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Original Research

Maternal high fructose and low protein consumption during pregnancy and lactation share some but not all effects on early-life growth and metabolic programming of rat offspring

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ARTICLE INFO

Article history:
Received 8 March 2016
Revised 20 June 2016
Accepted 23 June 2016

Keywords:
Rat
Fetal programming
Fructose
Low protein
Liver

ABSTRACT

Maternal nutritional stress during pregnancy acts to program offspring metabolism. We hypothesized that the nutritional stress caused by maternal fructose or low protein intake during pregnancy would program the offspring to develop metabolic aberrations that would be exacerbated by a diet rich in fructose or fat during adult life. The objective of this study was to characterize and compare the fetal programming effects of maternal fructose with the established programming model of a low-protein diet on offspring. Male offspring from Sprague-Dawley dams fed a 60% starch control diet, a 60% fructose diet, or a low-protein diet throughout pregnancy and lactation were weaned onto either a 60% starch control diet, 60% fructose diet, or a 30% fat diet for 15 weeks. Offspring from low-protein and fructose-fed dam showed retarded growth ($P < .05$) at weaning (50.3, 29.6 vs 59.1 ± 0.8 g) and at 18 weeks of age (420, 369 vs 464 ± 10.9 g). At 18 weeks of age, offspring from fructose dams expressed greater quantities ($P < .05$) of intestinal Pgc1a messenger RNA compared with offspring from control or low-protein dams (1.31 vs 0.89, 0.85; confidence interval, 0.78-1.04). Similarly, maternal fructose ($P = .09$) and low-protein ($P < .05$) consumption increased expression of Pgc1a in offspring liver (7.24, 2.22 vs 1.22; confidence interval, 2.11-3.45). These data indicate that maternal fructose feeding is a programming model that shares some features of maternal protein restriction such as retarded growth, but is unique in programming of selected hepatic and intestinal transcripts.

Abbreviations: AUC, area under the curve; BMI, body mass index; GTT, glucose tolerance test; HOMA-IR, homeostatis model assessment of insulin resistance; m, maternal background; Mat, main effects of maternal diet; mCT, 60% starch control diet; mFR, 60% fructose diet; mLP, low-protein diet; Pck1, phosphoenolpyruvate carboxykinase–cytosolic form; Pgc1a, peroxisome proliferator–activated receptor γ coactivater-1α; Ppara, peroxisome proliferator–activated receptor α; Pwn, postweaning nutrition; PCR, polymerase chain reaction; RT, reverse transcribed; w, postweaning; wCT, 60% starch control diet; wFAT, 30% fat diet; wFR, 60% fructose diet.

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http://dx.doi.org/10.1016/j.nutres.2016.06.014
0271-5317/

1. Introduction

An adverse early environment created by poor maternal nutrition programs offspring for increased risk of impaired glucose tolerance, insulin resistance, and dyslipidemia [1,2]. Maternal environments characterized by restricted food intake or restricted protein intake are well-established models of metabolic programming and predispose offspring to developing disturbances in insulin sensitivity [3,4] and increase risks for developing obesity [5,6], especially when challenged with a high-energy diet after weaning [3,7]. However, the connection between excessive postnatal energy intake as carbohydrate, such as fructose, and development of the metabolic disease is controversial [8,9].

Maternal low protein is the best characterized fetal programming model [10]. Maternal low protein causes intrauterine growth restriction as evidenced by reduced birth weight in low-protein offspring compared with control offspring [3,5,11]. Adult offspring of dams maintained on a low-protein diet during pregnancy exhibit impaired insulin-stimulated glucose uptake in muscle [12], increased relative fat mass, hyperglycemia, hypercholesterolemia, hyperleptinemia, and altered adipose tissue function [5]. Although low maternal protein intake has been extensively studied, knowledge of the impact and timing of maternal nutritional stress and the interaction with the postnatal environment is still incomplete.

Fructose consumption among adults in the United States has risen dramatically in the last 20 years [13-15]. Epidemiologic evidence and controlled laboratory studies have identified fructose as a significant dietary contributor to the current epidemic of obesity and type 2 diabetes [16]. Dietary fructose has been linked to symptoms of metabolic syndrome including dyslipidemia [17], impaired glucose homeostasis [15], insulin resistance [18,19], and hepatic fat accumulation [20]. Few studies have examined the role of gestational diabetes and postnatal diet in predisposing offspring to metabolic disease.

We previously established that fructose consumption during pregnancy and lactation causes hepatic steatosis and gestational glucose intolerance, mimicking gestational diabetes [21], and wanted to determine the effects on the offspring when compared with a maternal control or low-protein diet. We hypothesized that the metabolic stress associated with fructose or low protein intake during pregnancy would present an in utero challenge for the fetus and predispose them to the metabolic aberrations in adult life. We further hypothesized that postnatal diets rich in fructose or fat would exacerbate these preconditions. Therefore, the objective of this study was to characterize and compare the fetal programming effects of maternal fructose with the established programming model of a low-protein diet during pregnancy and lactation on offspring. In addition, we were interested in determining the role of postnatal high fat or high fructose consumption in offspring predisposed to higher risk for metabolic disease. We compared the consequences of fructose feeding and protein restriction during pregnancy and lactation in male offspring by measuring parameters that are established targets of programming including growth, serum lipid, and glucose profiles, as well as hepatic and intestinal abundance of transcripts involved in energy regulation.

2. Methods and materials

2.1. Feeding and management of dams

Six-week-old virgin Sprague-Dawley rats (Harlan Tekland, Indianapolis, IN, USA) were received within 3 days of confirmed mating and housed individually in standard polycarbonate rat cages (Ancare, Bellmore, NY, USA) containing wood shavings at a constant temperature (25°C), 40% to 50% relative humidity, and a 12-hour light/12-hour dark cycle. Dams were permitted ad libitum access to water and diet. Dams were fed either a control diet (mCT; n = 9), a diet containing 63% fructose (mFR; n = 6), or a diet containing 8% protein (mLP; n = 9) for the entire gestation and suckling phases (Table 1). Originally, 11 dams were assigned to each of the maternal diet groups; however, because of unsuccessful pregnancy, the total numbers of dams for each treatment are stated above. All procedures involving dams and offspring were approved by the Purdue Animal Care and Use Committee of Purdue University.

2.2. Feeding and management of offspring

Within 24 hours of parturition, all pups within the maternal diet group were pooled to a standardized litter size of 10 pups per dam. At 21 days of age, male pups were weighed and nose to anus length was recorded. Pups were randomized to either a control diet (wCT), a high-fructose diet (wFR), or a 30% fat diet (wFAT; Table 1). Diets were mixed from individual ingredients and fed as a powder in glass jars with metal lids. This resulted in a 3 by 3 factorial arrangement of 3 offspring diets (wCT, wFAT, wFR) nested within 3 maternal diet (mCT, mFR, mLP), totaling 9 treatment groups. The treatment groups are designated by maternal background (m) postweaning (w) diet: mCTwCT, mCTwFAT, mCTwFR, mFRwCT, mFRwFAT, mFRwFR, mLPwCT, mLPwFAT, and mLPwFR. The general experimental design is depicted in Fig. 1.

Weaned male offspring were housed individually in stainless steel wire bottom cages at a constant temperature (25°C), 40% to 50% relative humidity, and a 12-hour light/12-hour dark cycle. Food consumption was determined 3 times weekly by the difference in food offered and food remaining with an adjustment for any spillage. Offspring were weighed weekly. A measure of body mass index (BMI) at weaning (21 days) and harvest (18 weeks) was calculated using body weight (g) divided by the square of nose to anus distance (cm^2). Efficiency of growth was calculated as the difference between body weight at 18 weeks of age (g) and the body weight at weaning (g) divided by cumulative food intake during that interval of time.

2.3. Glucose tolerance test

A glucose tolerance test (GTT) was performed on the offspring at 17 weeks of age. Rats were fasted for 12 hours, and baseline samples (time = 0) of whole blood were taken from the tail vein. Glucose (200 g/L) in water was administered by intraperitoneal injection at a final dose of 2 g/kg body weight. At 10, 20, 30, 60, and 120 minutes after glucose load [22], blood samples were collected and glucose was measured using a handheld glucometer (ACCU-CHEK Advantage; Roche, Indianapolis, IN,