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RESEARCH ARTICLES

Postnatal overfeeding promotes early onset and exaggeration of high-fat diet-induced nonalcoholic fatty liver disease through disordered hepatic lipid metabolism in rats☆,☆☆

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Abstract

Exposure to overnutrition in critical or sensitive developmental periods may increase the risk of developing obesity and metabolic syndrome in adults. Nonalcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome, but the relationship among postnatal nutrition, lipid metabolism, and NAFLD progression during development remains poorly understood. Here we investigated in a rat model whether postnatal overfeeding increases susceptibility to NAFLD in response to a high-fat diet. Litters from Sprague-Dawley dams were culled to three (small litters) or ten (normal litters) pups and then weaned onto a standard or high-fat diet at postnatal day 21 to generate normal-litter, small-litter, normal-litter/high-fat, and small-litter/high-fat groups. At age 16 weeks, the small-litter and both high-fat groups showed obesity, dyslipidemia, and insulin resistance. Hepatic disorders appeared earlier in the small-litter/high-fat rats with greater liver mass gain and higher hepatic triglycerides and steatosis score versus normal-litter/high-fat rats. Hepatic acetyl-CoA carboxylase activity and mRNA expression were increased in small-litter rats and aggravated in small-litter/high-fat rats but not in normal-litter/high-fat rats. The high expression in small-litter/high-fat rats coincided with high sterol regulatory element-binding protein-1c mRNA and protein expression. However, mRNA expression of enzymes involved in hepatic fatty acid oxidation (carnitine palmitoyltransferase 1) and output (microsomal triglyceride transfer protein) was decreased under a high-fat diet regardless of litter size. In conclusion, overfeeding related to small-litter rearing during lactation contributes to the NAFLD phenotype when combined with a high-fat diet, possibly through up-regulated hepatic lipogenesis.

Keywords: Early overfeeding; Liver; High-fat diet; Lipid metabolism; Nonalcoholic fatty liver disease; Rat

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is characterized by excessive triglyceride (TG) accumulation in the absence of "significant" alcohol consumption [1] and is closely associated with metabolic dysregulation such as obesity, diabetes, hyperlipidemia, and cardiovascular disease. NAFLD is considered the hepatic manifestation of metabolic syndrome [2]. The prevalence of NAFLD ranges from 20–30% in the general population and 75–100% in obese adults [3,4], as well as having a range of 10–50% of obese children [5]. Although NAFLD is a "benign" condition, it may progress to nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and hepatocellular carcinoma and is associated with a significantly shorter survival [6]. Therefore, understanding the mechanisms underlying an increased susceptibility to NAFLD from obesity is crucial to developing strategies to prevent metabolic syndrome and NAFLD, especially in childhood.

Obesity is influenced by the interaction of genes, nutrition, and lifestyle. Additionally, increasing evidence from clinical and animal experimental studies shows that the way an individual grows and development early in life directly affects features of the metabolic syndrome in later life [5,7]. In particular, weight gain in the first years of life is important in programming body mass index in young adults [8], and the nutritional environment is another important factor. Maternal [9–11] and postnatal infant overfeeding [12,13] could increase the risk of obesity and associated metabolic disturbance and NAFLD in adulthood. In animal research, rats are ordinarily reared in litters of 8-12 pups (normal litter). Quantitative changes in the food

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supply of neonatal rats can be produced by altering litter size at birth, so that large and small litters are obtained [14]. It is usually expected that more milk would be available to the individual pups when litter size is decreased below that normally delivered, mainly by a high-TG maternal milk [15,16], thereby imposing a state of persistent postnatal overfeeding upon the suckling animals. Clearly, postnatal overfeeding using small litter (SL) rearing (3 pups) and a post-weaning high-fat (HF) diet in rats induces early onset and/or more pronounced obesity, insulin resistance, and lipid disorders at puberty [17]. However, the biochemical and molecular mechanisms underlying this increased susceptibility to metabolic disorders from postnatal overfeeding is unknown.

NAFLD is primarily a result of inappropriate fat storage; any mechanism leading to "ectopic" fat accumulation must involve persistent alterations in lipid metabolism [11]. Hepatic lipid metabolism includes circulating lipid uptake, de novo lipogenesis, fatty acid oxidation, and TG-rich lipoprotein secretion. Hepatic steatosis occurs when there is an imbalance in which lipid availability (uptake and synthesis) exceeds lipid disposal (oxidation and export) [18]. Enzymes regulating lipid metabolism are key in these processes. Hepatic lipoprotein lipase (LPL) and liver-type fatty acid-binding protein (L-FABP) are considered to play a central role in hepatic lipid uptake [18–20]. Acetyl-CoA carboxylase (ACC) is the rate-limiting enzyme involved in de novo lipogenesis [21], and carnitine palmitoyltransferase-1 (CPT1) and microsomal TG transfer protein (MTP) are the rate-limiting enzymes involved in fatty acid oxidation and export, respectively [22,23]. During energy overconsumption, LPL, L-FABP, and ACC mRNA expression in liver increase [24,25], but CPT1 and MTP decrease [26]. These alterations contribute to a hepatic lipid input (ACC, L-FABP, and LPL) that exceeds its output (CPT1 and MTP), favoring the occurrence of NAFLD [27–29]. Moreover, the activities of these enzyme systems are regulated by transcriptional factors, such as sterol regulatory element binding protein-1c (SREBP-1c) and peroxisome proliferator-activated receptor α (PPARα) [30,31]. In NAFLD, hepatic SREBP-1c and ACC mRNA expression is increased [24,32] whereas PPARα and CPT1 expression is decreased [33].

In addition, accumulating evidence indicates that exposure to malnutrition in the critical or sensitive periods of development may "program" the long-term or life-time structure or function of the organism [34]. Maternal protein restriction in pregnant rats reduces hepatocyte numbers [35], hepatic lipogenesis [10], and PPARα DNA methylation in the offspring [36]. A maternal HF diet up-regulates hepatic lipid intake (LPL) [37] and lipogenesis (ACC) [11], decreases β-oxidation (CPT1), and impairs hepatic mitochondrial metabolism in adult offspring [38]. Taken together, these observations strongly suggest that early malnutrition is linked to a derangement in liver development and function. In a previous study, SL rearing induced increased 11β-hydroxysteroid dehydrogenase type 1 activity and thus enhanced glucocorticoid action in peripheral tissue during puberty [17], which contributed to obesity and insulin resistance in adults. However, the effects of SL rearing on the lipid metabolic pathway in liver and possible association with NAFLD development have not been determined.

Our aim was to study the influence of neonatal overfeeding induced by SL rearing and a post-suckling HF diet on key enzymes and transcriptional factors involved in hepatic liver lipid metabolism. We also examined whether postnatal overfeeding increases susceptibility to NAFLD in response to a HF diet.

2. Methods and materials

2.1. Animals

All studies were approved by the University Committee on Use and Care of Animals and overseen by the Unit for Laboratory Animal Medicine at Nanjing Medical University (ID: 2008031801). Sprague–Dawley rats (Nanjing, Jiangsu, China) were maintained under controlled light (0600–1800 h) and temperature (22±2°C) conditions with free access to tap water.

2.2. Experimental design

The experimental setup was similar to that described in Boullu-Ciocca et al. [39]. Briefly, female rats were time-mated, and at postnatal day 3 (P3), male pups were redistributed to litter sizes of three (small litters, SL) or ten (normal litters, NL) to induce early postnatal overfeeding or normal feeding respectively [40,41]. At P21, rats were weaned to be fed either a standard laboratory diet or HF diet (Slac, Shanghai, China, Table 1) until postnatal week 16 (W16). Four groups were analyzed, namely NL, SL, NL-HF, and SL-HF. There were 24-36 rats in each group and 120 rats in the total experiment. All animals were housed three per cage, and body weight and food intakes were monitored throughout life. The animals were killed at W3, W6, W10, and W16 between 0830 and 1000 h after fasting overnight (12 h). Rats were anesthetized with chloral hydrate (300 mg/kg body weight, i.p.), and blood samples were obtained from the right ventricle. The blood was centrifuged (2000×*g*, 4°C, 15 min) and the separated serum stored at −70°C for subsequent determination of biochemical parameters. Liver, epididymal, and retroperitoneal fat pads were dissected out and weighed. All tissues were snap-frozen in liquid nitrogen and kept at −70°C until gene expression analysis.

2.3. Serum biochemistry

Total TG, total cholesterol (TC), alanine aminotransferase (ALT), and aspartic acid transaminase (AST) in the serum were measured using an Olympus AU400 analyzer with enzymatic reagents (Olympus America, New York, NY, USA).

2.4. Intraperitoneal glucose tolerance test

The intraperitoneal glucose tolerance test (IPGTT) was performed as described previously [42]. Briefly, at W3, W6, W10, and W16, rats were fasted overnight. A blood sample was then taken from a tail vein and the rats injected i.p. with 2.0 g D-glucose (50% stock solution in saline)/kg body weight. Blood samples were drawn at 30-, 60-, and 120-min intervals after the glucose injection, and glucose levels were measured by a glucose meter (Accu-Chek; Roche).

2.5. Hepatic lipid assays

Concentrations of TG in the liver were determined using tissue TG assay kits (Applygen, Beijing, China). Hepatic TG concentration was expressed relative to 1 g of liver protein. Hepatic protein concentrations were determined using a Pierce BCA protein assay kit with bovine serum albumin as the standard (Thermo Fisher Scientific, Rockford, IL, USA).

2.6. Hepatosomatic index

The hepatosomatic index was determined according to Wotton et al. [43] and calculated as follows: hepatosomatic index=(liver weight/body weight) * 100%.

2.7. Liver histological analyses

Portions of the left and right lobes were either flash frozen in isopentane (Oil Red O) or fixed in buffered formalin [hematoxylin and eosin (H&E)] for histological analyses.

2.8. NAFLD Activity Score (NAS)

The NAS is used to assess the severity of NAFLD, according to Kleiner et al. [44]. An activity score was generated by adding the individual scores for the following features: steatosis (<5%=0; 5–33%=1; 33–66%=2; >66%=3); lobular inflammation (none=0; <2 foci=1; 2–4 foci=2; >4 foci=3); and ballooning (none=0; few=1; prominent=2). A score of less than 3 correlates with mild nonalcoholic fatty liver, a score of 3–4

Table 1
Purified diet formula and composition [weight (%)].

	Standard diet (%)	High fat diet (%)
Casein	18.92	18.92
L-Cystine	0.28	0.28
Maltodextrin	3.32	3.32
Corn starch	48.34	39.34
Sucrose	13.00	13.00
Cellulose	4.74	4.74
Soybean oil	6.00	6.00
Lard	-	9.00
Mineral mix	4.26	4.26
Vitamin mix	1.14	1.14
Total	100.00	100.00
Energy (kcal/100 g)	392.60	438.24