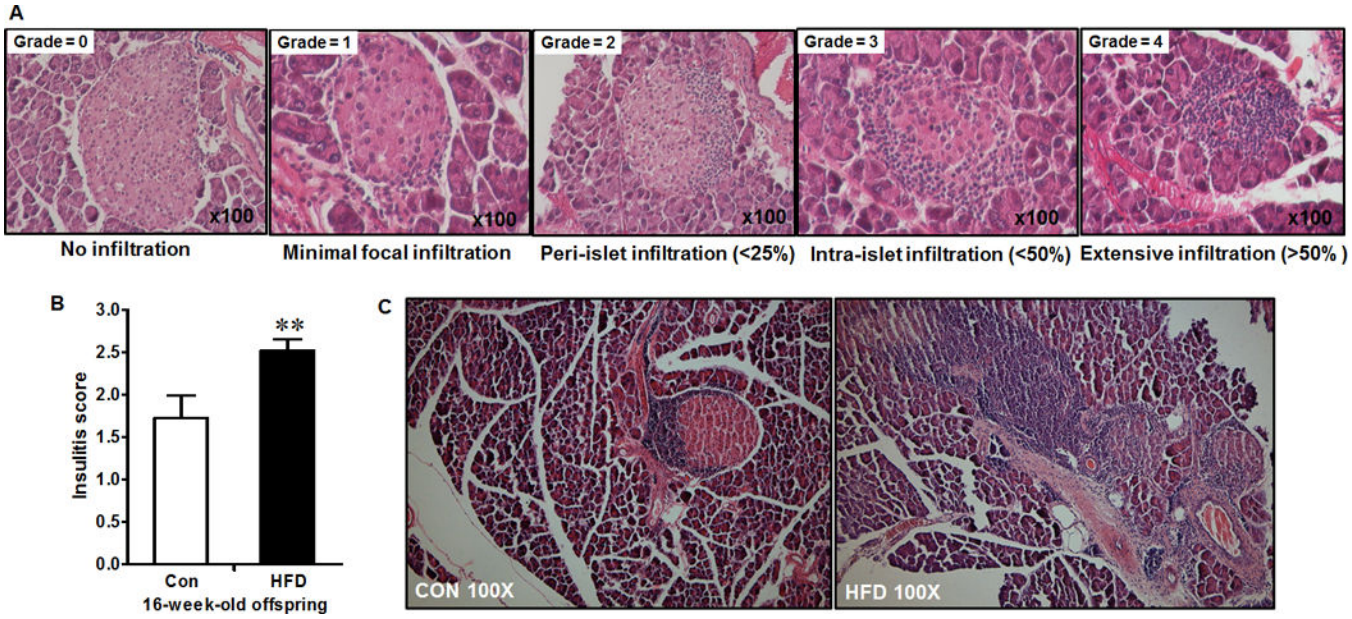

Figure 3.

Pancreatic pathobiological changes of female offspring mice (16-week-old) exposed to maternal CON or HFD diet. The paraffin embedded pancreatic tissues were sectioned at 5 μm thickness, and subjected to hematoxylin-eosin staining. A: Insulitis (lymphocyte infiltration in the islet of pancreas) scoring system; B: Pancreatic insulitis score; C: Representative images of pancreatic islets of CON and HFD offspring. (Mean ± SEM; **: $P < 0.01$; N = 10)

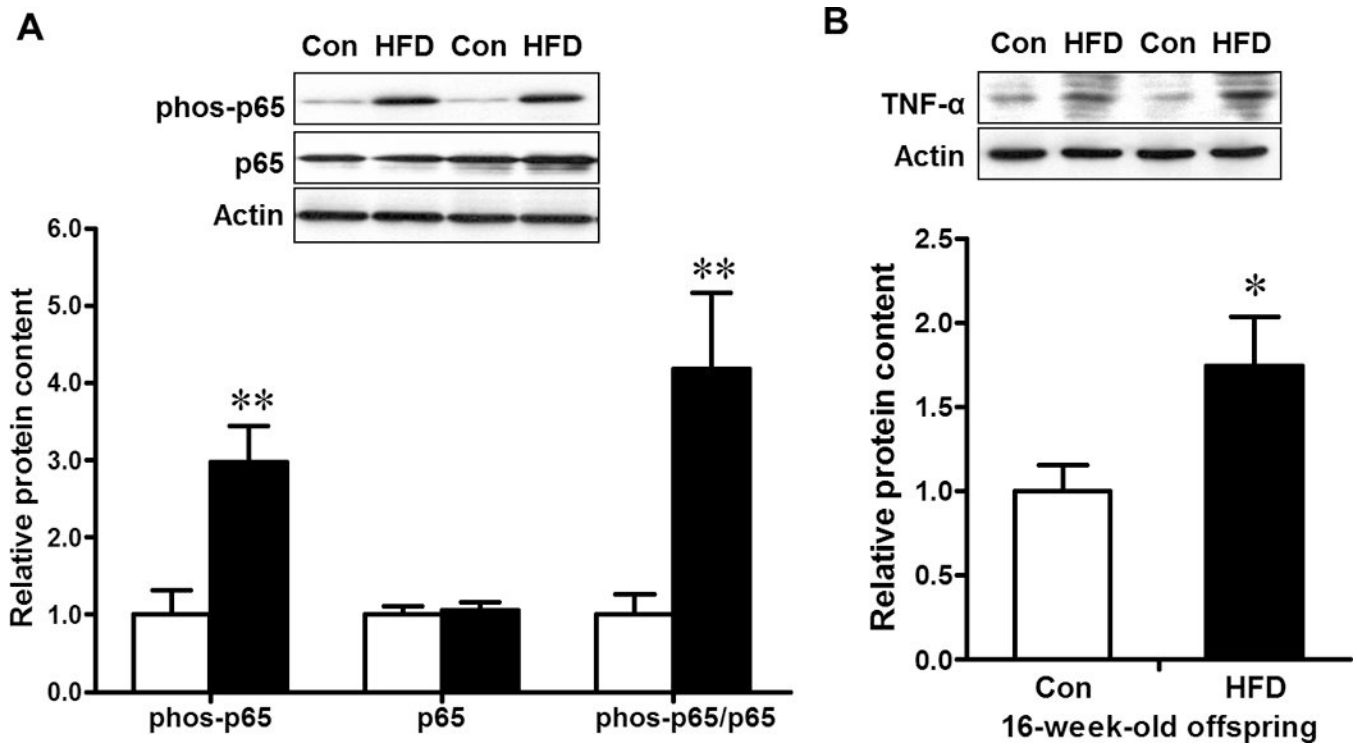

Figure 4.

Inflammatory signaling (A) and inflammatory cytokine content (B) in pancreas tissues of female offspring (16-week-old) exposed to maternal CON (□) or HFD (■) diet. Pancreatic tissues were subjected to immunoblotting. A: Elevation of NF-κB signaling shown by increased ratio of p-p65 to p65 level in HFD compared to CON offspring pancreas; B: Enhanced TNF-α protein level in HFD compared to CON offspring pancreas. (Mean ± SEM; **: $P < 0.01$; *: $P < 0.05$; N = 10)

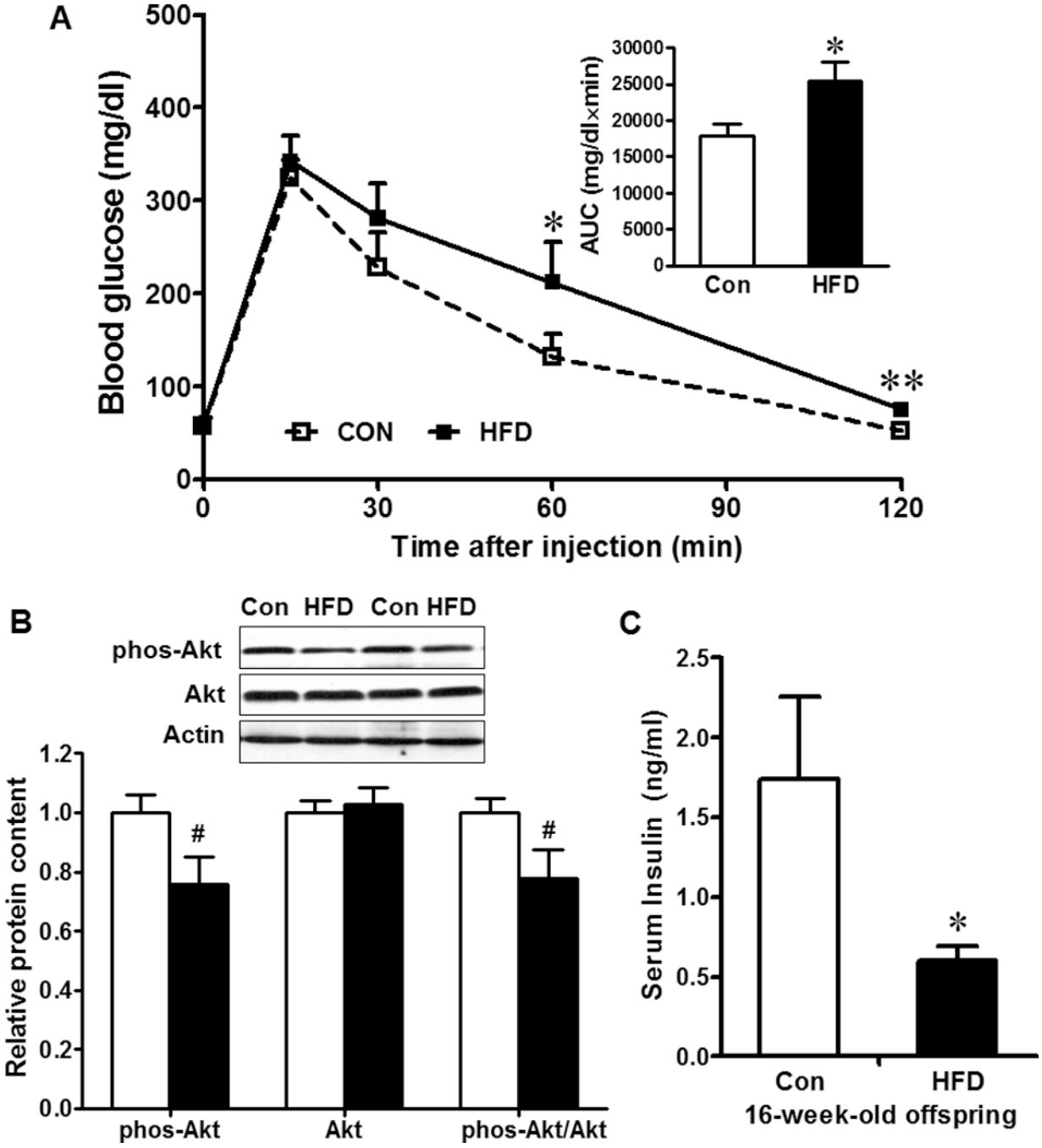

Figure 5.

Intraperitoneal glucose tolerance and insulin signaling of the pancreas of female offspring (16-week-old) exposed to maternal CON (□) or HFD (■) diet. A: Intraperitoneal glucose tolerance test with area under curve (AUC). Glucose was injected i.p. at 2 g/kg body weight. Blood glucose concentrations were measured at 0, 15, 30, 60, 90, and 120 min after glucose injection; B: Pancreatic phos-Akt and total Akt levels measured by immunoblotting; C.

Fasting blood serum insulin level. (Mean ± SEM; **: $P < 0.01$; *: $P < 0.05$; #: $P < 0.10$; N = 10)