Maternal high-fat diet programs rat offspring hypertension and activates the adipose renin-angiotensin system

Cristiane Guberman, MD; Juanita K. Jellyman, PhD; Guang Han, MD; Michael G. Ross, MD; Mina Desai, PhD

OBJECTIVE: A maternal high-fat diet creates an increased risk of offspring obesity and systemic hypertension. Although the renal renin-angiotensin system (RAS) is known to regulate blood pressure, it is now recognized that the RAS is also activated in adipose tissue during obesity. We hypothesized that programmed offspring hypertension is associated with the activation of the adipose tissue RAS in the offspring of obese rat dams.

STUDY DESIGN: At 3 weeks of age, female rats were weaned to a high-fat diet (60% k/cal; n = 6) or control diet (10% k/cal; n = 6). At 11 weeks of age, these rats were mated and continued on their respective diets during pregnancy. After birth, at 1 day of age, subcutaneous adipose tissue was collected; litter size was standardized, and pups were cross-fostered to either control or high-fat diet dams, which created 4 study groups. At 21 days of age, offspring were weaned to control or high-fat diet. At 6 months of age, body fat and blood pressure were measured. Thereafter, subcutaneous and retroperitoneal adipose tissue was harvested from male offspring. Protein expression of adipose tissue RAS components were determined by Western blotting.

RESULTS: The maternal high-fat diet induced early and persistent alterations in offspring adipose RAS components. These changes were dependent on the period of exposure to the maternal high-fat diet, were adipose tissue specific (subcutaneous and retroperitoneal), and were exacerbated by a postnatal high-fat diet. Maternal high-fat diet increased adiposity and blood pressure in offspring, regardless of the period of exposure.

CONCLUSION: These findings suggest that programmed adiposity and the activation of the adipose tissue RAS are associated with hypertension in offspring of obese dams.

Key words: angiotensinogen, lactation, pregnancy, rat, subcutaneous and retroperitoneal adipose tissue

Cite this article as: Guberman C, Jellyman JK, Han G, et al. Maternal high-fat diet programs rat offspring hypertension and activates the adipose renin-angiotensin system. Am J Obstet Gynecol 2013;209:262.e1-8.

Obesity and its associated health problems represent a worldwide epidemic.[1] Maternal obesity has been shown to increase the risk of offspring obesity and its related diseases, such as heart disease, stroke, and hypertension.[2-4] Hence, as the prevalence of obesity among pregnant women continues to rise, an increasing number of children are exposed to an "obese intrauterine environment" during development. It is clear that there is a strong correlation between the incidence of hypertension and obesity, more specifically visceral and abdominal obesity.[5] According to data from the Framingham Cohort, obesity by itself accounts for 78% and 65% of essential hypertension in men and women, respectively.[6] Although the mechanisms underlying these associations are not elucidated fully, adipose tissue clearly is a critical factor in the development of obesity-hypertension.

The renin-angiotensin system (RAS) is a classic endocrine system that is involved in blood pressure homeostasis. Angiotensinogen, the precursor of the bioactive peptide angiotensin II (Ang II), is synthesized mainly in the liver and cleaved enzymatically in the circulation by renin to angiotensin I and subsequently by the angiotensin-converting enzyme (ACE) to Ang II. The major vasopressor effects of Ang II are mediated through its receptor type 1 (AT1) whereas its interaction with receptor type 2 (AT2) modulates cell proliferation and renal sodium excretion.[7] In addition to the classic pathway of Ang II synthesis, adipose tissue has the ability to synthesize Ang II independently of the circulating RAS.[8] Recent observations indicate that all components of the RAS are expressed in white adipose tissue from rodents[9-11] and humans,[12-14] which suggests that the adipogenic RAS may be involved in the pathogenesis of obesity-related hypertension. Accordingly, studies have shown that adipose-derived angiotensinogen can contribute to approximately 20% of plasma angiotensinogen concentrations and can modulate blood pressure.[7] Angiotensinogen-knockout mice are hypotensive with hypotrophic adipocytes and adipose tissue–specific angiotensinogen gene expression in angiotensinogen-knockout mice limits angiotensinogen expression to the

From Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance (all authors), and Perinatal Research Laboratories, Department of Obstetrics and Gynecology, David Geffen School of Medicine, University of California, Los Angeles (Drs Ross and Desai), CA.

Received Feb. 25, 2013; revised April 30, 2013; accepted May 10, 2013.

Supported by the National Institutes of Health grant numbers R01DK081756 and R01HD054751.

The authors report no conflict of interest.

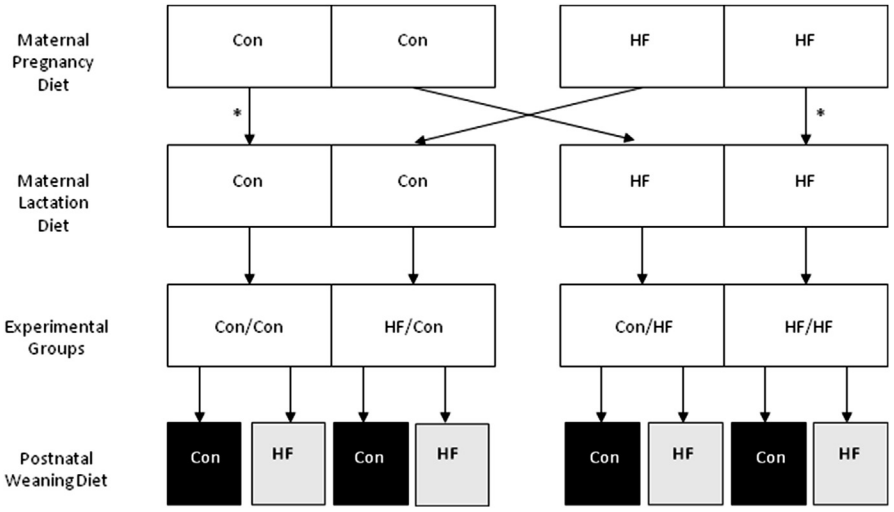
Presented at the 33rd annual meeting of the Society for Maternal-Fetal Medicine, San Francisco, CA, Feb. 11-16, 2013.

Reprints: Mina Desai, PhD, Department of Obstetrics and Gynecology, LABioMed at Harbor-UCLA Medical Center, 1124 West Carson St, Box 446, RB-1 Bldg, Torrance, CA 90502. mdesai@obgyn.humc.edu.

0002-9378/$36.00

http://dx.doi.org/10.1016/j.ajog.2013.05.023

FIGURE 1
Study design



Offspring from each experimental group were weaned onto either a control (*Con*) or high-fat (*HF*) postweaning diet. Experimental groups are (1) maternal control diet during pregnancy and lactation (*Con/Con*), (2) maternal high-fat diet during pregnancy and lactation (*HF/HF*), (3) maternal high-fat diet during pregnancy alone (*HF/Con*), and (4) maternal high-fat diet during lactation alone (*Con/HF*). To control for the cross-fostering effects, control and high-fat diet pups were cross-fostered similarly among dams of the same group (*asterisk*).

Guberman. Maternal high-fat diet programs offspring adipose RAS. Am J Obstet Gynecol 2013.

adipose tissue but results in systemic concentrations of angiotensinogen levels that are 20% of wild-type levels, which shows that angiotensinogen that is produced in adipocytes can enter the circulation.[15] Furthermore, overexpression of adipose angiotensinogen in mice induces hypertension with increased body fat and plasma angiotensinogen levels, which indicates that an increased adipose tissue mass may result in higher circulating angiotensinogen levels, a finding that has been confirmed in obese individuals.[15]

Increasing evidence suggests that adult cardiovascular and metabolic disorders can be "programmed" in utero[16] and in the early postnatal period.[17-19] Notably, both maternal under- or overnutrition results in offspring obesity and hypertension.[18,20,21] Despite this convincing evidence, there are no studies that have investigated the regulation of the adipogenic RAS in programmed, obesity-mediated hypertension. We hypothesized that the adipose tissue RAS is activated in the obese, hypertensive offspring that were exposed to maternal high-fat diet during pregnancy and/or lactation. We determined the protein expression of adipose RAS components in newborn rats and adult offspring that were exposed to maternal high-fat diet during pregnancy and/or lactation. We further investigated the changes in visceral and nonvisceral adipose tissue and the additive impact of a high-fat, postweaning diet on the adipose RAS.

Materials and Methods

A rat model of maternal obesity was created with a high-fat diet before mating and throughout pregnancy and lactation. Studies were approved by the Animal Care and Use Committee of the Los Angeles Biomedical Research Institute at Harbor—University of California, Los Angeles, and were in accordance with the American Association for Accreditation of Laboratory Care and National Institutes of Health guidelines. Sprague Dawley rats (Charles River Laboratories, Hollister, CA) were housed in a facility with constant temperature and humidity and a controlled 12-/12-hour light/dark cycle. Weanling female rats were fed a high-fat diet (60% k/cal fat, 20% k/cal protein, 20% k/cal carbohydrate; D12492; Research Diets Inc, New Brunswick, NJ; n = 6) or normal-fat control diet (10% k/cal fat, 20% k/cal protein, 70% k/cal carbohydrate; n = 6). At 11 weeks of age, rats were mated and continued on their respective diets during pregnancy and lactation.

Offspring

At birth, pups were culled to 8 per litter (4 male and 4 female) to normalize rearing and were cross-fostered, thereby generating 4 paradigms of maternal diets during pregnancy/lactation (Figure 1). To control for the cross-fostering effects, the control diet and high-fat diet pups similarly were cross-fostered among dams of the same group (Figure 1). At 3 weeks of age, offspring in each of the 4 groups were housed individually and weaned. To examine the effects of a high-fat diet, 1 male from each litter was weaned randomly to a normal-fat diet, and 1 male was weaned to a high-fat diet. Thus, there were 4 maternal feeding paradigms during pregnancy/lactation (control diet or high-fat diet) and 2 offspring feeding paradigms (control diet or high-fat diet) that were examined for male offspring (Figure 1). We elected to study males because females would have required estrus assessment, and estrogen is known to affect adiposity and lipid metabolism.[22]

Body weight and composition

Male offspring were weighed at 1 day and at 6 months of age. In addition, at 6 months of age, a noninvasive dual-energy X-ray absorptiometry scan was performed with the DXA system with a software program for small animals (6 males from 6 litters per group and postweaning diet; QDR 4500A; Hologic, Bedford, MA). An in vivo scan of whole body composition was obtained that allowed determination of the percentage of body fat.

Blood pressure

Measurements were undertaken in conscious animals with the use of noninvasive tail-cuff sphygmomanometry