European Journal of Obstetrics & Gynecology and Reproductive Biology 169 (2013) 39–44

Contents lists available at SciVerse ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

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

Maternal high-fat diets cause insulin resistance through inflammatory changes in fetal adipose tissue

N. Murabayashi [a], T. Sugiyama [b,*], L. Zhang [a], Y. Kamimoto [a], T. Umekawa [a], N. Ma [c], N. Sagawa [a]

[a] Department of Obstetrics and Gynaecology, Mie University Graduate School of Medicine, Mie, Japan
[b] Department of Obstetrics and Gynaecology, Tohoku University Graduate School of Medicine, Miyagi, Japan
[c] Faculty of Health Science, Suzuka University of Medical Science, Mie, Japan

ARTICLE INFO

Article history:
Received 30 July 2012
Received in revised form 10 November 2012
Accepted 5 February 2013

Keywords:
Offspring
Rodent
Adipose tissue
High-fat diet
Pregnancy

ABSTRACT

Objectives: Epidemiological and animal studies have shown that maternal obesity predisposes the offspring to obesity and the metabolic syndrome, possibly via late-onset metabolic programming of the fetus. Little is known, however, about the metabolic effect of maternal obesity on the fetus. This study investigated the effect of a maternal high-fat diet (HFD) on fetal growth and glucose metabolism using a diet-induced obesity mouse model.

Study design: Female mice (6 weeks old; C57BL/6N) were fed either a normal chow diet (NCD, 10 kcal% fat) or an HFD (60 kcal% fat) for 4 weeks before mating and throughout pregnancy. At 17 days of gestation, gene expression of inflammatory markers and adipokines in fetal subcutaneous adipose tissue was analyzed by quantitative real-time polymerase chain reaction.

Results: HFD mice were overweight, glucose intolerant and insulin resistant compared with NCD mice of the same gestational age. Although fetal body weight was not significantly different, fetal plasma glucose and insulin levels were higher in the HFD group than the NCD group. Furthermore, examination of fetal subcutaneous adipose tissue in the HFD group revealed hypertrophy with an increase in the levels of cluster of differentiation-68, chemokine receptor-2 and tumor necrosis factor-α mRNA, but a decrease in the level of glucose transporter-4 mRNA.

Conclusion: Maternal HFD causes inflammatory changes in the adipose tissue of offspring.

1. Introduction

Maternal obesity has an impact on intrauterine fetal life that extends to metabolic disorders in the offspring during infancy, adolescence and even adulthood [1,2]. Murine studies have shown that maternal diet-induced obesity leads to offspring adiposity, hypertension, and elevation of plasma insulin and glucose levels by 6 months [3]. Catalano et al. first demonstrated that maternal obesity causes fetal insulin resistance in humans [4], but the mechanism of action is not yet known. As adipose tissue is a key organ that is involved not only in both glucose and lipid metabolism but also insulin resistance [5], this study focused on adipose tissue in the offspring of dams fed a high-fat diet (HFD).

In addition to regulating systemic energy stores, adipocytes secrete many adipokines that have a major impact on energy homeostasis and insulin resistance [5]. In non-pregnant obese subjects, a chronic low-grade inflammatory state of adipose tissue has been implicated in the development of insulin resistance [6]. Obesity causes hypertrophy of adipocytes through the accumulation of triglycerides. Enlarged adipocytes release monocyte chemo-attractant protein 1 (MCP-1) [7], which activates blood monocytes through chemokine receptor 2 (CCR2) and induces monocyte migration to adipose tissue and differentiation into macrophages. CCR2 also plays a key role in monocyte/macrophage recruitment and the macrophage-dependent inflammatory response [8]. Macrophages in adipose tissue are activated in response to free fatty acids (FFA), whereby they are released from hypertrophied adipocytes, producing larger amounts of inflammatory factors such as tumor necrosis factor-α (TNF-α), interleukin 6, MCP-1 etc. [9]. The expression level of TNF-α mRNA in adipose tissue is significantly elevated in obesity and strongly correlated with the level of hyperinsulinaemia [10]. Subsequently, macrophages accumulate in the adipose tissue of obese animals as a likely direct response to the abnormal fat metabolism caused by the increased adiposity [11].

The authors previously found that hypertrophy from adipocyte tissue results in late pregnancy [12] and that such an inflammatory change occurs in late pregnancy [13]. These results lead to the

* Corresponding author at: Department of Obstetrics and Gynaecology, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi, Aoba-ku Sendai, Miyagi 980-8574, Japan. Tel.: +81 22 717 7254; fax: +81 22 717 7258.
E-mail address: sugiyama@med.tohoku.ac.jp (T. Sugiyama).

0301-2115/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ejogrb.2013.02.003

hypothesis that maternal obesity affects fetal insulin sensitivity through inflammatory changes and dysregulation of adipokines in fetal adipose tissue. Little is known, however, regarding the relationship between inflammatory changes in fetal adipose tissue and insulin resistance. This study therefore assessed the effect of maternal obesity produced by HFD-induced overeating on the expression of inflammatory markers and adipokines in the adipose tissue of offspring.

2. Materials and methods

2.1. Animals

This study was conducted in accordance with the principles and procedures outlined by the Ethics Committee for Animal Research of the Mie University Graduate School of Medicine. C57BL/6N mice were purchased from Charles River Japan, Inc. (Kanagawa, Japan). For 4 weeks, 6-week-old C57BL/6N female mice were randomly fed either a normal chow diet (NCD: 3.85 kcal/g, 10% fat, 20% protein, 70% carbohydrate, formula D12450B, Research Diets Inc., New Brunswick, NJ, USA) or an HFD (5.24 kcal/g, 60% fat, 20% protein, 20% carbohydrate, formula D12492, Research Diets Inc.). Mice were housed individually in a pathogen-free facility with a 12-h light, 12-h dark cycle, and were allowed free access to food and water. At 10 weeks of age, NCD and HFD C57BL/6N female mice were bred with C57BL/6N male mice for a single night. To improve the rate of successful mating, vaginal smears were checked before mating. The next day of mating was designated as Day 0 of gestation. All female mice remained on the same diet (NCD or HFD) throughout the entire experimental period. Body weight and food intake were monitored weekly from weeks 6 to 10, and also on days 0, 10 and 16 of gestation. Food intake was calculated by weight. On day 17 of gestation, after an overnight fast, dams were killed under pentobarbital anesthesia. The maternal abdomen was opened quickly, and the fetuses were removed from the uterus. Dams with fewer than eight fetuses or more than 10 fetuses were excluded from the study, as described previously [14]. Maternal blood was collected by cardiac puncture, and fetal blood was collected from each male fetus by cervical dislocation; both maternal and fetal blood samples were subsequently centrifuged and plasma was stored at −20 °C until analysis. Maternal subcutaneous adipose tissue, parametrial adipose tissue, and placenta and fetal abdominal subcutaneous adipose tissue were rapidly removed, weighed, frozen in liquid nitrogen, and stored at −80 °C. Some fetal subcutaneous tissue along with the attached skin was fixed as described previously [14].

2.2. Maternal glucose metabolism

Glucose tolerance and insulin sensitivity tests were performed by intraperitoneal administration of glucose (1 mg/kg body weight) or insulin (0.2 mU/g body weight) after an overnight fast on day 17 of gestation. The glucose and insulin tolerance tests were performed during the light phase. Blood samples were taken from the tip of the tail, and blood glucose concentration was determined using One Touch Ultra (Johnson & Johnson, Tokyo, Japan) [15].

2.3. Blood sample assays

Maternal and fetal plasma insulin were measured using an ultra-sensitive mouse insulin enzyme-linked immunosorbent assay (ELISA) kit (Morinaga Institute of Biological Science, Inc., Kanagawa, Japan) [16]. Maternal and fetal plasma triglyceride concentration and fetal plasma glucose concentration were determined using the Fuji DRI-CHEM system (Fuji Photo Film Co. Ltd., Tokyo, Japan) [15]. The litter was considered an experimental unit in the statistical analyses. Therefore, the fetal plasma was made up of all male fetuses' plasma from each litter. Maternal plasma FFA levels were determined using a NEFA C ELISA kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and maternal plasma TNF-α levels were determined using a mouse TNF-α ELISA kit (Shibayagi Co. Ltd, Gunma, Japan) [13]. The insulin intra-assay and interassay coefficients of variation (CV) were less than 20% and less than 10%, respectively. The TNF-α intra-assay and interassay CV were less than 10% and less than 15%, respectively. The plasma insulin, triglyceride, glucose, FFA and TNF-α assays were conducted according to the manufacturers' instructions.

2.4. Adipocyte size

A section of fetal subcutaneous abdominal adipose tissue was fixed in 4% formalin in phosphate-buffered saline, embedded in paraffin, and stained with hematoxylin and eosin. The fields of vision were selected at random, the diameter of each adipocyte in the field was measured manually, and the diameters of 100 adipocytes were measured microscopically by a single observer, as described previously [17].

2.5. Immunofluorescence staining

Immunofluorescence was performed to examine the localization of cluster of differentiation 68 (CD68) in subcutaneous adipose tissue of offspring. Paraffin-embedded sections were incubated with a goat polyclonal anti-CD68 antibody (Santa Cruz Biotechnology, Inc., Dallas, TX, USA) (1:400) overnight at 4°C. Next, the sections were incubated with fluorescent secondary antibody (Alexa 594-labeled donkey anti-goat IgG antibody, 1:400, Molecular Probes Inc., Eugene, Oregon, USA) for 3 h at room temperature. The sections were examined with a fluorescence microscope (BX53, Olympus, Tokyo, Japan).

2.6. RNA isolation and reverse transcriptase polymerase chain reaction

Two randomly selected fetuses were pooled from each dam, and total RNA from fetal subcutaneous abdominal adipose tissue was isolated using Sepasol-RNA1 Super according to the manufacturer's instructions (Nacalai Tesque, Kyoto, Japan). Reverse transcription of total RNA was conducted with TaqMan reverse transcription reagents and a TaqMan Gold reverse transcriptase polymerase chain reaction (RT-PCR) kit according to the manufacturer's instructions (Applied Biosystems, Foster City, CA).

2.7. Quantitative real-time polymerase chain reaction

RT-PCR products were analyzed by quantitative real-time PCR using TaqMan gene expression. Several target genes were assayed, including CD68 (Mm00839636_g1), MCP-1 (Mm00441242_m1), CCR2 (Mm99999051_gH), TNF-α (Mm00443258_m1) and glucose transporter-4 (GLUT-4) (Mm00436615_m1) (Applied Biosystems). The levels of gene expression were normalized to the gene expression of glyceraldehyde-3-phosphate dehydrogenase (4352932-0803020, Applied Biosystems). Gene expression was quantified as the second step of a two-step RT-PCR. Assays were completed in a 50-μl singleplex reaction mixture containing 25 μl TaqMan Universal PCR Master Mix, 2.5 μl 20 × TaqMan Gene Expression Assay Mix, 2 μl cDNA and 20.5 μl distilled water according to the manufacturer's instructions (Applied Biosystems). Reaction conditions consisted of pre-incubation at 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The C_T values were recorded automatically.