BBA - Molecular and Cell Biology of Lipids 1863 (2018) 126–131

Contents lists available at ScienceDirect

BBA - Molecular and Cell Biology of Lipids

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

Levels of palmitic acid ester of hydroxystearic acid (PAHSA) are reduced in the breast milk of obese mothers

Marie Brezinova[a,1], Ondrej Kuda[a,*,1], Jana Hansikova[a], Martina Rombaldova[a,b], Laurence Balas[c], Kristina Bardova[a], Thierry Durand[c], Martin Rossmeisl[a], Marcela Cerna[d], Zbynek Stranak[d], Jan Kopecky[a]

[a] Department of Adipose Tissue Biology, Institute of Physiology of the Czech Academy of Sciences, Videnska 1083, 142 20 Praha 4, Czech Republic
[b] Department of Analytical Chemistry, Faculty of Science, Charles University in Prague, Czech Republic
[c] Institut des Biomolécules Max Mousseron (IBMM), UMR 5247, CNRS, Université Montpellier, ENSCM, Faculté de Pharmacie, Montpellier Cedex 5, France
[d] Institute for the Care of the Mother and Child, Prague, Czech Republic

ARTICLE INFO

Keywords:
Breast milk
PAHSA
Chiral separation
Obesity
Lipogenesis
FAHFA

ABSTRACT

To achieve optimal development of a newborn, breastfeeding is extensively recommended, but little is known about the role of non-nutritive bioactive milk components. We aimed to characterize the fatty acid esters of hydroxy fatty acids (FAHFAs), namely palmitic acid hydroxystearic acids (PAHSAs)—endogenous lipids with anti-inflammatory and anti-diabetic properties, in human breast milk.

Breast milk samples from 30 lean (BMI = 19–23) and 23 obese (BMI > 30) women were collected 72 h postpartum. Adipose tissue and milk samples were harvested from C57BL/6J mice. FAHFA lipid profiles were measured using reverse phase and chiral liquid chromatography-mass spectrometry method.

PAHSA regioisomers as well as other FAHFAs were present in both human and murine milk. Unexpectedly, the levels of 5-PAHSA were higher relative to other regioisomers. The separation of both regioisomers and enantiomers of PAHSAs revealed that both R- and S-enantiomers were present in the biological samples, and that the majority of the 5-PAHSA signal is of R configuration. Total PAHSA levels were positively associated with weight gain during pregnancy, and 5-PAHSA as well as total PAHSA levels were significantly lower in the milk of the obese compared to the lean mothers.

Our results document for the first time the presence of lipid mediators from the FAHFA family in breast milk, while giving an insight into the stereochemistry of PAHSAs. They also indicate the negative effect of obesity on 5-PAHSA levels. Future studies will be needed to explore the role and mechanism of action of FAHFAs in breast milk.

1. Introduction

Obesity during pregnancy has both short- and long-term negative effects on the offspring's health [1]. To overcome these adverse consequences such as childhood obesity, breastfeeding is extensively recommended [2–4]. However, little is known about the role of non-nutritive bioactive milk components and how they could affect the growth and development of the newborn [5,6].

The breast is composed mainly of adipose tissue, but during pregnancy, it develops an extensive glandular/ductal system, and adipose tissue is replaced with functional glandular units. After birth, the mammary gland initiates the secretion of colostrum, which is rich in protein and bioactive components targeted for immunization and maturation of the gastrointestinal system of the newborn (reviewed in [3,7,8]). Later on, the lipid content in the milk increases [3] via mammary gland de-novo lipogenesis [3,9,10]. The most abundant lipid classes are triacylglycerols and phospholipids [3,5,11–13], but also other bioactive lipids like resolvins are important immunomodulatory players [14,15].

Fatty acid esters of hydroxy fatty acids (FAHFAs) are endogenous lipids with anti-inflammatory and anti-diabetic properties, including the enhancement of glucose tolerance, and insulin and glucagon-like peptide 1 (GLP-1) secretion while reducing inflammatory responses [16–19]. They consist of a fatty acid (e.g. palmitic acid, PA) esterified to the hydroxyl group of a hydroxy fatty acid (e.g. hydroxystearic acid, HSA), abbreviated as PAHSA. The position of the branching carbon

* Corresponding author at: Department of Adipose Tissue Biology, Institute of Physiology of the Czech Academy of Sciences, Videnska 1083, 142 20 Prague, Czech Republic.
E-mail address: ondrej.kuda@fgu.cas.cz (O. Kuda).
[1] These authors contributed equally.

https://doi.org/10.1016/j.bbalip.2017.11.004
Received 24 April 2017; Received in revised form 7 November 2017; Accepted 11 November 2017
Available online 14 November 2017
1388-1981/

defines a regioisomer (e.g. 5-PAHSA). There are several regioisomer families derived from palmitic, palmitoleic, stearic, oleic, linoleic, and docosahexaenoic acids with tissue-specific distribution documented so far [16–21]. Adipose tissue represents a major site of FAHFAs synthesis [16,17], but the biosynthetic enzymes involved are unknown. Serine hydrolase carboxyl ester lipase (CEL) [22] and threonine hydrolases [22] were identified as FAHFA-metabolizing enzymes. So far, FAHFAs were only detected in the serum and adipose tissues of mice and humans [16].

We hypothesized that since FAHFAs are linked to both anabolic metabolism and immunity, the production of these lipid mediators could be detected in human milk and affected by obesity. To test this hypothesis, we performed a lipidomic analysis of milk samples obtained from lean and obese mothers and compared the PAHSA levels of these groups. Experiments in mice were performed to characterize the bioavailability of orally administered PAHSA.

2. Materials and methods

2.1. Materials and reagents

All chemicals were purchased from Sigma-Aldrich (Czech Republic) unless stated otherwise. FAHFA standards (5-,9-,10-,12-,13-PAHSA, and 5-PAHSA-$^{2}H_{31}$, 9-PAHSA-$^{13}C_{4}$) were purchased from Cayman Pharma (Neratovice, Czech Republic). 9(S)-PAHSA and 9(R)-PAHSA were kindly provided by Cayman Pharma (Neratovice, Czech Republic). 5-PAHSA for the bioavailability experiment was prepared as before [19].

2.2. Study design and sample collection

The study was designed as a cross-sectional collection from 30 lean (BMI = 19–23) and 23 obese (BMI > 30) donors (Table 1). The inclusions criteria were: body mass index 19–23 and > 30 respectively, gestational age > 37 + 0 weeks, uncomplicated pregnancy and labor, maternal age > 18 years, no symptoms of acute infection (i.e. respiratory, urinary tract infection, otitis, chorioamnionitis), absence of any diabetes mellitus and sufficient amounts of breast milk to achieve adequate breastfeeding. Each donor provided a 5 mL sample of milk obtained during one breastfeeding session 72 h after giving birth. An experienced consultant for breastfeeding assisted in collecting a sample of fore milk. Milk samples were stored at − 20 °C in a milk bank. All samples were collected at the Institute for the Care of Mother and Child in Prague, Czech Republic. The study protocols were approved by the Research Ethics Board of the Institute (Approval number: 2016-12-19/3). All mothers gave informed written consent.

2.3. Murine samples

Female mice (C57BL/6J; Jackson Laboratory, ME, USA), maintained in a controlled environment (22 °C; 12 h light-dark cycle; light from 6.00 a.m.), were fed a standard chow diet and mated at 12 weeks [23]. Milk samples were obtained 5 days after parturition. Pups were separated from their mothers 2 h before the milk collection, each dam received 2 IU of oxytocin (Sigma) intraperitoneally, and milk was collected under isoflurane anesthesia into plastic tubes using a pipette and kept frozen at − 80 °C [24]. Adipose tissue samples were collected from adult male C57BL/6J mice. To characterize the bioavailability of orally administered 5-PAHSA, male mice were gavaged with 5, 10, and 20 mg 5-PAHSA/kg body weight or an equivalent volume of vehicle (50% PEG400, 0.5% Tween-80, 49.5% H_2O [18]) once daily for 3 days. After killing of the anaesthetized mice, levels of 5-PAHSA were quantified in plasma. All animal experiments were approved by the Animal Care and Use Committee of the Institute of Physiology of the Czech Academy of Sciences (Approval Number: 81/2016) and followed the guidelines.

Table 1
Basic characteristics of mothers who supplied milk.

		Lean	Obese
Mothers	Age [years]	32.0 ± 2.6	35.1 ± 4.3*
	BMI before [kg/m^2]	19.5 ± 1.4	33.2 ± 4.0*
	BMI after [kg/m^2]	24.3 ± 1.9	37.1 ± 3.7*
	Delta BMI [kg/m^2]	4.8 ± 1.4	3.9 ± 1.2*
	Gravidity	1.9 ± 1.0	1.7 ± 1.1
	Parity	1.2 ± 1.0	1.1 ± 1.0
Newborns	Birth weight [g]	3364 ± 512	3269 ± 454
	Length [cm]	49 ± 3	49 ± 2
	Weight at 72 h [g]	3209 ± 482	3100 ± 416
	Delta weight [g]	− 155 ± 89	− 169 ± 79

* Statistically significant difference at $p < 0.05$. BMI before and after pregnancy.

2.4. Lipid extraction

Before analysis, the stored milk samples were warmed to 25 °C in a water bath and homogenized for 10 s using an ultrasonic bath to ensure a consistent quality of samples. FAHFA extraction was performed based on the published method [16,17]. Murine tissues (50–100 mg) were homogenized using a MM400 bead mill (Retsch, Germany) chilled to − 20 °C in a mixture of citric acid buffer, methanol, and further extracted with dichloromethane (1:1:2 final ratio) rotating for 20 min at 4 °C. An internal standard of 9-PAHSA-$^{13}C_{4}$ was added to the homogenate (100 pg/sample). Milk samples (300 µL) were extracted following the same protocol, apart from the homogenization. The organic phase was collected, water phase re-extracted, organic phases pooled, dried in a Speed-vac (Savant SPD121P, Thermo, Massachusetts, USA), resuspended in dichloromethane and applied on HyperSep SPE columns (500 mg/10 mL, 40–60 µm, 70 Å, Thermo). FAHFAs were eluted from the SPE columns with ethylacetate, concentrated using a Speed-vac, resuspended in methanol and immediately analyzed using LC-MS as follows.

2.5. Liquid chromatography and mass spectrometry (LC-MS/MS/MS)

Chromatographic separation was performed in a UPLC Ultimate 3000 RSLC (Thermo) equipped with a Kinetex C18 1.7 µm 2.1 × 150 mm column (Phenomenex, California, USA) as before [17]. For structural characterization, an Accucore™ C30 2.6 µm 2.1 × 250 mm column (Thermo) and CHIRAL ART Amylose-SA, 3 µm 2.0 × 250 mm (YMC, Japan) were used. The flow rate was 600 µL/min at 50 °C and 400 µL/min at 25 °C, respectively, using isocratic elution with 93% acetonitrile and 7% water & 0.1% acetic acid for 30 min. UPLC was coupled to a QTRAP 5500/SelexION, a hybrid triple quadrupole linear ion trap mass spectrometer equipped with an ion mobility cell (Sciex, Massachusetts, USA). For chiral separation, samples were dissolved in the mobile phase. FAHFAs were detected in negative ESI mode as before [17].

2.6. Statistics

Based on published differences in total serum PAHSA concentrations [16] between insulin-sensitive and insulin-resistant patients, the sample size of 17 per group was calculated using G*Power software (power 0.95, $\alpha = 0.05$) [25]. Statistical analysis was performed with SigmaStat and $p < 0.05$ was considered significant.

3. Results

3.1. Targeted lipidomics of FAHFAs using LC-MS/MS/MS

We improved our targeted LC-MS/MS/MS lipidomic methodology for the separation and identification of FAHFA positional isomers [17] using a C30 chromatographic column and isocratic elution (Fig. 1A).