Abstract. – OBJECTIVE: We established an animal model of diet-induced obesity pregnant rats and their offsprings, and explored the effect of high-energy feeding on oxidative stress in filial rat liver, as well as the underlying mechanism.

MATERIALS AND METHODS: Pregnant female Sprague-Dawley (SD) rats were randomly assigned into two groups: control group and palatable high-energy diet (PHED) group. Liver tissues were obtained 12 days after offspring rats were born for further study. The expressions of malondialdehyde (MDA), reduced glutathione (GSH) and antioxidative enzyme activities were measured, and pathological change of liver tissues was examined by HE staining. In addition, the expressions of inflammatory factors, tumor necrosis factor-α (TNF-α), IL-1β, and mRNA level of Heme oxygenase-1 (HO-1), were examined by ELISA and RT-PCR, respectively. Furthermore, COX-2 and nuclear factor kappa B (NF-κB) expressions were also examined by Western Blot.

RESULTS: Offspring rats of PHED group displayed a significantly higher level of MDA than the control group, and significantly lower level of GSH. Significant reductions in the activities of a number of antioxidant enzymes, such as glutathione S-transferase (GST), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), were found in the PHED group offspring rats compared to the control group offspring rats. HE staining showed the liver cells in the control group offspring rats showed normal histopathological appearance, such as well-aligned cell patterning and unchanged cellular structure, but in the PHED group offspring rats showed slight structure deformation and misalignment. HO-1 mRNA in PHED group offspring rats is significantly higher than that in the control group offspring rats. Furthermore, COX-2 and p-NF-κB-p65 in PHED group offspring rats is also significantly higher than that in the control group offspring rats.

CONCLUSIONS: Palatable high-energy intake of obesity pregnant rats could lead to reduced antioxidant function in offspring rat liver, even increase the chance of chronic liver disease in the early ages of offsprings. The underlying mechanism is associated with the activity of NF-κB protein.

Key Words: Obesity pregnant rat, Palatable high-energy diet (PHED), Rat offspring, Oxidative stress, Nuclear factor kappa B (NF-κB).

Abbreviations
Palatable high-energy diet (PHED); malondialdehyde (MDA); glutathione (GSH); heme oxygenase-1 (HO-1); nuclear factor kappa B (NF-κB); reactive oxygen species (ROS); triphosphopyridine nucleotide (NADPH); Sprague-Dawley (SD); nuclear factor (erythroid-derived 2)-like 2 (Nrf2); tumor necrosis factor-α (TNF-α); glutathione S-transferase (GST); superoxide dismutase (SOD); catalase (CAT); glutathione peroxidase (GSH-Px).

Introduction
Obesity, as a globally widespread public health problem, has become a chronic disease which has huge influence on the quality of life for many people. The incidence of obesity has reached epidemic proportions all over the world and it is predicted to reach 70% in 2025. Excess body fat will not only affect people’s quality of life, but also will increase many healthcare problems and even risk of death. Obesity has been shown to be associated with the development of many health
issues, such as cardiovascular complications, diabetes mellitus, sleep disorders, asthma, renal dysfunction, hepatic dysfunction, infertility and cancer. Recent researches have shown that there are close connections between obesity and oxidative stress. Obesity will cause systemic oxidative stress via different mechanisms, like the generation of superoxide from oxidative phosphorylation, triphosphopyridine nucleotide (NADPH) oxidases, the activation of protein kinase C, glyceraldehyde auto-oxidation, and polyol and hexosamine pathways. Some other factors also connect oxidative stress to obesity, like low antioxidant defense and hyperleptinemia, chronic inflammation, tissue dysfunction, as well as postprandial reactive oxygen species (ROS) production.

In the United States, nearly 2/3 women of childbearing age is overweight or obese, more than half of which have excess gestational weight gain when becoming pregnant. The increase in the prevalence rates of obesity and diabetes during pregnancy has become an important public healthcare issue; it not only enhances potential cardiometabolic disease risk in mothers, but also brings higher risks to offspring in developing some chronic diseases like obesity, type 2 diabetes, non-alcoholic fatty liver disease, metabolic syndrome, and even possibly autism. However, the mechanism through which the maternal obesity portends higher risk for chronic diseases to offspring has not been well investigated. We established an animal model using diet-induced obesity pregnant Sprague-Dawley (SD) rats and analyzed the influence of palatable high-energy diet on offspring oxidative stress in liver.

**Materials and Methods**

**Experimental Animals**

Healthy male and female Sprague-Dawley (SD) rats were purchased from Suzhou Industrial Park Ireland Metall Technology Co., Ltd., (Suzhou, Jiangsu, China), under permit certificate SCXK 2014-0007. The protocol for animal use was approved by the Institutional Animal Care and Use Committee (IACUC) of Yantaishan hospital.

Animals were housed in a pathogen-free animal house over 1 week for acclimatization, then mixed in a ratio of 1:2 (male to female) to mate. 20 selected pregnant rats were randomly divided into two groups (n=10): control group were provided with basic feed 100%, and another group was provided with palatable high-energy diet (PHED, which contains basic feed 80%, 10% lard, 1.5% cholesterol, 0.5% bile salts, 8% sucrose) for the whole pregnancy. 12 days after the offspring rats were born, they were separated from the adult rats and sacrificed by decapitation under ether anesthesia. The liver tissue was collected and homogenized with saline (1:10, w/w). The supernatant was kept at -40°C for experimental measurements.

**Measurement of Malondialdehyde (MDA) and Glutathione (GSH) Levels and Anti-oxidant Enzymes’ Activities**

To analyze the influence of PHED in diet-induced obesity pregnant rats on offspring oxidative stress in liver.

Blood was collected from offspring rats in both control and HPED groups, and kept at 4°C overnight for stratification. Serum IL-1β and TNF-α levels were determined using ELISA kit (R&D systems, Minneapolis, MN, USA) following the manufacture’s instruction. The activity of SOD was determined using a method similar to that developed by Habig et al. CAT activity was carried out by using Aebi’s method. GSH-Px activity was measured by the Kondo et al method. The activity of SOD was determined by following the procedures reported by Sun et al. The kits used in above experiments were obtained from the Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). The procedures were following as specified in the manuals.

**Histopathological Examination**

The influence of palatable high-energy diet in obesity pregnant rats on the offspring rats liver cells, liver tissues from both control and PHED groups offspring rats were fixed using 10% neutral formalin for 24 h, and embedded into paraffin. 5-μm sections were prepared and then stained with hematoxylin and eosin (HE) using the HE Staining Kit (Baihao Biological Technol-
ogy, Tianjin, China) according to the manufacturer’s instructions. Finally, the stained sections were observed under a light microscopy (Olympus, Tokyo, Japan).

Expression of HO-1 mRNA Determined by RT-PCR

To measure the levels of HO-1 mRNA, the total liver RNA was extracted from offspring rat liver tissue with TRIzol (TaKaRa, Otsu, Shiga, Japan) following the protocol provided by the manufacturer. The concentration of total RNA was quantified by the optical density at 260 nm (OD260). 1000 ng of total RNA were applied as a template for reverse transcription reaction using M-MuLV and the Master Mix Reverse Transcription Kit (TaKaRa, Otsu, Shiga, Japan), following the manufacturer’s instruction. β-actin was selected as the internal reference. The sequences of primers used in PCR were listed in Table I. The PCR products were amplified using the following cycling parameters: for HO-1, 95°C for 5 min, then 30 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s, and finally a single cycle at 72°C for 10 min. For β-actin, 94°C for 5 min, then by 30 cycles of 94°C for 40 s, 55°C for 40 s, and 72°C for 40 s, and finally a single cycle at 72°C for 10 min. 10 μL PCR product were mixed with 2 μL loading buffer and applied to 1.5% agarose gel containing 0.05 μg/mL ethidium bromide for 50 min under 70V. 2−ΔΔCt method was used to calculate the relative level of HO-1 mRNA using β-actin mRNA as internal reference.

Western Blot

For Western blot analysis, rat liver tissue from both control and PHED groups were lysed with 20 mM Tris-HCl (pH 8.0) containing 1% NP-40, 1 mM EDTA, 150 mM NaCl, 0.1% β-mercaptoethanol, 10% glycerol, 0.5 mM dithiothreitol, and a cocktail of proteinase inhibitors (Sigma-Aldrich, St. Louis, MO, USA). β-actin served as an internal control. 50 μg protein samples were loaded per lane, separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (12% polyacrylamide gels) under 100 V voltage, which were then transferred onto a polyvinylidene difluoride (PVDF) membrane. The membranes were then blocked with 5% non-fat dry milk dissolved in saline with 0.1% Tween 20 in 50 mM Tris at pH 7.4 with 150 mM NaCl for 1.5 h at 4°C overnight, and then incubated overnight at 4°C with the primary antibodies, polyclonal anti-rat COX-2 (Ab52234, Abcam, Cambridge, MA, USA; 1:1000), and polyclonal anti-rat NF-κB (Ab16502, Abcam, Cambridge, MA, USA; 1: 1000), and polyclonal anti-rat p-NF-κB (Ab86299, Abcam, Cambridge, MA, USA; 1: 2000) were added, as well as β-actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA, 1:500). The polyvinylidene difluoride (PVDF) membrane was washed 3 times with Tween 20, each time 6 min, after which the secondary antibody goat anti-rabbit IgG (Ab6721, Abcam, Cambridge, MA, USA; 1:3000) were added and then incubated for 2 h. The signal was measured with the Amersham ECL system (Amersham-Pharmacia Biotech, Piscataway, NJ, USA). The relative expression of COX-2, NF-κB and p-NF-κB was determined densitometrically using the Image-Pro Plus software, and calculated with β-actin bands as reference.

Statistical Analysis

Statistical analysis was performed using the SPSS software, v19.0 (IBM Corp., Armonk, NY, USA). Data were reported as the mean ± standard deviation (SD). Statistical comparision for two groups was performed with Student t-test (two tailed). p < 0.05 was considered to be significant.

Results

Influence of PHED on MDA and GSH Levels and Antioxidant Enzymes Activities

PHED group offspring rats displayed a significantly higher level of MDA (p < 0.05) than the control group offspring rats, and significantly

Table I. Primers used in RT-PCR experiments.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers</th>
<th>Length (bp)</th>
</tr>
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<tbody>
<tr>
<td>HO-1</td>
<td>Forward 5’-ATGGAGCGCCCCACAGTCGAC-3’</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Reverse 5’-GGTAGCGGGTATATGCGTGGG-3’</td>
<td>21</td>
</tr>
<tr>
<td>β-actin</td>
<td>Forward 5’-CTTCCTGGGCATGGAGTCCTG-3’</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Reverse 5’-GGAGCAATGATCTTGTCTTC-3’</td>
<td>21</td>
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lower level of GSH \( (p < 0.01) \). SOD, CAT, GSH-Px and GST are important antioxidant enzymes, and their activities were compared between the PHED group offspring rats and the control group offspring rats. Significant reductions in the activity of GST, CAT, SOD and GSH-Px were found in the PHED group offspring rats compared to the control group offspring rats \( (p < 0.05 \text{ or } p < \) 

**Figure 1.** Changes of antioxidant enzymes expression levels in the offspring rat liver. The results are presented as: \( A) \) MDA, \( B) \) GSH, \( C) \) SOD, \( D) \) CAT, \( E) \) GSH-Px and \( F) \) GST. *\( p < 0.05 \) and **\( p < 0.01 \) represent significant difference compared to the control group rats at \( p < 0.05 \) and \( p < 0.01 \), respectively.
Increased Level of Serum IL-1β and TNF-α Induced by PHED

Both TNF-α and IL-1β are pro-inflammatory factors. To evaluate the influence of PHED on inflammation in liver of offspring rats, the concentration of TNF-α and IL-1β in serum was measured through ELISA. The serum concentration of TNF-α (27.2%) in PHED group offspring rats was higher than that of the control group offspring rats, and the serum concentration of IL-1β (23.2%) in PHED group offspring rats was higher than that in the control group (Figure 2). In both cases, there was statistically significant difference between two groups ($p < 0.01$).

Influence of PHED in Offspring Rat Liver Cell Detected by HE Staining

The structure of liver tissue was examined by HE staining. The liver cells in control group offspring rats showed normal histopathological appearance, like well-aligned cell patterning and unchanged cellular structure, but in the PHED group offspring rats, the cell structure of the liver tissue showed some structure deformation and misalignment (Figure 3). There are some large and continuous gaps between cells, indicating the destructive influence of PHED on liver cells in offspring rats.

![Figure 2. Changes in the blood inflammatory factors in offspring rats. A) TNF-α; B) IL-1β. These indicated that the TNF-α and IL-1β in PHED group offspring rats are significantly higher than that in the control group rats. **Represent significant difference compared to the control group rats $p < 0.01$ level.](image)

![Figure 3. Histopathological changes in the offspring rat liver. The left is the control group (HE, x100), which showed normal cell structure. The right is the PHED group (HE, x100), which showed some deformation in cell structure.](image)
offspring rats.

**Increased Level of HO-1 mRNA in Offspring Rat Liver Induced by PHED**

As the most inducible antioxidant enzyme in liver, HO-1 mRNA level was measured in offspring rat liver tissue by RT-PCR. The mRNA level of HO-1 in PHED group offspring rats was increased very significantly compared to the mRNA level in the control group offspring rats (p < 0.01) (Figure 4). This suggests that PHED condition in rats will cause an increased HO-1 level in offspring liver.

**Enhanced Expression of COX-2, NF-κB and p-NF-κB in PHED Offspring Rats**

Over-expression of COX-2 will inhibit the activity of antioxidant enzymes and promote production of oxidants. Expression of COX-2 in rat liver tissue was measured by Western-blot method. The mRNA expression of COX-2 (around 72%) in PHED group offspring rats liver was significantly higher than that in the control group offspring rats liver (p < 0.01) (Figure 5). Additionally, the expression of both NF-κB and p-NF-κB in rat liver tissue was also measured by Western-blot method. The expression of p-NF-κB-p65 in PHED group offspring rats liver was 38% higher than that in the control group offspring rats liver (p < 0.01) (Figure 5). Also, expression of NF-κB-p65 in PHED group offspring rats was also significantly higher than that in the control group offspring rats (p < 0.05).

**Discussion**

We used diet-induced obesity pregnant rats to investigate the influence of palatable high-energy diet on oxidative stress of offspring liver. It was reported that obesity is closely connected to the induction of ROS. In the current research, the experiment results indicated the production of ROS in PHED group rat offspring was increased. Excessive amount of ROS will result in lipid peroxidation, for which MDA is an important indicator. We showed that MDA level was increased in the liver of PHED group rat offspring, which is consistent with the enhanced level of ROS. This will lead to imbalance between ROS and the antioxidants, eventually causing oxidative stress. The data presented here also showed that the enzymatic activities of many antioxidant enzymes including GST, SOD, CAT, and GSH-PX were decreased in HPED group rat offspring, and the GSH contents in offsprings’ liver were also decreased. CAT and SOD are well-known antioxidant enzymes, which play important functions in the elimination of ROS. GSH, as a non-enzymatic scavenger, has also been shown to be involved in the scavenging of ROS, and the dysfunction of GSH could aggravate the organ injury. Another enzyme, GSH-PX, was found to promote the reaction between GSH and H2O2 to achieve the purpose of eliminating peroxide. Therefore, the decreased activity of GSH-PX should be closely correlated to the GSH reduction. The above results clearly indicated that HPED in pregnant rats offspring can induce the production of ROS, and also inhibit mRNA expression of some antioxidant enzymes in the liver. The oxidative damage caused by oxidative stress will then promote the development of many related illnesses and diseases. HO-1 is one of the important proteins that play important roles in relieving oxidative stress and protecting the body against various inflammatory diseases. HO-1 can inhibit the expression of some pro-inflammatory mediators such as COX-2 and iNOS, which can reduce the production of PGE2 and NO. In this work, both HO-1 mRNA and COX-2 expression in PHED group offspring rats are more increased than the control group offspring rats (p < 0.01). During oxidative conditions, nuclear factor (erythroid-derived 2)-like 2 (Nrf2) will translocate to the nucleus, where it will bind to

![Image](image_url)
the element of antioxidant response and then regulate the mRNA level of some antioxidant genes such as HO-1. Therefore, the NF-κB and the Nrf2/HO-1 signaling pathways are two key targets for the anti-inflammatory response. The increased COX-2 expression as well as increased pro-inflammatory cytokines (TNF-α and IL-1β) induction, indicates the HPED in pregnant rat offspring may activate the Nrf2/HO-1 anti-inflammation pathways. In addition, NF-κB pathway activation is also highly related to ROS generation during obesity and inflammation. It was found that ROS could mediate inhibitor of NF-κB kinase phosphorylation and also release free NF-κB dimers. TNF-α, as a bona fide NF-κB activator, could mediate a redox-dependent activation of protein kinase A. Moreover, the DNA binding activity of the NF-κB p50 unit was found to be reduced when oxidized at Cys62. We also showed that p-NF-κB-p65 and NF-κB-p65 in PHED group offspring rats liver were significantly higher than that in the control group offspring rats liver. Taking together, we suggest that the palatable high-energy food can lead to excessive production of ROS in offspring rat liver, which causes oxidative stress during obesity and inflammation.

**Conclusions**

We showed that palatable high-energy food taken by obesity pregnant rats will lead to reduction in antioxidant function of offspring rat liver, further increasing the chance of chronic liver disease of offspring in their early ages. The mechanism is associated with the activity of NF-κB.
The influence of PHED in diet-induced obesity pregnant rats on offspring oxidative stress in liver

κB protein.

Acknowledgements
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Conflict of Interest
The Authors declare that they have no conflict of interests.

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