Available online at www.sciencedirect.com
ScienceDirect
Nutrition Research 30 (2010) 410–417
Nutrition Research
www.nrjournal.com

Maternal protein restriction during pregnancy and lactation in rats imprints long-term reduction in hepatic lipid content selectively in the male offspring

Rani J. Qasem[a], Ganesh Cherala[b], Anil P. D'mello[a,*]

[a]Department of Pharmaceutical Sciences, University of the Sciences in Philadelphia, Philadelphia, PA 19104, USA
[b]Department of Pharmacy Practice, College of Pharmacy, Oregon State University & Oregon Health and Science University, Portland, OR 97239, USA

Received 27 January 2010; revised 7 May 2010; accepted 26 May 2010

Abstract

Maternal protein restriction during pregnancy and lactation reduces whole body lipid stores and alters lipid homeostasis in the adult offspring. Lipid homeostasis in the body is regulated, in part, by the liver via the metabolic processes of synthesis and utilization of lipids. The present study tested the hypothesis that maternal protein restriction will imprint changes in hepatic lipid metabolism and thereby alter the hepatic lipid content of the adult offspring. Pregnant rats were fed purified diets containing 19% protein (control group) or 8% protein (low-protein group) throughout pregnancy and lactation. On day 28, pups from both groups were weaned onto regular laboratory chow. On days 65 and 150, male and female pups from each litter in both groups were killed and blood and liver collected. Maternal protein restriction was found to reduce birth weight and produce long-term reduction in the body weight of the offspring. On day 65, liver triglyceride content was decreased by 40% in the male offspring that were fed a low-protein diet. The reduction in liver triglyceride content persisted until day 150, at which time it was accompanied by decreases in hepatic cholesterol content. No such changes were observed in the female offspring. To determine if the alterations in liver lipid content resulted in compensatory changes in liver carbohydrate stores, hepatic glycogen content was measured in male offspring. Hepatic glycogen content was similar between the 2 groups on days 65 and 150. In conclusion, the present study in rats showed that maternal protein restriction during pregnancy and lactation imprints long-term changes in hepatic lipid content selectively in the male offspring.

Keywords: Rats; Diet, protein restricted; Prenatal exposure delayed effects; Triglycerides; Lipids; Glycogen; Ketone bodies; Cholesterol

Abbreviations: ANOVA, analysis of variance; VLDL, very low density lipoprotein.

1. Introduction

Epidemiologic studies have shown an association between low birth weight and increased susceptibility to developing one or more components of the metabolic syndrome during adulthood [1,2]. To explore this finding in more mechanistic detail, a variety of animal models of intrauterine growth restriction have been developed. The most commonly used model involves exposure of rats to low-protein diets during pregnancy and lactation. Maternal protein restriction during pregnancy and lactation reduces birth weight of the pups and produces long-term reduction in the body weight of the offspring [3-5]. A review of the literature reveals that this model has been predominantly used to demonstrate the effect of a restricted maternal nutritional milieu and the resultant fetal growth retardation on altering glucose tolerance and insulin resistance in the offspring [6-8].

* Corresponding author. Tel.: +1 215 596 8941.
E-mail address: a.dmello@usp.edu (A.P. D'mello).

0271-5317/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.nutres.2010.05.008

Recent studies have examined the effects of maternal low-protein diets on lipid metabolism in the offspring of rats. Maternal low-protein diets during pregnancy and lactation in rats alter whole body lipid homeostasis in the offspring as evidenced by lower plasma triglyceride and cholesterol levels [4,9] and decreased whole body lipid content [4] in the adult offspring. An important organ involved in the regulation of whole body lipid homeostasis is the liver [10]. The liver participates in lipid homeostasis by synthesizing lipids in the absorptive state and by using lipids via oxidation or export to peripheral tissues in the postabsorptive state. Disturbances in whole body lipid homeostasis in low-protein offspring could result, in part, from the imbalance between hepatic lipid synthesis and utilization. Insulin is the primary regulator of hepatic lipogenesis via its actions on sterol regulatory element binding protein 1c, a transcription factor that induces the expression of a number of lipogenic genes [11,12]. Low protein offspring exhibit lower plasma levels of insulin that can decrease hepatic lipid synthesis and account for the lower plasma triglyceride levels in these animals [4,13,14]. Interestingly, low-protein offspring exhibit an increased activity of carnitine palmitoyltransferase 1, a key rate-limiting enzyme in fatty acid oxidation [15]. The anticipated increase in hepatic fatty acid oxidation and consequent increased utilization of lipids could account, in part, for the decrease in the whole body lipid content in these rats.

We hypothesized that decreased hepatic lipid synthesis coupled with increased hepatic lipid oxidation is partially responsible for the altered lipid homeostasis in adult low-protein offspring and the imbalance will be reflected in reduced hepatic lipid content in these animals. Therefore, the primary objective of the present study was to determine the effect of maternal protein restriction during pregnancy and lactation on hepatic lipid content in the adult offspring. The next objective was to assess the status of lipid synthesis and utilization in low-protein offspring by measurement of plasma concentrations of select lipids, their biosynthetic precursors, and products of lipid oxidation. Furthermore, it is known that alterations in lipid metabolism in organs and tissues often provoke compensatory responses in carbohydrate metabolism [16,17]. These responses are reflected in perturbations in a number of biochemical parameters including organ/tissue glycogen content. Therefore, the final objective of the present study was to determine if putative changes in hepatic lipid content induced by maternal protein restriction are accompanied by changes in hepatic glycogen content in the adult offspring.

2. Methods and materials

2.1. Animals and experimental design

The study was approved by the Institutional Animal Care and Use Committee of the University of the Sciences in Philadelphia. Ten- to twelve-week-old, approximately 275-g virgin female Sprague-Dawley rats were mated by housing 1 male with 2 females. Day 1 of pregnancy was confirmed by the appearance of sperm in the daily morning vaginal smear. Pregnant rats were randomly assigned to be fed a modified version of the AIN 76 A purified diet (control group, n = 7) containing 19% protein or its corresponding low-protein formulation AIN M76 A (low-protein group, n = 8) containing 8% protein. The diets are isoenergetic and their detailed compositions are described in Table 1. Pregnant rats were fed the diets throughout pregnancy and lactation. At birth, pups were weighed and sexed and at 72 hours post-birth, litters were randomly culled to 8 pups (4 males and 4 females) to ensure a standard litter size for each dam. On day 28 post-birth, offspring were weaned onto normal laboratory chow and subsequently kept on this diet for the entire duration of the study. It is important to note that the different dietary treatments were only confined to the gestation and lactation period. Body weight of the pups was periodically measured throughout the duration of the study. Morning fed-state blood samples (150 μL) were collected from the offspring by tail snip on days 65 and 150 post-birth, and plasma separated and stored at −20°C. On days 65 and 150 post-birth, one male and one female offspring from each litter in both groups were killed in the fed state by decapitation and livers dissected, blotted, weighed, and snap frozen in liquid nitrogen and stored at −80°C. Body weights of the remaining male and female offspring from each litter in the 2 groups were measured until day 180 when the study was terminated.

2.2. Measurement of plasma triglycerides, ketone bodies, and free fatty acids

Plasma triglycerides, β-hydroxybutyrate, and free fatty acids were assayed using commercial kits available from

Table 1
Composition (grams per kilogram) of the control and low-protein diets

	Control	Low protein
Casein—vitamin-free	210	88
Dextrinized cornstarch	436	434
Sucrose	150	274
Lard	50	50
Corn oil	50	50
Powdered cellulose	30	30
RP vitamin mix no. 10[a]	20	20
RP mineral mix no. 10[b]	50	50
DL-methionine	1.5	0.63
Choline chloride	2	2
Gross energy (kJ/g)	17.07	17.28

[a] Provided per kilogram of diet: thiamin 20 mg, riboflavin 20 mg, pyridoxine 20 mg, nicotinic acid 90 mg, D-calcium pantothenate 60 mg, folic acid 4 mg, biotin 0.4 mg, cyanocobalamin 20 μg, retinyl acetate 22000IU, tocopheryl acetate 50 IU, cholecalciferol 2200 IU, menadione sodium bisulfite complex 20 mg.

[b] Provided per kilogram of diet: calcium 6 g, phosphorus 4 g, sodium 2.1 g, potassium 4 g, magnesium 0.69 g, manganese 65 mg, iron 60 mg, copper 15 mg, zinc 20 mg, iodine 0.6 mg, selenium 0.2 mg, chromium 3 mg, chloride 2.4 g, sulfate 1.2 g, cobalt 3.2 g, fluoride 5 mg, molybdenum 0.8 g.