Respiratory Physiology & Neurobiology 207 (2015) 1–6

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Maternal high-fat diet feeding during pregnancy and lactation augments lung inflammation and remodeling in the offspring

Ye Song[a,b,d], Yang Yu[a,c,d], Dongjuan Wang[a,b,d], Shuiqin Chai[a,c,d], Dan Liu[a,d], Xiaoqiu Xiao[a,c,d,e,*], Ying Huang[b,d,**]

[a] Ministry of Education Key Laboratory of Child Development and Disorders, Children's Hospital, Chongqing Medical University, Chongqing 400014, China
[b] Department of Respiratory Medicine, Children's Hospital, Chongqing Medical University, Chongqing 400014, China
[c] Chongqing Key Laboratory of Translational Medical Research in Cognitive Development and Learning and Memory Disorders, Children's Hospital, Chongqing Medical University, Chongqing 400014, China
[d] Key Laboratory of Paediatrics in Chongqing, Children's Hospital, Chongqing Medical University, Chongqing 400014, China
[e] Chongqing International Science and Technology Cooperation Centre for Child Development and Disorders, Children's Hospital, Chongqing Medical University, Chongqing 400014, China

ARTICLE INFO

Article history:
Accepted 4 December 2014
Available online 11 December 2014

Keywords:
High-fat diet
Obesity
Inflammation
Fibrogenic factors
Lung remodeling

ABSTRACT

Accumulating evidence suggests that maternal obesity increases the risk of their offspring developing noncommunicable diseases later in life, but the potential mechanisms, especially those resulting in abnormal respiratory conditions, are not thoroughly understood. Here, we used maternal high-fat diet (HFD) feeding during premating, pregnancy, and lactation to investigate the effect of maternal HFD on offspring lung development. Offspring birth weight and body weight and composition were measured. Serum leptin levels were measured by ELISA. Hematoxylin–eosin (H&E) and Masson's staining were used in paraffin-embedded lung sections. Levels of transfer growth factor-β (TGF-β) and α-smooth muscle actin (α-SMA) were examined by immunohistochemistry and western blot, respectively. Maternal HFD feeding during pregnancy and lactation lead to higher birth weight, final body weight, fat accumulation and hyperleptinemia in offspring. Maternal HFD feeding aggravated lung inflammatory response in the offspring, resulting in inflammatory cell infiltration and collagen deposition potentially via the enhanced expression of TGF-β and α-SMA in the offspring.

1. Introduction

The global prevalence of overweight and obesity increased by 27.5% in adults and 47.1% in Children between 1980 and 2013 (Ng et al., 2014). A growing body of evidence suggests that obesity contributes to an increased risk for diseases later in life, such as cancer, heart disease, stroke, diabetes, and asthma, as well as increasing morbidity from these diseases (Mokdad et al., 2003; Shore, 2010). Overweight or obesity may result partly from excessive consumption of a high-fat diet (HFD) in conjunction with a sedentary lifestyle. Exposure to HFD is increasingly thought to be a principal cause of several diseases including diabetes, hypertension, and cancer (Innis, 2007). Maternal HFD exposure has already been extensively investigated in animal studies and found to be important in mediating susceptibility to overweight and obesity in offspring, and also in terms of the timing of this exposure during development (Gniuli et al., 2008; Lucas, 1998; Parente et al., 2008; Shi et al., 2007; Symonds et al., 2007). This is consistent with the notion of developmental origin of health and disease (Gillman, 2005), or the concept that a stimulus or insult during a critical period of development in early life causes deviations in normal growth and development.

Exposure to HFD may also play a role in chronic inflammatory disease development. Remodeling is a hallmark of a number of chronic lung inflammatory diseases, which are thought to be initiated and perpetuated by chronic inflammation (Broide, 2008; Ge et al., 2013; Maddox and Schwartz, 2002). Studies have revealed that HFD exposure can result in lung fibrosis development via

* Corresponding author at: Ministry of Education Key Laboratory of Child Development and Disorders, Children's Hospital, Chongqing Medical University, 136 Zhongshan Er Road, Yuzhong District, Chongqing 400014, China.
Tel.: +86 023 6363 7863; fax: +86 023 6363 3751.
** Corresponding author at: Department of Respiratory Medicine, Children's Hospital, Chongqing Medical University, Chongqing 400014, China.
Tel.: +86 023 6362 4074; fax: +86 023 6362 4074.
E-mail addresses: xiaoxq@cqmu.edu.cn (X. Xiao), yinghuangchcqm@163.com (Y. Huang).

http://dx.doi.org/10.1016/j.resp.2014.12.003
1569-9048/

the elevation of transfer growth factor-β (TGF-β) (Ge et al., 2013; Naura et al., 2009) and plasminogen activator inhibitor-1 levels (Ge et al., 2013). In addition, HFD increases the production of circulating inflammatory mediators, which may continuously slip through the interstitial spaces of vascular endothelial cells into the alveolar septum, thus leading to chronic inflammation and remodeling (Ge et al., 2013; Naura et al., 2009). It should be noted that lung remodeling in apolipoprotein-deficient (ApoE−/−) mice who are on a regular diet is similar to that seen in those who are on an HFD, and remodeling takes lesser time in the HFD group. In other words, ApoE−/− mice undergo lung remodeling while on a regular diet given adequate time. These results indicate that dietary and/or genetic effects on lipid metabolism might cause inflammation, concomitant remodeling, and contribute to lung pathologies, such as those associated with chronic obstructive pulmonary disease and asthma. Maternal HFD exposure can alter the development of numerous organs and thus predispose the offspring to disease in later life. The effect of maternal HFD exposure in utero and during lactation on the offspring has been mainly studied in terms of metabolic disorders rather than potential influence on respiratory disease and development. Accordingly, in this study, we aimed to investigate the effect and mechanism of maternal HFD exposure on lung inflammation and remodeling in offspring.

2. Materials and methods

2.1. Animals

This study was approved by the Ethical Principles in Animal Research Board at the Chongqing Medical University for Animal Experimentation. Maternal Sprague–Dawley (SD) rats were weaned at 3 weeks, housed under a 12 h light/dark cycle, and maintained on either a high-fat rodent diet (45% kcal from fat) (D12451, Research Diets, Inc., USA) or a standard rodent diet (10% kcal from fat) (D12450B, Research Diets, Inc., USA), and had free access to water until their offspring were weaned (Fig. 1). At 12-week old, maternal SD rats were crossed, and pregnant SD rats were maintained on an HFD or standard rodent diet and housed individually until delivery. At least 10 male SD rat offspring in both groups were maintained on a standard rodent diet and housed under a 12 h light/dark cycle for 3 months. Rats were euthanized by using 10% chloral hydrate (0.4 ml/100 g body weight) via intraperitoneal injection and sacrificed.

2.2. Body composition measurement

Body composition of SD-O (offspring from mother fed on standard diet) and HFD-O (offspring from mother fed on high fat diet) rats were measured with an EchoMRI 100 (Echo Medical Systems, Houston, TX, USA) as previously described (Liu et al., 2013). Briefly, unanesthetized rats were weighed first before they were put in a rat holder and inserted in MR analyzer. Reading of body fat mass and body lean mass were given within 1 min.

2.3. Enzyme-linked immunosorbent assay (ELISA) for leptin

The ELISA kit was provided by Uscn Life Science Inc. (SEA084Ra, WuHan, HuBei, China) and used according to manufacturer instructions. The ELISA kit specifications were follows: detection range 0.312–20 ng/mL, intra-assay variation <10%, inter-assay variation <12%.

2.4. Histological and immunohistochemistry evaluation

Paraffin-embedded peribronchial sections were deparaffinized, rehydrated through a graded alcohol series, cleared with dimethylbenzene, and stained with hematoxylin–eosin (H&E) and Masson's stain. Paraformaldehyde-fixed paraffin-embedded lung sections were deparaffinized, followed by 3 min of heat-induced antigen retrieval in a microwave in 6.0 pH citric acid buffer, repeated 3 times. Endogenous peroxidase activity inactivation was achieved with incubation in 3% H_2O_2 for 30 min at 37 °C. After 3 washes in phosphate-buffered saline (PBS), the slides were incubated with 10% bovine serum albumin for 1 h at room temperature. Following PBS washing, the sections were incubated overnight in a 1:200 dilution of primary polyclonal rabbit anti-α-smooth muscle actin (α-SMA) (ab5694, Hong Kong, China) and polyclonal rabbit anti-TGFβ antibody (18978-1-A, proteintech™, China). The sections were visualized using a Nikon Eclipse 80i with a Nikon Digital Camera DXM 1200. α-SMA/TGF-β expression was evaluated utilizing the Image-Pro Plus (Version 6.0, Media Cybernetics, USA).

2.5. Western blot analysis

Protein extracted from lung tissue was subjected to 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the separated proteins were transferred to polyvinylidene difluoride membranes. The following primary antibodies were used: polyclonal rabbit anti-α-SMA (ab5694, Hong Kong, China) and polyclonal rabbit anti-TGFβ antibody (18978-1-A, proteintech™, China). The membranes were incubated with rat anti-rabbit IgG, followed by horseradish peroxidase complex, and visualized using enhanced chemiluminescence reagents (Cat.# 170-5060, Bio-Rad, USA).

The densitometry of the bands was quantified with Image J (version 1.47, Wayne Rasband, USA) software. The densitometry of the bands was adjusted by subtracting the background densitometry readings. In this study, blots detected by antibodies against α-SMA/TGF-β were developed together for tissue lysates from each study subject.

2.6. Statistical analysis

Results were expressed as mean ± SEM. Statistical analysis was conducted with SPSS, version 19.0 (Sun Microsystems, USA). Differences in variables between the 2 groups were analyzed using the Student's *t*-test. A P value <0.05 was considered significant.

3. Results

3.1. Maternal HFD exposure in utero and during lactation could accelerate the offspring's birth weight and ultimate body weight

As shown, when compared with standard diet, maternal HFD before mating and during pregnancy could significantly increase birth weight (Fig. 2A) and maternal HFD during lactation further accelerate accumulation of fat (Fig. 2D) in offspring, which might lead to a higher final body weight (Fig. 2B). Also, maternal HFD during pregnancy and lactation could significantly enhanced the serum leptin levels (Fig. 2D).

3.2. Maternal HFD could enhance inflammatory cell accumulation in peribronchial and alveolar septa

H&E staining showed that maternal HFD during pregnancy and lactation could cause significant inflammatory cell infiltration into the perivascular, peribronchial, and alveolar septa (Fig. 3).

3.3. Maternal HFD could aggregate collagen deposition in offspring lung tissue

Masson's stain, used to observe the effect of maternal HFD exposure on the offspring's predisposition to lung remodeling, showed