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

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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

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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),

and free fatty acids (FFA). For a more detailed and informative molecular characterization of the detected lipids, high resolution tandem-MS information obtained from a high-throughput IDA approach was exploited for an acyl-, alkyl-, or alkenyl- chain assignment to all detected phospholipids. These data are fully provided as a supplement to this manuscript (Table S-1).

To gain an initial overview, a multivariate principal component analysis (PCA) of the lipid composition of the different tissues was performed. Since cell size could be a factor influencing the tissue content of membrane or neutral lipids, we performed PCAs using firstly the concentration data, as obtained by normalization to internal standards and tissue weight, and secondly the relative lipid composition. The latter was obtained by normalizing the content of individual lipids to the total abundance of the respective lipid class. Performing PCA with both concentration- and compositional data resulted in a pronounced separation of brown and white fat as well as a distinct sex-dependent difference in the lipid profiles of BAT. The PCA plot from the lipid compositional data is exemplarily shown in Fig. 1.

Next, we compared the summed amounts of the individual lipid classes between the different depots by heatmap visualization. Fig. 2 shows that the phospholipids LPC, PC, LPE, PG, PI, PE and FFA were higher in BAT. A less pronounced difference was visible for PC-O, PS and SM which were highest in BAT but also tended to be higher in SAT than in GAT. On the other hand, TAG and DAG levels were visibly higher in WAT. The most prominent characteristic of GAT was its high TAG levels in conjunction with a slightly, but significantly lower content of several phospholipid classes (Table 1). The levels of the two detected species from the low-abundant hexCER class of lipids were specifically elevated in SAT only, while being similarly low in BAT and GAT (Table 1).

To identify individual lipids distinguishing BAT from WAT, we compared BAT to both subcutaneous and gonadal fat. In total, 184 lipids showed a significant difference between BAT and both WAT depots in both sexes (Fig. S-1). Interestingly, the magnitude of difference between BAT and WAT was much greater for BAT-characteristic than for WAT-characteristic lipids, due to an extremely low abundance of several lipids in WAT (Fig. 3A and Supplementary Table S-2), as exemplarily shown for PE(22:6/18:1) in Fig. 3B. Two of the eleven top BAT-characterizing lipids were PC species and nine were PE species (Fig. 3A). Interestingly, six of these lipids contained at least one docosahexaenoic acid (DHA) moiety. The lipids with lowest levels in BAT compared to WAT, in contrast, were TAGs and three CER species (Supplementary Table S-2). The latter finding is specifically noteworthy given that total CER levels were not different between BAT and WAT (Table 1).

3.2. The lipid profile of BAT is sex-specific

In addition to tissue-specific differences in lipid content, the PCA scores plot (Fig. 1) and the heatmap in Fig. 2 also revealed sex-specific differences which were most obvious for BAT. At the level of tissue content, the most notable difference between male and female adipose tissue was evident for TAG levels which were almost twice as high in BAT of male mice: 111 ± 12 μmol/g compared to only 73 ± 7 μmol/g in female BAT. On the other hand, phospholipid levels appeared to be higher in female BAT, with PG and PI levels reaching statistical significance (Table 1); a similar trend could be observed for PE and SM levels. The sum of all phospholipids amounted to 26 ± 3 μmol/g tissue in female and to 22 ± 2 μmol/g in male BAT (Table 1). Only in BAT, the ratio of PE to PC was pronouncedly higher in female mice, 2.1 versus 1.5 ($p = 0.0015$, data not shown). WAT did not exhibit this sex-specific difference and had much lower PE/PC ratios, 0.5 in subcutaneous and 0.4 in gonadal fat.

As delineated above, differences in the total content of individual lipid classes could in principle be caused by differences in cell size. Thus, we also investigated the lipid composition, the abundance of individual lipids normalized to the total abundance of the respective lipid class. In the PCA plot generated from these normalized data (Fig. 1), samples from female and male BAT separated into two clusters. To identify the lipids contributing most to the separation of female and male BAT, we performed a PLS-DA analysis and selected for each sex the 15 variables with the highest discriminating power according to VIP (variable importance). These variables were predominantly phospholipids, with the exception of four TAGs, two of which were associated with either sex, and CER(d18:1/16:0) that was higher in female BAT (Table 2). Interestingly, the phospholipids characterizing male and female BATs were dominated by different sets of fatty acid residues: In females, stearic (18:0) and arachidonic (20:4) acid were the most common residues, while palmitic (16:0), palmitoleic (16:1) and linoleic (18:2) acid prevailed in males (Table 2). This acyl chain bias appeared to be a general phospholipid characteristic: Arachidonic and stearic acid were the most abundant residues in phospholipids of female BAT, while palmitic and linoleic acid dominated in males (Supplementary Table S-3). Compared to BAT, the phospholipid composition of WAT

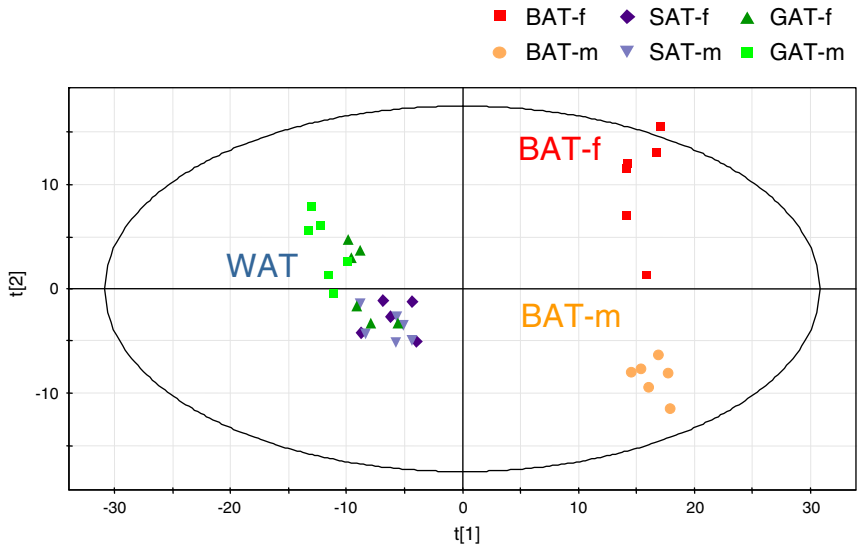

Fig. 1. Score plot of a multivariate principal component analysis (PCA) of the lipid composition of brown (BAT), subcutaneous white (SAT) and gonadal white (GAT) adipose tissue from female (f) and male (m) C57BL/6 N mice.

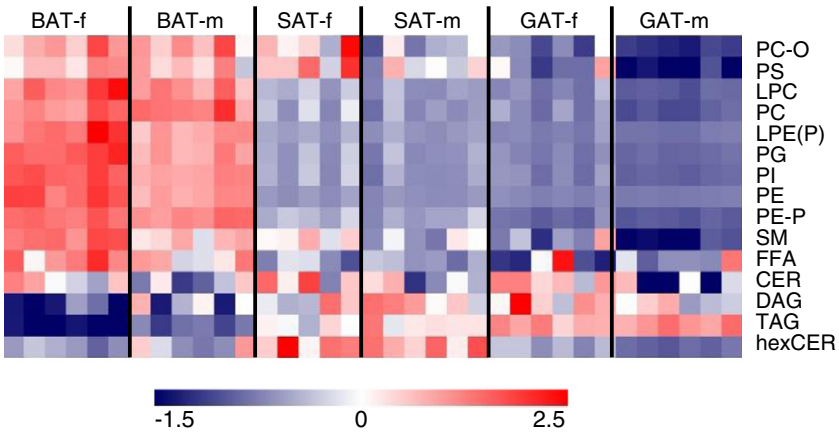

Fig. 2. Heatmap visualization of the distribution of the different lipid (sub)classes in brown (BAT), subcutaneous white (SAT) and gonadal white (GAT) adipose tissue of female (f) and male (m) C57BL/6 N mice. In total, 329 lipid species were detected: 6 PC-O, 11 PS, 7 LPC, 38 PC, 7 LPE, 2 LPE-P, 11 PG, 13 PI, 1 LPI, 40 PE, 23 PE-P, 13 SM, 32 FFA, 9 CER, 8 DAG, 106 TAG, and 2 hexCER species. LPE and LPE-P were summarized as LPE(P); the single LPI species detected was not included in the heatmap. Each column represents values from an individual animal.

was less sex-specific. Palmitic acid was the most abundant residue in SAT and GAT of both male and female mice (Supplementary Table S-3).

4. Discussion

By combining MTBE-based extraction and high-resolution LC-tandem-MS analysis, we generated detailed lipid profiles of brown and two white fat depots of female and male mice. In total, 329 lipid species from 17 (sub)classes, covering DAG, TAG and different phospho- and sphingolipid classes as well as FFA, could quantitatively be detected. An asset of these data is the provision of acyl/alkyl/alkenyl chain compositions for all detected phospholipid species, in addition to summed carbon and double bond numbers. Although current lipidomics tools enable global lipid characterization on the scale of molecular species, information on acyl/alkyl/alkenyl composition is scarce and usually limited to a small number of lipids. The fatty acid composition of individual lipids might be indicative of fatty acid metabolism or remodeling among different lipid pools and could reflect potential roles of lipids in (patho)physiological conditions. This is specifically relevant for phospholipids as major components of cellular membranes that determine membrane fluidity, dynamics and homeostasis while interacting with membrane-associated proteins [15].

In accordance with their respective metabolic functions, energy-storing WAT contained significantly more TAG while heat-generating BAT with its high density of mitochondria contained more phospholipids and sphingomyelin. A detailed comparison of BAT and the two WAT depots revealed a set of phospholipids, nine PE- and two PC-species (PC(18:2/22:6), PC(22:6/22:6), PE(14:0/16:1), PE(34:4) consisting of the isomers PE(16:1/18:3) and (16:2/18:2), PE(14:0/20:4), PE(16:1/20:5), PE(20:4/20:0), PE(22:6/14:0), PE(22:6/18:2), PE(22:6/18:1), and PE(22:6/18:3)) to have the highest specificity for BAT in both sexes. Interestingly, this group of lipids appears to overlap with a lipid cluster recently identified to be common to heart and skeletal muscle in a lipidomics study of various mouse tissues [19]. This finding is in line with previous reports that brown, but not white, adipocyte precursors are related to the myogenic lineage [20,21]. Due to our tandem-MS approach, we could unravel the fatty acid composition of these phospholipids and identify DHA to be contained in six of the eleven BAT-discriminant lipids. DHA amounted to about 8% of residues in BAT compared to only 5% in all WAT depots (Supplementary Table S-3), thus showing the same trend as the individual BAT-specific phospholipids. Of note, the fatty acid composition of a lipid class can only approximately be calculated based on LC-MS data where, as in other "omics" approaches, the number of analytes exceeds the number of internal standards. However, a trend towards higher DHA levels in BAT appears reasonable as it is in line with a previous study in mice showing that

Table 1
Content of different lipid (sub)classes in brown (BAT), subcutaneous white (SAT) and gonadal white (GAT) adipose tissue of female (f) and male (m) C57BL/6 N mice.

[μmol/g]	BAT-f		BAT-m		SAT-f		SAT-m		GAT-m		GAT-m		BAT-WAT	SAT-GAT	BAT f-m	SAT f-m	GAT f-m
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD					
PC-O	0.042	0.008	0.042	0.009	0.039	0.016	0.023	0.007	0.019	0.006	0.011	0.002	**				
PS	0.781	0.092	0.755	0.110	0.820	0.174	0.647	0.108	0.566	0.152	0.353	0.059		*			
LPC	0.398	0.081	0.332	0.054	0.127	0.021	0.103	0.019	0.089	0.024	0.054	0.010	**	*			
PC	6.476	0.784	6.799	0.951	2.733	0.631	2.215	0.383	2.152	0.392	1.387	0.214	**				
LPE(P)	0.436	0.102	0.311	0.043	0.071	0.013	0.061	0.012	0.042	0.014	0.025	0.007	**	**			
PG	0.509	0.053	0.359	0.047	0.093	0.034	0.073	0.031	0.049	0.017	0.021	0.007	**		*		
PI	1.434	0.085	1.097	0.103	0.359	0.100	0.280	0.073	0.247	0.067	0.142	0.016	**		*		
PE	13.685	1.577	9.756	1.120	1.408	0.323	1.179	0.248	0.903	0.235	0.552	0.076	**	*			
PE-P	2.320	0.136	2.134	0.201	0.693	0.111	0.591	0.137	0.305	0.103	0.193	0.037	**	**			
SM	0.425	0.021	0.351	0.032	0.333	0.029	0.289	0.033	0.278	0.056	0.208	0.025	**				
FFA	5.939	0.937	4.930	0.931	3.437	0.530	3.464	0.279	3.641	2.009	3.820	1.187	**				
CER	0.119	0.015	0.104	0.014	0.127	0.022	0.108	0.015	0.127	0.014	0.092	0.027					
DAG	0.192	0.034	0.254	0.058	0.294	0.055	0.327	0.047	0.336	0.076	0.281	0.038	*				
TAG	73.108	7.201	110.844	11.849	166.415	19.592	189.011	30.630	220.541	19.332	233.051	17.499	**	*	*		
hexCER	0.009	0.003	0.016	0.011	0.039	0.019	0.034	0.010	0.011	0.003	0.005	0.001		**			

LPE and LPE-P were summarized as LPE(P); the single LPI species detected is not included. Shown are mean values ± SD ($n = 6$).
* Significant difference ($p < 0.05$).
** Highly significant difference ($p < 0.005$) according to Student's *t*-test with Bonferroni correction.

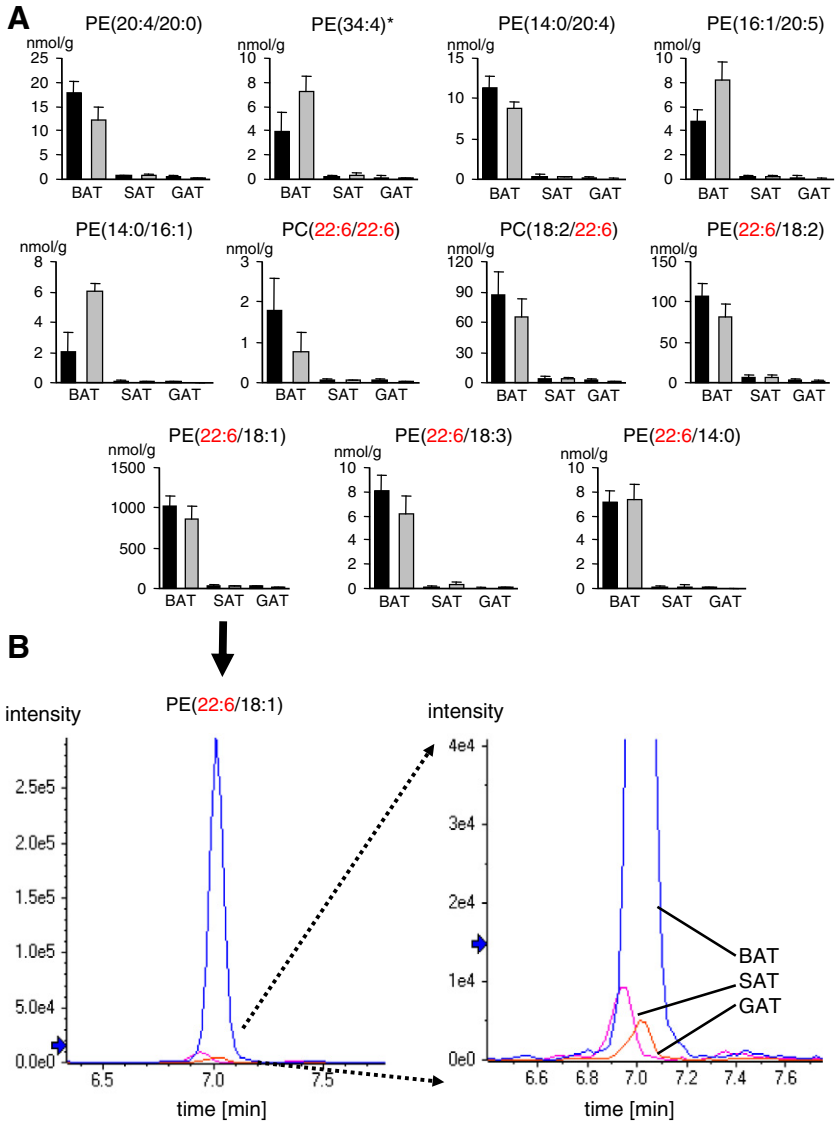

Fig. 3. A: Lipid species showing the most prominent increase in brown compared to both white adipose tissue depots in both sexes. Values are means ± SD of $n = 6$ mice. Black bars represent female and grey bars male mice. *PE(34:4) was composed of the co-eluting isomers PE(16:1/18:3) and PE(16:2/18:2). B: Extracted ion chromatogram of a representative BAT-characteristic phospholipid, PE(22:6/18:1).

DHA-enriched lipoproteins are preferentially targeted to BAT, in addition to heart and muscle [22]. The content of DHA-containing phospholipids in muscle mitochondria can be increased by dietary DHA supplementation [23] and BAT could possibly also be responsive to such a dietary intervention. The omega-3 fatty acid DHA is an important contributor to membrane fluidity [24]. Increasing the DHA content of phospholipids has been shown to improve the ADP sensitivity of human skeletal muscle [23]. In addition, DHA counteracts Ca^{2+}-induced transition pore opening [25], a mechanism that has been associated with its cardioprotective effects and that could also improve the stress tolerance of BAT mitochondria. In an in vitro approach, mitochondria were fused with liposomes enriched in one DHA-containing phospholipid species, causing increased proton movement [26]. This interesting experimental approach could, possibly, also be employed to study additional mitochondrial parameters and more complex alterations in phospholipid composition. Another difference revealed in this study, the higher FFA content of BAT compared to WAT, might also be related to the higher mitochondria content of BAT. Unbound fatty acids are required to activate UCP1 and since the largest fraction of the FFA pool is protein-bound, relatively high total FFA concentrations are required for uncoupling [27]. Another phospholipid class that could be expected to discriminate BAT and WAT are the mitochondria-specific cardiolipins which were, however, not covered by the applied LC-MS approach.

The differences between the two WAT depots were comparatively smaller, but still significant. Here, the main findings were a higher amount of TAG and a lower content of several phospholipid classes in GAT which could at least partly be explained by differences in cell size as phospholipids are mainly present in cell membranes. A previous study reported a slightly larger adipocyte area in epididymal than in subcutaneous fat of male mice [28] and our data suggest that a similar difference also exists between SAT and the ovarian depots of female animals.

An additional noteworthy finding was pronounced sex-specific differences in the lipid composition of murine BAT. Despite the fact that female and male GAT differ in their anatomical location and likely, biological function, we observed less sex-specific differences in lipid composition for GAT than for BAT. Male BAT had a higher TAG content, and one explanation for this finding could be a larger cell size. However, previous data on BAT cell size, which to date has only been reported for rats, suggests a similar [29] or even smaller [30] size of male BAT adipocytes. BAT of female rats appears to have larger mitochondria with a greater cristae density [30,31] which could, independent of cell size,

Table 2
Top 15 lipid species with the highest discriminating power for female (f) and male (m) brown adipose tissue (BAT) according to multivariate partial least square discriminant analysis (PLS-DA) variable importance.

Lipid	BAT-f		BAT-m		Fatty acid residues	
	Mean	SD	Mean	SD		
Higher in female BAT						
PC 36:2	17.66%	1.29%	12.76%	0.48%	18:0	18:2
PC 38:4	3.39%	0.31%	1.61%	0.08%	18:0	20:4
PC 40:4	0.10%	0.01%	0.06%	0.01%	18:0	22:4
PC 40:6	1.27%	0.23%	0.67%	0.07%	18:0	22:6
PC O-36:5[a]	28.99%	2.21%	22.69%	1.30%	20:4	16:1e[a]
PC O-38:4[a]	5.55%	0.43%	3.65%	0.61%	20:4	18:0e[a]
PE 38:4	26.45%	1.26%	17.34%	1.35%	18:0	20:4
PE P-38:4[a]	13.13%	0.75%	9.57%	0.38%	20:4	18:0p[a]
PE P-40:4[a]	1.38%	0.14%	0.89%	0.07%	20:4	20:0p[a]
PI 38:4	74.60%	4.17%	58.09%	3.95%	18:0	20:4
PS 40:6	34.74%	1.64%	29.43%	0.91%	18:0	22:6
LPC 18:0	39.63%	1.90%	30.84%	1.45%	18:0	
TAG 47:0	0.22%	0.03%	0.13%	0.02%		
TAG 48:0	1.52%	0.15%	0.90%	0.04%		
CER(d18:1/16:0)	4.03%	0.55%	2.35%	0.13%		
Higher in male BAT						
PC 30:0	0.64%	0.05%	1.03%	0.06%	14:0	16:0
PE 30:1	0.01%	0.01%	0.06%	0.00%	14:0	16:1
PE 32:0	0.06%	0.01%	0.16%	0.02%	16:0	16:0
PE 32:1	0.41%	0.18%	1.28%	0.12%	[b]	[b]
PE 32:2	0.20%	0.07%	0.58%	0.05%	[b]	[b]
PE 34:3	1.74%	0.50%	3.66%	0.13%	16:1	18:2
PE 34:4	0.03%	0.01%	0.07%	0.01%	[b]	[b]
PE 38:7	0.81%	0.12%	1.57%	0.09%	16:1	22:6
PE 42:8	0.57%	0.06%	0.84%	0.06%	20:2	22:6
PE P-32:1[a]	0.49%	0.18%	1.47%	0.07%	16:1	16:0p[a]
PE P-34:2[a]	5.68%	0.99%	9.71%	0.51%	18:2	16:0p[a]
PI 38:3	1.52%	0.26%	4.03%	0.67%	18:0	20:3
PI 40:4	0.25%	0.04%	0.64%	0.09%	18:0	22:4
TAG 46:3	0.05%	0.00%	0.07%	0.01%		
TAG 48:3	0.34%	0.06%	0.57%	0.03%		

Shown are mean relative contents ± SD ($n = 6$).
[a] P/p denotes plasmalogens (i.e., alkenyl/acyl), O/e denotes (alkyl/acyl) ether lipids.
[b] The fatty acid composition of co-eluted isomers is listed in the Supplementary Table S-1.

contribute to the higher amount of membrane-building lipids and the lower amount of TAG in female BAT observed in our study. FFA, which activate mitochondrial uncoupling, were similarly high in BAT of both sexes.

Another sex-specific difference that was most pronounced for BAT was the composition of the fatty acid chains contained in phospholipids. A set of residues, including arachidonic and stearic acid, occurred at higher levels in females while others, including linoleic and palmitic acid, were pronouncedly lower. Since all animals had been fed the same standard diet, the shifted fatty acid profile could be a result of biochemical desaturation and elongation processes that might take place at a higher rate in female mice, as delineated in Supplementary Fig. S-2. Arachidonic acid is preferentially incorporated by liver and BAT [32]. Our finding of major sex-specific differences in lipid composition only in BAT, but not in WAT, may imply that these hormonal effects are directly related to a higher demand of female BAT for arachidonic acid-containing phospholipids. A higher desaturation degree leads to increased membrane fluidity, and this is likely counterbalanced in female BAT by more long-chain acyl chains and an increased PE/PC ratio that promote membrane rigidity [33]. Given the high mitochondrial content of this tissue, the sex-specific phospholipid composition of BAT likely reflects differences in the composition of mitochondrial membranes which may, in turn, be related to the increased mitochondrial size and cristae density of BAT mitochondria from female rats described by Rodriguez-Cuenca et al. [30] who found these to resemble the morphological changes of mitochondria from cold-acclimated rats [30,34]. Similarly, a lower rate of TAG to phospholipids, as observed in female BAT in our study, also has been detected in BAT following cold acclimation [35,36]. These findings suggest a higher thermogenic activity of female BAT, and at least in rats this could, indeed, be the case [37]. Although highly speculative, it is interesting to note in this context that both female rodents [38,39] and pre-menopausal women [40,41] have been reported to be less prone to develop glucose intolerance or type 2 diabetes.

The key mediator of these sex-specific differences appears to be estrogen which has been found to protect against the metabolic syndrome in mouse models and post-menopausal women [42,43]. In addition to other, more widely known central and local actions, estrogen may also facilitate the conversion of linoleic to arachidonic acid, resulting in a detectably higher content of PUFA in liver and plasma [44,45]. Another study failed to show estrogenic effects, but indicated that progesterone upregulates the expression of elongases and desaturases in cultured hepatocytes [46] that could accelerate the conversion of linoleic to arachidonic acid. Thus, sex hormones may regulate not only BAT activity and mitochondrial biogenesis [47,48] but also the lipid composition of its membranes. In rats, the differing hormonal regulation results in a higher thermogenic capacity and greater BAT mass relative to body weight of the female animals [30]. Interestingly, metabolically active BAT also has a higher prevalence and glucose uptake activity in human females compared to males [49,50]. Although the difference is less pronounced than in mice, serum PUFA compositions are also higher in women than in men [51,52], and there is compelling evidence for a decisive role of sex-steroid hormones in the regulation of human energy homeostasis and fat distribution [53–55] which is particularly obvious in pathophysiological conditions such as the polycystic ovary syndrome [56]. Thus, despite the comparatively smaller amount of BAT in humans, differences in BAT metabolism might still contribute to sex-specific differences in human whole-body metabolism and energy homeostasis. Due to the small amounts of tissue required, lipidomics could even be a conceivable approach to unravel such sex-specific differences in humans.

5. Conclusions

To summarize, we generated lipid profiles from BAT and two WAT depots from female and male mice. This is, to the best of our knowledge, the first study to elucidate the differences in lipid composition between female and male BAT. Our high resolution tandem-MS approach enabled us to obtain the fatty acid composition of all phospholipids, and these data might serve as a reference lipid bank for future studies on murine adipose tissues. A key finding of this study is that differences between the lipid profiles of BAT and WAT were dominated by DHA-containing phospholipids which are likely to regulate the thermogenic function of BAT as components of the mitochondrial membrane. In addition, we identified arachidonic acid as the phospholipid residue dominating the sex-specific lipid profile of BAT from female mice. This finding may prompt further studies aiming to understand sex-specific differences in BAT metabolism.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbalip.2014.08.003.

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