Biochimica et Biophysica Acta 1841 (2014) 1563–1570

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

The lipid profile of brown adipose tissue is sex-specific in mice

Miriam Hoene [a,1], Jia Li [d,1], Hans-Ulrich Häring [a,b,c], Cora Weigert [a,b,c], Guowang Xu [d,*], Rainer Lehmann [a,b,c,**]

[a] Division of Clinical Chemistry and Pathobiochemistry, Department of Internal Medicine IV, University Hospital Tübingen, Tübingen, Germany
[b] Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Centre Munich at the University of Tübingen, Tübingen, Germany
[c] German Center for Diabetes Research (DZD), Tübingen, Germany
[d] Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China

ARTICLE INFO

Article history:
Received 2 June 2014
Received in revised form 31 July 2014
Accepted 5 August 2014
Available online 14 August 2014

Keywords:
Brown adipose tissue
White adipose tissue
Gonadal fat
Subcutaneous fat
Female and male mice
Tandem-MS lipidomics

ABSTRACT

Brown adipose tissue (BAT) is a thermogenic organ with a vital function in small mammals and potential as metabolic drug target in humans. By using high-resolution LC-tandem-mass spectrometry, we quantified 329 lipid species from 17 (sub)classes and identified the fatty acid composition of all phospholipids from BAT and subcutaneous and gonadal white adipose tissue (WAT) from female and male mice. Phospholipids and free fatty acids were higher in BAT, while DAG and TAG levels were higher in WAT. A set of phospholipids dominated by the residue docosahexaenoic acid, which influences membrane fluidity, showed the highest specificity for BAT. We additionally detected major sex-specific differences between the BAT lipid profiles, while samples from the different WAT depots were comparatively similar. Female BAT contained less triacylglycerol and more phospholipids rich in arachidonic and stearic acid whereas another set of fatty acid residues that included linoleic and palmitic acid prevailed in males. These differences in phospholipid fatty acid composition could greatly affect mitochondrial membranes and other cellular organelles and thereby regulate the function of BAT in a sex-specific manner.

1. Introduction

The last decade has seen an unprecedented interest in brown adipose tissue (BAT), triggered by the detection of BAT depots in adult humans and fueled by an increasing incidence of type 2 diabetes and related diseases that calls for novel strategies to combat the obesity pandemic. Long before advances in medical imaging enabled its detection in adult humans [1–4], BAT had been recognized for its occurrence and role in small mammals and human neonates [5,6] where it fulfills the vital function of producing non-shivering heat by energy dissipation [7,8]. Cold-induced signaling via the sympathetic nervous system increases the breakdown of triglycerides and oxidation of fatty acids in this mitochondria-rich tissue. Its high thermogenic capacity is due to the presence of uncoupling protein 1 (UCP1), a BAT-specific inner mitochondrial membrane protein that dissociates oxidative phosphorylation from ATP production [9]. Besides having more and bigger mitochondria, brown adipocytes are characterized by multilocular lipid droplets [10]. BAT is therefore distinct from white adipose tissue (WAT) that stores fatty acids in the form of triacylglycerol (TAG) and releases them without producing significant amounts of heat. However, besides serving as energy stores, lipids are also the central constituents of cellular and organellar membranes. Thus, understanding the differences in lipid composition that distinguish BAT from WAT is a prerequisite for understanding its specialized function. A comparison of BAT and WAT is, however, complicated by the fact that WAT itself is not a homogenous tissue but dispersed as several depots. Subcutaneous adipose tissue (SAT), which has a double function as energy store and thermal insulator, is more sensitive to antilipolytic hormones and less prone to inflammation than visceral fat and

Abbreviations: ACN, acetonitrile; BAT, brown adipose tissue; CER, ceramide; DAG, diacylglycerol; DHA, docosahexaenoic acid; FFA, free fatty acids; FDR, false discovery rate; GAT, gonadal adipose tissue; hexCER, hexosyl-ceramide; IPA, isopropyl alcohol; LPC, lyso-phosphatidylcholine; LPE, lyso-phosphatidylethanolamine; LPI, lyso-phosphatidylinositol; MS, mass spectrometry; MTBE, methyl-*tert*-butylether; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; PCA, principal component analysis; PLS-DA, partial least square-discriminant analysis; PUFA, poly-unsaturated fatty acids; SAT, subcutaneous adipose tissue; SD, standard deviation; SM, sphingomyelin; TAG, triacylglycerol; UCP1, uncoupling protein 1; UPLC, ultra performance liquid chromatography; WAT, white adipose tissue

* Correspondence to: G. Xu, Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457 Zhongshan Road, Dalian 116023, China. Tel./fax: +86 411 84379559.
** Correspondence to: R. Lehmann, Division of Clinical Chemistry and Pathobiochemistry, Department of Internal Medicine IV, University Hospital Tübingen, Otfried-Müller-Straße 10, 72076 Tübingen, Germany. Tel.: +49 7071 29 83193; fax: +49 7071 29 5348.
E-mail addresses: miriam.hoene@med.uni-tuebingen.de (M. Hoene), muranmyself@126.com (J. Li), hans-ulrich.haering@med.uni-tuebingen.de (H.-U. Häring), cora.weigert@med.uni-tuebingen.de (C. Weigert), xugw@dicp.ac.cn (G. Xu), rainer.lehmann@med.uni-tuebingen.de (R. Lehmann).
[1] Miriam Hoene and Jia Li contributed equally to this study.

http://dx.doi.org/10.1016/j.bbalip.2014.08.003

may even be protective by preventing fatty acids from being stored in ectopic fat depots [11,12]. The gonadal adipose tissue (GAT) pads are the largest visceral fat depots in non-obese mice. In mice, where SAT develops pre- and GAT postnatally, the two depots have also been shown to respond differently to a prolonged high-fat diet. Hyperplasia eventually takes place in GAT, but not in SAT, following a period of hypertrophy [13]. Finally, the GAT depots are directly attached to the reproductive organs, to the ovaries in female and to the epididymis in male animals, and it is unclear how these anatomical and functional specializations translate to sex-specific differences in GAT lipid profiles. Thus, a comparative study of BAT and WAT requires the inclusion of different WAT depots from both sexes. Modern mass spectrometric (MS) techniques have greatly advanced the state-of-the-art lipidomics approach, which serves as a powerful tool for the qualitative characterization and quantification of a wide spectrum of structurally distinct lipids [14,15]. To obtain a deeper insight into the lipid patterns distinguishing BAT from WAT, we performed a global lipidomics analysis of BAT, SAT and GAT from male and female C57BL/6 N mice, with a special focus on sex-specific differences. To maximize the conclusions about potential lipid metabolic processes and functions, individual fatty acid chains were assigned to phospho- and sphingolipids by using high-resolution tandem-MS.

2. Materials and methods

2.1. Chemicals and internal standards

Liquid chromatography grade acetonitrile (ACN) and isopropyl alcohol (IPA) were purchased from Merck (Darmstadt, Germany). Ammonium acetate was from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water was obtained from a Milli-Q system (Millipore, MA, USA). Internal standards (d4-palmitic acid, ceramide (CER) (d18:1/17:0), diacylglycerol (DAG) (14:0/14:0), lyso-phospatidylcholines (LPC) (15:0) and (19:0), phosphatidylcholine (PC) (17:0/17:0) and (19:0/19:0), phosphatidylethanolamine (PE) (17:0/17:0), sphingomyelin (SM) (d18:1/12:0) and triacylglycerol (TAG) (17:0/17:0/17:0) and (15:0/15:0/15:0) were purchased from Avanti Polar Lipids, Inc. (Alabaster, Alabama, USA) or Sigma-Aldrich (Taufkirchen, Germany).

2.2. Animal procedures and lipid extraction

The animal experiment was conducted in accordance with the national guidelines of laboratory animal care and approved by the local governmental commission for animal research (Regierungspraesidium Tuebingen, Baden-Wuerttemberg, Germany). Male and female C57Bl/6 N mice ($n = 6$ of each sex) were purchased from Charles River (Sulzfeld, Germany) and studied at 11 weeks of age. After anesthesia with an intraperitoneal injection of ketamine (150 mg/kg body weight) and xylazine (10 mg/kg body weight), the animals were killed by decapitation. Interscapular BAT, SAT from the femerogluteal region and GAT were quickly excised and snap-frozen in liquid nitrogen for later processing. One female SAT sample was lost during sample processing. For the lipid extraction, 10 mg of frozen fat was homogenized twice for 2 min at 20 Hz with 1.6 ml of cold 75% ethanol in a TissueLyser (Qiagen, Hilden, Germany) and further extracted with 4 ml methyl-*tert*-butylether (MTBE) as previously described [16].

2.3. Lipidomics analyses

Non-targeted lipidomics analysis was performed by a Waters ACQUITY ultra performance liquid chromatography (UPLC) system (Waters, USA) coupled with an AB Sciex tripleTOF™ 5600 plus mass spectrometer (Applied Biosystems, USA) via a Duospray ion source. Briefly, adipose lipid extracts were separated using an ACQUITY UPLC BEH C_8 column (2.1 × 100 mm, 1.7 μm) (Waters, USA) by gradient elution at 55 °C, as described previously with modifications [16]. The elution started with 68% mobile phase A (ACN:H_2O = 6:4, 10 mM ammonium acetate) and 32% mobile phase B (IPA:ACN = 9:1, 10 mM ammonium acetate) and maintained for 1.5 min. Mobile phase B was then linearly increased to 85% during 10 min and further to 97% in the next 0.1 min followed by maintenance for 1.5 min. Afterwards it was decreased to 32% B in 0.1 min and kept for 2 min till the next injection. The flow rate was 0.26 ml/min. The total run time for each injection was 20 min. In both ESI(+) and ESI(−) modes, TOF MS full scan and information-dependent acquisition (IDA) were performed in parallel to acquire high resolution MS and tandem-MS data simultaneously. In the positive mode, ion source gas 1 and gas 2 were set to 50 psi, curtain gas to 35 psi, temperature to 500 °C, ion spray voltage floating (ISVF) to 5500 V, declustering potential (DP) to 80 V, and collision energy (CE) to 35 V with a collision energy spread (CES) of ± 15 V. In the negative mode, ion source gas 1 and gas 2 were set to 60 psi, curtain gas to 35 psi, temperature to 600 °C, ISVF to −4500 V, DP to −100 V, and CE to −45 V with CES of ± 15 V. In the IDA setting, candidate ions with top 10 intensity were selected and subjected to high resolution tandem-MS analysis.

CER analysis was performed on an UHPLC (1290 Infinity, Agilent Technologies, Santa Clara, CA, USA)–electrospray ionization–Q-TOF system (6540 UHD accurate–mass Q-TOF LC/MS, Agilent Technologies, Santa Clara, CA, USA) in the positive mode. The LC method was as described above. The gas temperature was set to 325 °C, drying gas to 8 L/min, nebulizer to 35 psig, Vcap to 3500 V, fragmentor to 200 V, and skimmer to 65 V.

The identification of lipid species, facilitated by the LipidView ™ software (Version 1.2, AB Sciex, USA), was based on accurate *m/z*, retention behavior and tandem-MS fragmentation pattern. High resolution tandem-MS was utilized to enhance lipid identification. All detected lipids were quantified by normalization to the corresponding internal standard and tissue weight. The lipid nomenclature was based on the LIPID MAPS recommendations, where P denotes plasmalogens, i.e., (alkenyl/acyl) and O denotes (alkyl/acyl) ether lipids [17].

2.4. Statistical analysis

Data are presented as means ± standard deviation (SD), either quantitatively per unit of wet tissue weight or as relative abundance. The relative abundance or composition of a lipid class was calculated by normalizing individual values to the summed abundance of all lipids within a given class. Statistical analysis was performed using Student's *t*-test with Bonferroni-adjustment or Mann–Whitney test with Benjamini–Hochberg correction for the false discovery rate (FDR). A *p*-value <0.05 was considered significant, a *p*-value <0.1 as a trend. Multivariate principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA) were performed with SIMCA-P 11.5 (Umetrics AB, Umeå, Sweden). Relative abundance data were autoscaled for multivariate analyses. The open-source MultiExperiment Viewer software [18] was employed for heatmap generation using autoscaled concentration data.

3. Results

3.1. Comparative lipidomics of brown and subcutaneous and gonadal white adipose tissues

Lipidomics analyses were performed using samples of BAT and subcutaneous and gonadal WAT depots from female and male C57BL/6 N mice, resulting in the identification of 329 lipid species from the 17 (sub)classes ceramide (CER), diacylglycerol (DAG), hexosyl-ceramide (hexCER), lyso-phosphatidylcholine (LPC), lyso-phosphatidylethanolamine (LPE) and LPE-P, lyso-phosphatidylinositol (LPI), phosphatidylcholine (PC) and PC-O, phosphatidylethanolamine (PE) and PE-P, phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylserine (PS), sphingomyelin (SM), triacylglycerol (TAG),