BBA - Molecular and Cell Biology of Lipids 1863 (2018) 1488–1497

Contents lists available at ScienceDirect

BBA - Molecular and Cell Biology of Lipids

journal homepage: www.elsevier.com/locate/bbalip

Maternal n-3 PUFA supplementation promotes fetal brown adipose tissue development through epigenetic modifications in C57BL/6 mice

Rong Fan[a], Ashley Mulcahy Toney[a], Yura Jang[b], Seung-Hyun Ro[b], Soonkyu Chung[a,*]

[a] Department of Nutrition and Health Sciences, University of Nebraska, Lincoln, NE 68583, USA
[b] Department of Biochemistry, University of Nebraska, Lincoln, NE 68583, USA

ARTICLE INFO

Keywords:
BAT
N-3 PUFA
Fish oil
Thermogenesis
Maternal nutrition

ABSTRACT

Brown adipose tissue (BAT) is a crucial regulator of energy expenditure. Emerging evidence suggests that n-3 PUFA potentiate brown adipogenesis in vitro. Since the pregnancy and lactation is a critical time for brown fat formation, we hypothesized that maternal supplementation of n-3 PUFA promotes BAT development in offspring. Female C57BL/6 mice were fed a diet containing n-3 PUFA (3%) derived from fish oil (FO), or an isocaloric diet devoid of n-3 PUFA (Cont) during pregnancy and lactation. Maternal n-3 PUFA intake was delivered to the BAT of neonates significantly reducing the n-6/n-3 ratio. The maternal n-3 PUFA exposure was linked with upregulated brown-specific gene and protein profiles and the functional cluster of brown-specific miRNAs. In addition, maternal n-3 PUFA induced histone modifications in the BAT evidenced by 1) increased epigenetic signature of brown adipogenesis, i.e., H3K27Ac and H3K9me2, 2) modified chromatin-remodeling enzymes, and 3) enriched the H3K27Ac in the promoter region of *Ucp1*. The offspring received maternal n-3 PUFA nutrition exhibited a significant increase in whole-body energy expenditure and better maintenance of core body temperature against acute cold treatment. Collectively, our results suggest that maternal n-3 PUFA supplementation potentiates fetal BAT development via the synergistic action of miRNA production and histone modifications, which may confer long-lasting metabolic benefits to offspring.

1. Introduction

Brown adipose tissue (BAT) is a specialized fat that dissipates excess energy into heat (non-shivering thermogenesis) through mitochondrial uncoupling protein 1 (UCP1) [1]. Current research renews the metabolic function of BAT by revealing BAT as a crucial regulator in maintaining energy balance by increasing thermogenic energy expenditure. A significant amount of BAT is found in healthy adults as well as most children and adolescents [2–4], but not in the obese adults, suggesting that loss of active BAT depots is a contributing factor to obesity. Childhood obesity predisposes adults with metabolic susceptibility to obesity and type 2 diabetes (T2D) [5–7]. Therefore, identifying early regulatory factors to prevent childhood obesity is critical to combat the current obesity epidemic. The fetal and neonatal stages are critical for fetal BAT development, which are expected to have long-term impacts on offspring BAT function [8]. However, a limited amount of studies have been conducted regarding the effects of maternal nutrition on prenatal BAT development. Despite the well-established physiological relevance of BAT in obesity outcome, it is unclear whether the amount of BAT at birth or the rate of BAT loss (either by degeneration or by transdifferentiation into WAT) are associated with susceptibility to obesity in later life. In particular, it is poorly understood whether prenatal BAT development through dietary intervention could be a manageable target to attenuate the risk for childhood obesity.

Human BAT depots are found in the deeper neck (cervical), supraclavicular, paravertebral, perirenal, and axillary areas [9–11] and possesses 50-times greater respiratory activities than white adipose

Abbreviations: ARA, (arachidonic acid); Cidea, (cell death-inducing DNA fragmentation factor α-like effector A); DHA, (docosahexaenoic acids); EHMT1, (euchromatic histone N-lysine methyltransferase 1); EPA, (eicosapentaenoic acids); eWAT, (epididymal white adipose tissue); FO, (fish oil); GPR120, (G-protein-coupled receptor 120); HF, (high fat); iBAT, (interscapular brown adipose tissue); iWAT, (inguinal white adipose tissue); Jmjd1, (JmjC domain-containing protein 1); Jmjd3, (JmjC domain-containing protein 3); miRNAs, (microRNAs); Prdm16, (PR domain containing 16); n-3 PUFA, (omega-3 polyunsaturated fatty acids); *Pgc1-α*, (peroxisome proliferator-activated receptor gamma coactivator 1-alpha); PTM, (post-translational modifications); sWAT, (subcutaneous white adipose tissue); UCP1, (uncoupling protein 1)

* Corresponding author at: Department of Nutrition and Health Sciences, University of Nebraska-Lincoln, 110 Ruth Leverton Hall, Lincoln, NE 68588, USA.
E-mail address: schung4@unl.edu (S. Chung).

https://doi.org/10.1016/j.bbalip.2018.09.008
Received 19 June 2018; Received in revised form 24 August 2018; Accepted 23 September 2018
Available online 25 September 2018
1388-1981/

tissue (WAT) [12]. These BAT depots are comprised of 1.5% of total body mass (roughly 5% of total fat mass), and up to 90% depots could be activated BAT [13]. An image-guided mapping of rodent adipose depots reveals the topological analogy of BAT between rodents and humans [14], which provides the feasibility to use rodent in studying human BAT. Also, the comparable functional analysis demonstrated that human supraclavicular BAT features functional similarity with rodent interscapular BAT regarding mitochondrial activity and thermogenic potential [12]. Regarding the timeline for BAT development, late pregnancy (at the last trimester) is the critical time for human BAT formation [15,16]. The human brown adipocytes in the interscapular are rapidly lost after birth via either degeneration and replacement with white adipocyte or transdifferentiation into white adipocytes. However, brown adipocytes reside in the deeper neck and supraclavicular regions, remain active to adolescence and adulthood until they lost thermogenic potential with the progression of obesity, type 2 diabetes, or aging [6]. Given these developmental similarities, the regulation of interscapular BAT in rodents during pregnancy seems to be translatable to BAT around the neck and supraclavicular in humans.

BAT development is modulated by epigenetic modifications that are heritable and reversible changes in gene expression occur without altering DNA sequences through DNA methylation, chromatin histone remodeling, and noncoding RNAs such as miRNAs [17–19]. Nearly a dozen miRNAs have been identified in promoting the transcriptional program of brown adipogenesis [20]. On the other hand, the site-specific acetylation (Ac) and methylation (Me) status on the lysine (K) residues of histone tails, especially at H3K9 and H3K27, play essential roles in adipogenesis by controlling gene activation or repression [21,22]. Maternal nutrition is a key epigenetic modulator for fetus development. However, it is largely unknown whether the BAT epigenome is a viable target for obesity control through maternal nutrition.

Accumulating evidence has supported that n-3 PUFA promotes brown adipogenesis and adaptive thermogenesis [23–27]. We have previously demonstrated that eicosapentaenoic acids (EPA) promote the brown adipogenic program through a miRNA-dependent epigenetic mechanism in the murine primary brown adipocytes [28], suggesting that maternal n-3 PUFA nutrition may be effective in reinforcing embryonic BAT developmental program during pregnancy. This study aimed to investigate the impact of early n-3 PUFA exposure through maternal nutrition on the fetal BAT development. Here, we demonstrate that maternal n-3 PUFA intake during pregnancy and lactation enhanced brown transcriptional programming through miRNA and histone modification-mediated epigenetic regulations. These results open a new research avenue emphasizing that 'boosting prenatal BAT development' could be a novel therapeutic target for attenuating childhood obesity through thermogenic energy expenditure.

2. Material and methods

2.1. Animals

All animal experiments were conducted according to the protocols approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Nebraska-Lincoln. C57BL/6 male and female mice were purchased at 8–10 weeks of age from the Jackson Laboratory. After 3 days of acclimatization period, mice were put into the breeding colony. Female mice were randomly assigned to two diet groups receiving either a diet containing 3% of n-3 PUFA from the fish oil (FO) or a diet devoid of n-3 PUFA (Cont). The AIN-93G rodent formulation was modified for the fat composition containing 15% of total calorie from fat either 10% palm oil (Cont) or fish oil (FO), the other 5% of total calorie from soybean oil as a source of essential FA. Gas Chromatography (GC) was used to analyze fatty acid profiles of each diet and dietary composition in Supplement Table 1. The same diets were maintained throughout gestation and lactation. The pregnancy of the female mice last for 19–21 days, and the pups (n = 16 each group) were weaned (3 weeks postpartum). Necropsy was conducted at weaning, to collect blood, liver, interscapular BAT (iBAT), and inguinal (iWAT) and epididymal WAT (eWAT). Tissue samples were snap-frozen in liquid nitrogen and kept at −80 °C for further analysis.

2.2. Energy expenditure by metabolic cages

To measure the effect of maternal n-3 PUFA intake on energy expenditure, pups (n = 5 each group) were individually placed into the metabolic cage (TSE systems) for six days (two days of adaptation and four days of measurement). Indirect oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were used to calculate metabolic parameters. Energy expenditure (EE) and respiratory exchange rate (RER = VCO_2/VO_2) were calculated and obtained from the TSE systems software and plotted into a figure with hourly time point.

2.3. Cold exposure, measurement of rectal temperature and heat release

To measure the long-term effect of maternal n-3 PUFA supplementation on the offspring's thermogenic potential, male pups from both maternal fish oil or control diet (n = 6 per group) were switched to a standard AIN-93G diet (no additional n-3 PUFA). At week 11, mice were exposed to cold temperature (6 °C) acutely (1 − 3 h) or for 24 h (See Fig. 5A study design). To measure the core body temperature, a rectal thermometer (Kent Scientific Corp) was used. The probe was positioned into the anal ducts of the mice and three readings of each time point were recorded. Infrared (IR) camera (A655sc, FLIR Systems) was used to detect thermal release and to capture images of the surface body temperature. FLIR Research IR program software was used to display surface heat release via color palette representing temperatures between 22 and 34 °C.

2.4. Gas Chromatography (GC) for fatty acid analysis

To determine FA profiles in the red blood cells, whole blood was collected, and red blood cells were precipitated by centrifugation (6000 × g for 15 min). A 200 μl of the packed volume of red blood cells were transferred to a fresh glass vial and total lipids were extracted. They were then subjected to FA methylation by 14% boron trifluoride (BF_3)-methanol reagent (Sigma, USA) at 100 °C for 1 h to form fatty acid methyl ester (FAME). Agilent Technologies HP-88 column (100 m × 0.25 mm × 0.2 μm film thickness) was used. The individual FA peak was identified by comparing its relative retention times with the commercial mixed-FA standard (NuCheck PreP), and the area percentages for all resolved peaks were analyzed using the GC Chemstation software.

2.5. Blood chemistry

To measure plasma glucose, insulin, and cAMP levels, immunoassays were conducted by using mouse glucose assay (Crystal Chem), ultra-sensitive mouse insulin ELISA kit (Crystal Chem), and mouse cAMP parameter assay kit (R&D Systems), respectively, in accordance to the manufacturer's protocol.

2.6. qPCR of mRNA and microRNA analysis

Total RNA was extracted using Trizol® reagent (Invitrogen) from homogenized tissues. RNA was purified using DNase treatment & removal kit (Invitrogen), and 2 μg of RNA was converted into cDNA (iScript, BioRad) via reverse transcription. Relative gene expressions were determined based on the $2^{-\Delta\Delta CT}$ method with normalization of the raw Ct value to 18 s. For miRNA analysis, miRNA was converted to cDNA using the miScript reverse transcription kit (Qiagen) according to the manufacturer's instructions. MiRNA was measured by using the