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ScienceDirect

Journal of Nutritional Biochemistry 26 (2015) 433–440

Journal of Nutritional Biochemistry

Early-life exposure to high-fat diet may predispose rats to gender-specific hepatic fat accumulation by programming *Pepck* expression

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Received 8 April 2014; received in revised form 28 September 2014; accepted 16 October 2014

Abstract

Phosphoenolpyruvate carboxykinase (PEPCK) produces phosphoenolpyruvate during glyceroneogenesis. We previously demonstrated that a high-fat diet during pregnancy induced *Pepck* mRNA expression in neonatal rat pups, which is characterized by histone modifications in specific regions of the gene (Strakovsky RS, Zhang X, Zhou D, Pan YX. Gestational high fat diet programs hepatic phosphoenolpyruvate carboxykinase gene expression and histone modification in neonatal offspring rats. *The Journal of Physiology* 2011;589:2707–17). In the present study, we investigated whether these alterations persistent in adult offspring. Dams were fed either control or high-fat diet throughout gestation and lactation. Offspring were placed on control diet after weaning, generating C/C and HF/C groups. Liver was collected at 12 weeks of age. Hepatic nicotinamide adenine dinucleotide (reduced) (NADH) level was increased in both genders, but fat accumulation occurred only in liver of female offspring in HF/C group. This was accompanied by a significant increase of *Pepck* and fatty acid synthase (*Fasn*) mRNA expression in only female liver. The induction of *Pepck* gene expression in females was associated with increased dimethylated histone H3 lysine 4 level in multiple regions of the gene. Meanwhile, acetylated histone H3 and trimethylated histone H3 lysine 4 were induced at a specific coding region in HF/C, accompanied by decreased trimethylated histone H3 lysine 9 level at the promoter of female offspring. In conclusion, maternal high-fat diet programs *Pepck* expression through histone modifications in adult female offspring. Persistent *Pepck* induction in females may contribute to increased triglyceride synthesis, together with induced *Fasn* expression and NADH levels, which may lead to increased fat deposition in a gender-specific manner.
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Keywords: Epigenetic modification; Maternal programming; PEPCK; Glyceroneogenesis; Fatty liver

1. Introduction

Fetal growth and postnatal development indispensably rely on perinatal nutrition. Dietary disturbances during the period of gestation and lactation program the offspring to have long-term physiological and pathological outcomes [1,2]. As the Western diet has become more and more prevalent and accessible in the past decades, cross-generational health issues of consuming this diet have been raised and brought to public attention. In order to unravel the potential health risks and mechanisms underlying this phenomenon, animal studies utilizing a high-fat dietary treatment have been extensively conducted, revealing a multitude of biological consequences for the offspring. Thus far, numerous health outcomes in the offspring have been characterized in the maternal high-fat model, including behavior changes [3], thyroid dysfunction [4] and more frequent incidence of obesity [4,5]. Importantly, many of these observations were made in a gender-dependent manner [4,6]. Indeed, it was recently shown that only 10% of the gene expression that was altered by a maternal high-fat diet overlapped in male and female pups, highlighting the sex-specific nature of the response to early-life nutrition [7].

PEPCK controls two critical signaling pathways regulating both glucose and fatty acid metabolism. The function of PEPCK is well defined in mammalian liver, kidney and adipose tissue [8]. It is activated only after birth and catalyzes the irreversible step of oxaloacetate decarboxylation to produce phosphoenolpyruvate [9], which is an essential substrate for both gluconeogenesis and glyceroneogenesis pathways. It can either be converted to glucose during carbohydrate deprivation and/or under stress or be detoured into glycerol-3-phostate synthesis to integrate with nonesterified fatty acids in support of triglyceride (TAG)/fatty acid cycle [10,11].

Abbreviations: Pol II, RNA polymerase II; H3Ac, acetylated histone H3; H4Ac, acetylated histone H4; H3K9Me3, trimethylated histone 3 at lysine 9 residues; H3K27Me3, trimethylated histone 3 at lysine 27 residues; H3K36Me3, trimethylated histone 3 at lysine 36 residues; H3K4Me3, trimethylated histone 3 at lysine 4 residues; H3K4Me2, dimethylated histone 3 at lysine 4 residues; HF, high fat; PCR, polymerase chain reaction; ChIP, chromatin immunoprecipitation; TAG, triglyceride; NADH, nicotinamide adenine dinucleotide (reduced).

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http://dx.doi.org/10.1016/j.jnutbio.2014.10.009
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Hence, as an essential enzyme for all aspects of energy metabolism, expression of PEPCK should be tightly controlled under all circumstances.

The transcription of PEPCK responds to both hormonal signals and nutritional stimulus, and is thus the primary target of regulation. A handful of transcription factors binding at the upstream of PEPCK have been revealed — such as cAMP regulatory element binding protein (CREB), CAAT/enhancer binding protein (C/EBP) family and sterol regulatory element binding protein-1c (SREBP-1c) — that respond to amino acid availability, insulin signals or fatty acids [12]. The coactivator proteins peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1) is also known to be a key activator of PEPCK through interacting with the CREB element at the promoter regulatory region [13]. More recently, the importance of epigenetic modifications is gradually becoming recognized and addressed due to their intricate but distinctive role in regulating gene expression. Multiple lines of evidences have shown that patterns of histone modifications, such as that of trimethylated histone 3 at lysine 27 residues (H3K27Me3) and acetylated histone H3 (H3Ac), are highly associated with PEPCK gene transcription [14,15]. Histone modifications also reciprocally interact with transcription factors [16], the combination of which determines PEPCK gene expression levels.

Our previous publication reported that *in utero* exposure to a high-fat diet during early development induced hepatic PEPCK gene expression in neonates through epigenetic modifications [14]. In the current study, we investigated the long-term effects of maternal high-fat diet on gene expression and epigenetic programming of *Pepck* as well as its associated pathophysiological outcomes. We report for the first time that PEPCK-mediated glyceroneogenesis may greatly contribute to the fat accumulation in the liver of female offspring in this model.

2. Methods and materials

2.1. Animal and diet

Timed-pregnant Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA, USA) about 10–12 weeks old (nondiabetic) were separated into two dietary groups, either control (16% fat) or high fat (45% fat) throughout gestation and lactation [17]. Rats were kept individually in standard polycarbonate cages in a humidity- and temperature-controlled room on a 12-h light–dark cycle, with *ad libitum* access to food and drinking water. Body weight and food intake were recorded every 3 days during gestation. Offspring body weight and liter size were recorded right after delivery. Twenty-four hours after birth, both groups of dams were randomly assigned 10 pups (5 male and 5 female coming from the same gestational diet) until weaning at day 21. After weaning, pups from both groups were housed individually and maintained on a control diet generating C/C and HF/C groups until they were sacrificed at 12 weeks of age. The left lobe of liver was snap-frozen in liquid nitrogen and stored at −70°C for future use. We certify that all applicable institutional and governmental regulations regarding the ethical use of animals were followed during this research (University of Illinois Institutional Animal Care and Use Committee approval no. 09112).

2.2. RNA isolation and two-step real time quantitative polymerase chain reaction

Total RNA was isolated using TRI reagent (Sigma, St. Louis, MO, USA). Reverse transcription was performed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The mRNA mixed with reverse transcriptase was incubated in the 2720 Thermal Cycler (Applied Biosystems) under the procedure of 25°C for 10 min, 37°C for 2 h and 85°C for 5 s. A serially diluted standard curve and all cDNA samples were amplified using SYBR Green (Quanta Biosciences, Inc.) in a 7300 Real-Time PCR System (Applied Biosystems, ABI) at 95°C for 10 min, followed by 35 cycles of 95°C for 15 s and 60°C for 1 min. A dissociation curve was included to check the purity of the polymerase chain reaction (PCR) product. Primers used for quantitative PCR are shown in Table 1.

2.3. Protein extraction and Western blotting

Liver tissues were lysed with 1× Laemmli Buffer [62.5 mmol/L Tris–HCl, pH 6.8, 2% sodium dodecyl sulfate (SDS), 10% glycerol v/v, 0.01% bromophenol blue, 5% 2-mercaptoethanol, 1× protease inhibitors (Roche, Indianapolis, IN, USA) and 1× phosphatase inhibitors (Sigma-Aldrich)]. For longer storage, the protein samples were placed in −70°C. Lowry assay was performed to determine protein concentration. Samples containing 30 μg of protein were resolved by 10% SDS polyacrylamide gel electrophoresis. A wet transfer protocol was performed to complete the transfer. A polyvinylidene difluoride membrane (0.2 μm; Bio-Rad, Hercules, CA, USA) was blocked in 10% milk in TBS/T (30 mM Tris base, pH 7.6, 200 mM NaCl and 0.1% Tween 20) for 1 h at room temperature. The membranes

Table 1
Primer sequences used in real-time PCR analysis of genes.

Gene name (Ensembl ID #)	Sequences
L7a mRNA	Forward 5'-GAGGCCAAAAAGGTGGTCAATCC-3'
(ENSRNOT00000006754)	Reverse 5'-CCTGCCCAATGCCGAAGTTCT-3'
Actin mRNA	Forward 5'-GAGACCTTCAACACCCCAGC-3'
(ENSRNOT00000042459)	Reverse 5'-CAGTGGTACGACCAGAGGCA-3'
PEPCK mRNA	Forward 5'-AGGAGGAAGAAAGGTGGCACCAG-3'
(ENSRNOT00000031586)	Reverse 5'-GGCAGAGAAGTCCAGACCATTATGC-3'
PEPCK pre-mRNA	Forward 5'-CGAACGCCATTAAGACCATCCAG-3'
(ENSRNOT00000031586)	Reverse 5'-CCAGTAAACACCCCCATCACTTGTC-3'
G6Pase mRNA	Forward 5'-CTCCAGCATGTACCGCAAGA-3'
(ENSRNOE00000433052)	Reverse 5'-AACGGAATGGGAGCGACTT-3'
FBP1 mRNA	Forward 5'-GATCCCCTCGATGGCTCAT-3'
(ENSRNOE00000169019)	Reverse 5'-CCAAAGATGGTTCCGATGGA-3'
PFK-1 mRNA	Forward 5'-CCACCATCAGCAACAATGTC-3'
(ENSRNOT00000001625)	Reverse 5'-TTGATGCGGTCACAACTCTC-3'
ACC1 mRNA	Forward 5'-GGTGATAATGAACGGCTCGTGTGT-3'
(ENSRNOE00000344921)	Reverse 5'-TGTAACTGCTGCCATCGTAGGACA-3'
FASN mRNA	Forward 5'-CTTTGTGAGCCTCACCGCCAT-3'
(ENSRNOG00000045636)	Reverse 5'-ATGCCATCAGGTTTCAGCCCC-3'
DGAT1 mRNA	Forward 5'-TCAATCTGTGGTGCCGCCAG-3'
(ENSRNOT00000039795)	Reverse 5'-CCCACTGACCTTCTTCCCTGCA-3'
GPAM mRNA	Forward 5'-AACTGAGACGAATCTGCCG-3'
(ENSRNOE00000145287)	Reverse 5'-AGGATGAAGGTGAGCAGCA-3'
AGPAT6 mRNA	Forward 5'-TCTGCCACTCAGGATTGCTC-3'
(ENSRNOT00000024392)	Reverse 5'-GCAGGTATCCAACCACGGTA-3'
C/EBPα mRNA	Forward 5'-AGTCGGTGGATAAGAACAGCAACG-3'
(ENSRNOE00000102880)	Reverse 5'-GCTGTTTGGCTTTATCTCGGCTC-3'
C/EBPβ mRNA	Forward 5'-AGAACGAGCGGCTGCAGAAGA-3'
(ENSRNOE00000218848)	Reverse 5'-GAACAAGTTCCGCAGCGTGC-3'
SREBP1-c mRNA	Forward 5'-GCTTCTCTGGGCTCCTCTCTGG-3'
(ENSRNOE00000310938)	Reverse 5'-CAGTGGGTGCCGATGGTGG-3'
PGC1-α mRNA	Forward 5'-TGGCGTCATTCAGGAGCTGG-3'
(ENSRNOE00000042081)	Reverse 5'-CAACCAGGGCAGCACACTCTATG-3'
CPT1-α mRNA	Forward 5'-GAGCGACTCTTCAATACTTCCC-3'
(ENSRNOT00000019652)	Reverse 5'-TGTGCCTGCTGTCCTTGATA-3'
LDHD mRNA	Forward 5'-AATACCCACCTGCGTAACAGC-3'
(ENSRNOT00000044844)	Reverse 5'–TGCCACACAGAGAAGCATCTG-3'
PEPCK −4 kb	Forward 5'-AGATGCCTGGACTCAACCTACC-3'
(ENSRNOT00000031586)	Reverse 5'-GCTACCCTACCGAGTGACCAAT-3'
PEPCK −2 kb	Forward 5'-TCTCCTCCTCCATCATTGGTCTG-3'
(ENSRNOT00000031586)	Reverse 5'-CATCCAAGGTCTCTCTGCTGTGA-3'
PEPCK −1 kb	Forward 5'-ACTCCAGTGAGCAACTTCTC-3'
(ENSRNOT00000031586)	Reverse 5'-AGCAGCGAGACCTTTATC-3'
PEPCK promoter	Forward 5'-TGTTAGGTCAGTTCCAAACCGTGC-3'
(ENSRNOT00000031586)	Reverse 5'-TGGACCTGGAGGCTCGCC-3'
PEPCK +1 kb	Forward 5'-GTGACTTTTTCAGTTGGCTG-3'
(ENSRNOT00000031586)	Reverse 5'-TGCTCTTGGGTAATGATGAC-3'
PEPCK +3 kb	Forward 5'-ACTGATCCCTGTCCCTTCAGATCC-3'
(ENSRNOT00000031586)	Reverse 5'-GCTGCCAGGTATTTCTTCTTGCC-3'
PEPCK +5 kb	Forward 5'-ATTCTGGGAGAAGGAGGTGGA-3'
(ENSRNOT00000031586)	Reverse 5'-TATTTCGTAAGGGAGGTCGGC-3'
PEPCK +7 kb	Forward 5'-TCTCAGTGTGCCTTAGTGTG-3'
(ENSRNOT00000031586)	Reverse 5'-TTACAGCCAGGGAAGTTG-3'

Table 2
List of antibodies.

Antibody name		Catalog number	Source
Name	Modification		
PEPCK	H-300	Sc-32879	Santa Cruz
H3Ac	Ac-K9, 14	06-599	Millipore
H4Ac	Ac-K5, 8, 12, 16	06-866	Millipore
H3K4Me2	M-K4	07-030	Millipore
H3K4Me3	M-K4	07-473	Millipore
H3K9Me3	M-K9	07-442	Millipore
H3K27Me3	M-K27	07-449	Millipore
H3K36Me3	M-K36	Ab9050	Abcam
IgG	N/A	sc-2027	Santa Cruz
Pol II	N/A	05-623	Millipore

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