Original article

MOLECULAR METABOLISM

Sexually dimorphic brain fatty acid composition in low and high fat diet-fed mice

Carlos Rodriguez-Navas [1], Eugenia Morselli [2,**,4], Deborah J. Clegg [3,*,4]

ABSTRACT

Objective: In this study, we analyzed the fatty acid profile of brains and plasma from male and female mice fed chow or a western-style high fat diet (WD) for 16 weeks to determine if males and females process fatty acids differently. Based on the differences in fatty acids observed in vivo, we performed in vitro experiments on N43 hypothalamic neuronal cells to begin to elucidate how the fatty acid milieu may impact brain inflammation.

Methods: Using a comprehensive mass spectrometry fatty acid analysis, which includes a profile for 52 different fatty acid isomers, we assayed the plasma and brain fatty acid composition of age-matched male and female mice maintained on chow or a WD. Additionally, using the same techniques, we determined the fatty acid composition of N43 hypothalamic cells following exposure to palmitic and linoleic acid, alone or in combination.

Results: Our data demonstrate there is a sexual dimorphism in brain fatty acid content both following the consumption of the chow diet, as well as the WD, with males having an increased percentage of saturated fatty acids and reductions in ω6-polyunsaturated fatty acids when compared to females. Interestingly, we did not observe a sexual dimorphism in fatty acid content in the plasma of the same mice. Furthermore, exposure of N43 cells to the ω6-PUFA linoleic acid, which is higher in female brains when compared to males, reduces palmitic acid-induced inflammation.

Conclusions: Our data suggest male and female brains, and not plasma, differ in their fatty acid profile. This is the first time, to our knowledge, lipidomic analyses has been used to directly test the hypothesis there is a sexual dimorphism in brain and plasma fatty acid composition following consumption of the chow diet, as well as following exposure to the WD.

Keywords Obesity; N43; Palmitic acid; Linoleic acid; Central nervous system; Western diet; ω6-fatty acids

1. INTRODUCTION

In the central nervous system, lipids represent more than 50% of the brain's dry weight, making the brain second only to adipose tissue with respect to its lipid content [1]. Fatty acids (FAs), the principal constituents of brain lipids [2], can be synthesized by de-novo lipogenesis in brain cells. Essential FAs however are the exception, and must be provided through the diet. FAs are taken up from circulating blood into the brain through the blood brain barrier (BBB) [3,4].

FAs are key components of cellular membranes and precursors for biosynthesis of phospholipids and sphingolipids [4]. FAs play essential roles in signaling and influencing neuronal function [4]. The biological properties of lipid bi-layers depend on their FA composition; correspondingly, FA concentrations are tightly regulated [5]. Additionally, FAs provide an important energy source for cells via mitochondrial β-oxidation and through the generation of metabolic intermediates [4,6].

Brain lipids are rich in polyunsaturated fatty acids (PUFAs) containing double bonds at the ω3 and ω6 position, such as arachidonic (AA;

[1]Department of Molecular Genetics, University of Texas Southwestern Medical Center, 75390-8857, Dallas, TX, USA [2]Department of Physiology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Avda. Libertador Bernardo OHiggins 340, 8331150, Santiago, Chile [3]Cedars-Sinai Diabetes and Obesity Research Institute, Department of Biomedical Research, 8700 Beverly Blvd, Thalians E101, 90048, Los Angeles, CA, USA

[4] Eugenia Morselli and Deborah Clegg share senior authorship.

*Corresponding author. 8730 Alden Drive, Thalians E101, Los Angeles, CA 90048, USA. Tel.: +1 (310) 967 2787; fax: +1 (310) 967 3869. E-mail: Deborah.clegg@cshs.org (D.J. Clegg).

**Corresponding author. Tel.: +56 2 354 2871; fax +56 2 3541850. E-mail: emorselli@bio.puc.cl (E. Morselli).

Abbreviations: AA, arachidonic acid; ACC, acetyl-CoA carboxylase; B2m, beta-2 microglobulin; BBB, blood brain barrier; BSA, bovine serum albumin; C, Chow diet; CNS, central nervous system; DHA, docosahexaenoic acid; F, female; FAs, fatty acids; FABP, fatty acid binding protein; FAT/CD36, fatty acid transporter; FATP1, fatty acid transport protein 1; FAS, fatty acid synthase; FFAs, free fatty acids; IL6, interleukin 6; LA, linoleic acid; M, male; MCD, malonyl-CoA decarboxylase; MSFD2a, membrane protein major facilitator super family domain containing 2a; MUFAs, monounsaturated fatty acids; NF-κB, Nuclear Factor-κ Beta; PA, palmitic acid; PUFAs, polyunsaturated fatty acids; SatFAs, saturated fatty acids; TFAs, total fatty acids; TNFα, Tumor Necrosis Factor α; UnsatFAs, unsaturated fatty acids; WD, western diet; WT, wild-type

Received June 13, 2016 • Revision received June 24, 2016 • Accepted June 27, 2016 • Available online 30 June 2016

http://dx.doi.org/10.1016/j.molmet.2016.06.014

20:4ω6) and docosahexaenoic (DHA; 22:6ω3) fatty acids [1]. In contrast with other organs or plasma, AA and DHA precursors, which are the essential FAs linoleic (18:2ω6) and α-linolenic (18:3ω3) acids, represent less than 1% of the lipid content of the brain [7]. As previously indicated, essential PUFAs cannot be synthesized by the brain and are derived from the diet, and they are critical mediators of normal brain development and function [8]. Importantly, decrements in dietary consumption of ω3-PUFAs and alterations in lipid metabolism are implicated in neuropsychiatric diseases including neurodegenerative diseases [9,10] such as cognitive decline, Alzheimer's disease and neuroinflammation [11–13]. High concentrations of dietary saturated long chain fatty acids (SatFAs) have also been associated with neurological dysfunction [5]. Unfortunately, diets consumed by westernized civilizations are typically rich in SatFAs and are low in PUFAs [14]. Consumption of westernized diets is associated with the development of obesity, cognitive dysfunction, as well as cancer.

Research has focused on understanding lipid and FA metabolism; however, due to the complexity with hundreds of different enzymes, activators, and substrates leading to tens of thousands of different lipid species, a full understanding FA metabolism remains elusive. Not only is little known about the impact of dietary FA consumption on brain lipid uptake and assimilation, but even less is known if there is a sexual dimorphism in lipid processing. What is known is that the prevalence of many diseases associated with the metabolic syndrome and cognitive function differs based on sex [7,15,16]. Therefore, it is important to begin to address if the brain varies with respect to uptake and response to dietary FAs.

Recently, we reported males and females differ with respect to their metabolic response to a high fat, westernized diet [17,18]. We further demonstrated that the SatFA content of the brain differs between the sexes, with increased concentrations of SatFAs in the brains of male mice when compared to female mice [17]. Here, we sought to extend our initial findings, and determine in a more comprehensive FA profile analysis of brain and plasma, if there are other sexually dimorphic differences in FAs. To this end, we analyzed age-matched wild-type (WT) male and female mice fed a low fat (chow) or western-style high fat diet (42% of the calories derived from fat, WD) for 16 weeks. Importantly, this type of diet was chosen because it is similar in nutrient composition to the human diet, which based on the latest statistics published by the National Institute of Health, contains approximately 35–40% of the calories derived from SatFas as well as is high in simple sugars [21]. Our data demonstrate there is a sexual dimorphism in brain-FA content both following consumption of the low fat/chow diet as well as following consumption of the WD. Specifically, we found males have an increased percentage of SatFAs and reductions in ω6-PUFAs when compared to females. To begin to elucidate how the FA milieu may impact brain inflammation, *in vitro* experiments were performed and our findings suggest ω6-PUFA linoleic acid (18:2ω6, LA), which is higher in female brains, has an anti-inflammatory role and reduces palmitic acid (16:0, PA)-induced inflammation.

2. METHODS

2.1. Animals and body weight

Animal care and procedures were approved by the University of Texas Southwestern Medical Center. All the methods were in accordance with the approved guidelines. C57BL/6 mice, purchased from the Jackson Laboratory (The Jackson Laboratory, Bar Harbor, MA, USA) were housed in groups of two to five per cage, in a temperature-controlled environment at 22°C–24 °C using a 12-hour light/dark cycle. Mice were fed standard chow (#2916, Harlan-Teklad, Madison, WI), or exposed to 42% WD (#88137, Harlan Teklad) at 8 weeks of age with free access to water. Animals were food restricted for 3 h before sacrifice, which occurred 3 h following the onset of the light phase.

2.2. Tissues collection

Brains were collected from mice following cervical dislocation, weighed and quickly frozen in liquid nitrogen and stored at −80 °C. Blood was collected from anesthetized mice through eye bleeding and centrifuged twice at 8,000 rpm at 4 °C to collect plasma. Plasma aliquots were stored at −80 °C.

2.3. Fatty acid analysis

52 different FA isomers were analyzed using pentafluorobenzoyl bromide by GC-ECNI-MS as previously described [19,20]. Half of each of the brains was homogenized while still frozen in 5 ml of methanol. To avoid lipase activity, which might overestimate the free fatty acid (FFA) fraction, these homogenates were kept on dry ice at all times and processed immediately after homogenization. For the quantification of the total fatty acids (TFAs), samples were hydrolyzed and extracted as previously described [17]. For the FFA quantification samples were directly extracted from the homogenized brain solution by liquid–liquid extraction, using a 1:1:2 mixture of MeOH:PBS:isooctane under acidified conditions (25 mM HCL) as described in [20]. For the plasma analysis, 10 ul samples were assayed for the quantification of both TFA and FFA (separately), following the same extraction protocols used for brains. In the analysis of FFAs, no fatty acyl-CoAs or other mono-acyl lipids are measured [19].

2.4. Cell culture

N43 cells were purchased from CELLutions Biosystem Inc. (Cedarlane, Burlington, NC, USA) and maintained in HyClone™ DMEM medium (Thermo Scientific, Waltham, MA) containing 10% fetal bovine serum (Gemini, West Sacramento, CA) 100 units/mL penicillin G sodium and 100 μg/mL streptomycin sulfate and 100 mg/L sodium pyruvate (Thermo Scientific). Cells were grown for 24 h before treatments with medium containing 2% fetal bovine serum. Cells were pre-treated for 1 h with 30 μM LA (Sigma, St-Louis, MO) conjugated with fatty acid free bovine serum albumin (BSA) (MP Biomedicals, LLC, Solon, OH) and then treated for 5 h with 30 μM LA or 100 μM PA (Matreya, Pleasant Gap, PA) conjugated with BSA alone or in combination.

2.5. qPCR

For the analysis of gene expression in the cell culture experiments, cells were washed twice with ice-cold PBS and lysed in 1 ml of TRIzol® (Ambion, Life Technologies). RNA was extracted from cells using RNeasy Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Total RNA (1 μg) was reverse-transcribed using the SuperScript® III First-Strand Synthesis System (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Assay-on-demand kits with TaqMan® Universal Master Mix II (Applied Biosystems, Foster City, CA, USA) were used according to manufacturer's protocol and analyzed with the ABI PRISM 7700 Sequence Detection System (Applied Biosystems). Relative mRNA expression levels for interleukin 6

