Triple-knockout, synuclein-free mice display compromised lipid pattern

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Abstract

Background: Recent studies have implicated synucleins in several reactions during the biosynthesis of lipids and fatty acids in addition to their recognised role in membrane lipid binding and synaptic functions. All members of the synuclein family interact robustly with lipid membranes, and appear to be important for the physiological functions of proteins while influencing the pathological aggregation of α-synuclein.

Methods: The following tissues were used for lipid and fatty acid analysis: plasma, liver and two brain areas (cortex and midbrain). Lipid classes were separated using thin-layer chromatography. Fatty acids were analysed using gas chromatography.

Results: We describe the importance of long-chain polyunsaturated fatty acids (LCPUFA) and palmitic acid in liver and plasma, reduced triacylglycerol (TAG) accumulation in liver and circulated plasma non-esterified fatty acids in synuclein free mice. In midbrain, observed changes in the relative concentrations of phosphatidylcholine (PC) and cerebrosides (CER) were counterbalanced. In midbrain, we recorded a notable reduction in ethanolamine plasmalogens in synuclein free mice and consider this an important finding considering the abnormal ether lipid metabolism usually associated with neurological disorders.

Conclusions: In summary, our data demonstrate that synuclein deficiency can result in alterations of PUFA synthesis, storage lipid accumulation in liver, and reduction of plasmalogens and CER, those polar lipids which are principal compounds of lipid rafts in many tissues. An ablation of all three synuclein family members resulted in more pronounced lipid modifications then previously showed by us γ-synuclein deficiency. Possible mechanisms by which synuclein deficiency may govern the reported modifications of lipid metabolism in TKO mice are proposed and discussed.

Background

The synucleins are small, highly conserved proteins, expressed primarily in the nervous system of vertebrates. The synuclein family comprises three closely related proteins, α-, β- and γ-synucleins with distinctive functions that share sequence homology with other proteins such as the class A2 lipid-binding domains of the apolipoproteins, 14-3-3 chaperones and several small heat-shock-proteins [1, 2, 3, 4, 5].

Interest in this protein family is first and foremost associated with their special role in human neurodegenerative diseases, collectively termed “synucleinopathies”, and largely with a potential involvement of α-synuclein aggregates in Parkinson’s disease (PD). Many studies show that oligomers, protofibrils, some intermediates and fibrils of α-synuclein are toxic agents [6, 7, 8, 9, 10, 11]. Moreover, it has been suggested that α-synuclein fibrils are transmittable (*via* exosomes and tunneling nanotubes) to induce the aggregation of endogenous α-synuclein in primary neurons via “prionoid” mechanism [9, 12, 13].

α-Synuclein was originally identified as a protein associated with synaptic vesicles (SV) [14]. It is widely expressed in various brain regions but localizes specifically to the nerve terminal where it modulates synaptic functions. Several non-neural tissues, e.g. red blood cells also express this protein. Like α-synuclein, β-synuclein also localizes at presynaptic terminals but it is not involved in the etiology of PD. Instead, β-synuclein has been shown to inhibit the aggregation of α-synuclein both *in vitro* and *in vivo* [5, 16]. Mutations in β-synuclein have been linked to case of dementia with Lewy bodies [17, 18]. In contrast to α- and β-synucleins, γ-synuclein is predominantly expressed in sensory neurons and motoneurons of the peripheral nervous system. It has not been associated with the onset or suppression of PD. This family member is also expressed in retina and in a variety of cancer cells [1, 2].

Full length of α-synuclein is 140 amino-acid protein and its sequence comprises three overlapping regions including the N-terminal region, the NAC (non-Aβ component) region and C-terminal region. The positively charged N-terminal region contains seven 11-residue repeats with the structural features similar to those of apolipoprotein-like class A2 amphipathic α-helix. This terminal also retains one of two conserved fatty acid binding protein (FABS) motifs. N-regions of β- and γ-synucleins show a high structural homology to that of α-synuclein [2].

The hydrophobic NAC region is also involved in membrane binding and serves as the building block of α-synuclein aggregates during its conformational change from random coil to β-sheet structure to form amyloid-like protofibrils and fibrils. β- and γ-synucleins are distinct form α-synuclein by these features due to their lack of most NAC sequence [19, 20, 21]. These differences have been linked to the various propensities of synucleins to aggregation, with α-synuclein having the highest proneness to form aggregates while β-synuclein is the last prone [22]. The negatively charged C-terminal region is highly unstructured and has been found to retain unstructured in the amyloid fibril. Post-translation phosphorylation and nitration and of some sites of this region changes the net charge of the C-terminal region and affect the α-synuclein aggregation and membrane binding. Moreover, C-truncated form of α-synuclein exacerbates aggregation [1, 2, 23, 24]. From these findings, it has been suggested that this region may play a role in regulation of α-synuclein function and conformation. Moreover, this tail mediates interactions between α-synuclein and soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) complex proteins [23, 25]. This domain is less conserved among α, β- and γ-isoforms.

α-Synuclein constitutes about 1% of the total brain proteins in healthy brain tissue and its normal physiological state is still debating (26). Either monomeric form of α-synuclein or the formation of α –helically folded tetramers and related multimers has been demonstrated [24, 27, 28, 29, 30, 31]. In general, it is believed that the monomeric N-terminal acetylated form represents the functional form of the protein [32].

The functions of α-synuclein have been thoroughly reviewed elsewhere [2, 5, 8, 21, 33, 34, 35]. They include modulation of neuronal plasticity and neurotransmission, synaptic vesicle pool maintenance and trafficking, dopamine metabolism, SNARE regulation, vesicle recycling and synaptic integrity.

All members of the synuclein family interact robustly with lipid membranes, and appear to be important for the physiological functions of proteins while influencing the pathological aggregation of α-synuclein. Many *in vitro* experiments have demonstrated the affinity of synucleins towards membranes containing negatively charged lipids, esterified with oleic acid and polyunsaturated fatty acids, suggesting that it may specifically recognize the membrane microdomains that differs in fluidity and charging [8, 16, 32]. α-Synuclein also associates with the membranes composed of zwitterionic phospholipids with varied reports of strong or weak binding [36, 37, 38].

Upon membrane binding, synucleins can adopt a range of structural architectures, such as a pair of anti-parallel curved α-helices or a single curved α-helix, encompassing approximately the entire N-terminal region [32, 39]. The α-synuclein helix has been shown to extend parallel to the curved membrane; its conserved N-terminal residues are attached to the twitterionic headgroups of the outer membrane leaflet, while uncharged residues penetrate into acyl chain region ensuring a strong interaction of synuclein monomers and membrane bilayer [39, 40, 41, 42]. Membrane-induced amphipathic helices can act as membrane curvature sensors and modulators by binding preferentially to hydrophobic lipid packing defects enriched in curved surfaces. This property implies the functions of α-synuclein in synaptic vesicle (SV) exocytosis, endocytosis, and vesicle recycling [43]. This protein not only senses curvatures but also a curvature-inducing. Two other synucleins are also able to interact with lipid bilayers and generate membrane curvature [18, 44]. This interaction induces a large scale of membrane remodeling, such as tubulation and vesiculation; it also may reorganize membrane bilayer through lifting and lateral expansion of lipids leading to the membrane warping and structural destabilization [33, 41, 45, 46, 47, 48]. Moreover, oligomeric species formed during self-assembly of α-synuclein enhanced membrane permeability, and induced substantial increases in the intracellular reactive oxygen species production and, thus, neuronal toxicity [22, 49]. In contrast, aggregation of α-synuclein may be neuroprotective by sequestering its toxic oligomer.

Furthermore, synucleins respond to the presence of lipid rafts. Post-translational modifications, such as the addition of glycosylphosphatidylinositol anchors, palmitoylation and/or myristoylation, have been suggested to localize proteins to rafts [50, 51, 52]. In general, a greater number of pre- and post-synaptic proteins, involved in neuronal communication, are localised to lipid rafts. These cholesterol/sphingomyelin-enriched microdomains are well-established as plasma membrane signaling assemblies [50, 51]. In lipid rafts, a special role of long chain polyunsaturated fatty acids, namely docosahexaenoic acid (C22:6n3, DHA), has been shown in modulation of the architecture of lipid raft domains with potential implications for signaling network [53]. It has been suggested that α-synuclein interacts with PUFAs to stabilize its three-dimensional structure, whereas PUFA peroxidation products, which are associated with the brain mitochondrial oxidative stress and neurodegeneration, may modify α-synuclein and induce its toxic oligomerization [54]. In spite of some controversy, the majority of findings support the connection between oligomeric α-synuclein and PUFA oxidation in dopaminergic toxicity, which is increasing when α-synuclein interacts with PUFA and their oxidation products. Insofar, many studies revealed the molecular basis of interactions between α-synuclein and PUFA and their importance in both, neuroprotection and neurodegeneration *in vitro* and *in vivo*, including many PD cell and animal

models [31, 32, 55–59]. Lipids influence the structure and, consequently, the functions of synucleins (and vice versa, of note!) through complex interplaying and overlapping mechanisms. As an interplay, numerous recorded changes in lipids (and lipid metabolism in cells and various synuclein mice models) were associated to either, the synuclein loss-of-function or a toxic gain-of-function hypothesis (summarised recently by Alza and co-authors [59]. In summary, they include: uptake of fatty acids into cells; changes in brain cardiolipin and its fatty acid acyl chains [2]; an increased level of brain triacylglycerol (TAG) content in α-synuclein mice mutant model associated with increased fatty acid synthase expression and acyl-CoA synthase activity, with no variations in TAG lipase activity or in fatty acid β-oxidation [60]. α-Synuclein-null animals showed decreased palmitate uptake and the altered metabolism of this FA in brain, and turnover of arachidonate and docosahexanoate in brain phospholipids [61, 62]. Our previous work on γ-synuclein null mice showed significant brain region-specific changes in phosphatidylserine and docosahexaenoic acid when compared to wild-type mice [63]. This member of the family has been demonstrated to be directly involved in lipid metabolism by enhancing TAG lipolysis in brown and white adipose tissue where γ-synuclein was highly-expressed [64, 65]. In white fat adipocytes isolated from TKO mice, TAG synthesis was further boosted by high fat diet or reduced by caloric restriction [64, 65].

In this work, we studied how deficiency of all three synucleins affected lipid composition in the brain regions of young adult triple knockout (TKO) mice that at this age do not display any signs of pathological changes in the nervous system [66, 67]. We suggested that triple ablation would eliminate a well-known effect of functional redundancy and compensatory ability for loss of functions of the absent synuclein family member(s). In addition, in order to get a broader view on possible lipid modulations in transgenic mice, lipids and fatty acid composition of plasma and liver were also investigated.

Material And Methods

Animals

A line of synuclein-free mice that are null mutants for the all three genes coding for α-, b- and γ-synuclein was described early [4]. The colony was kept on C57Bl6J (Charles River) background and wild-type control mice on the same C57Bl6J (Charles River) background were bred in the Cardiff University Transgenic Animal Unit. Mice were maintained in conventional open-lid cages with ad libitum access to standard chow and water. Animals were fed special diet DOI 58Y2 with energy from fats (labDiets). Fatty acid composition of the diet is given in our previous publication [63]. Genotyping was carried out by conventional PCR as described previously [4]. Animals were sacrificed by cervical dislocation and tissues were collected on ice and processed. All animal work was carried out in accordance with the United Kingdom Animals (Scientific Procedures) Act (1986).

Lipid analysis

The following tissues were used for lipid and fatty acid analysis: plasma, liver and two brain areas (cortex and midbrain). Lipids were extracted immediately by Folch's method [68], and lipid extracts were stored at

-20 °C under nitrogen atmosphere prior to further analysis. Non-polar lipids were separated using one-dimensional TLC on 10 × 10 cm silica gel G plates (Merck KGaA, Darmstadt, Germany) in the solvent system hexane/diethyl ether/acetic acid (80:20:1, v/v/v). Polar lipids were separated by two-dimensional TLC on 10 × 10 cm 1.2% boric acid-impregnated silica gel plates using chloroform/methanol/ammonium hydroxide (65:25:4, v/v/v) in the first dimension and *n*-butanol/acetic acid/water (90:20:20, v/v/v) in the second direction. Lipids were identified by reference to authentic standards and by using specific colour reagent [69]. In addition, several lipid identifications were performed using HPLC-MS-MS (details can be found in our previous publication [63]. Plates were sprayed with 0.2% (w/l) 8-anilino-4-naphtholenesulphonic acid in dry methanol and viewed under U.V. light to reveal lipids. Individual lipids were scraped from the plates and their fatty acid compositions and contents were determined by gas chromatography (GC) with heptadecanoate (C17:0) as an internal standard.

Fatty Acid Analysis

Aliquots of the total lipid extracts and individual lipid classes separated by TLC were used for fatty acid methyl esters (FAMEs) preparation. FAMEs were prepared using a solution of 2.5% H_2SO_4 in dry methanol/toluene (2:1, v/v). Samples were heated to 70 °C for 2 h, with occasional vortexing. FAMEs were extracted by two additions of 3 ml of hexane. The hexane fractions were transferred into clean glass tubes, evaporated under a stream of nitrogen and reconstituted in 50 µl of hexane. All reagents for fatty acid extraction and derivatisation were of HPLC quality.

Gas Chromatography

FAMEs were analysed using a Clarus 500 GC with a flame ionisation detector (Perkin-Elmer, Norwalk, CT, USA). A Perkin-Elmer Elite 225 column (30 m x 0.32 mm x 0.25 µm) was used with a nitrogen carrier gas constant pressure of 20 psi. The oven temperature program began at 170 °C for 3 min, was ramped to 220 °C at 4 °C/min and was then held for 30 min. FAMEs were identified by comparison of the peak retention times with those of a GC-411 standard mixture (Nu-Chek Prep. Inc., Elysian, MN, USA). Perkin-Elmer TotalChrom software was used for data acquisition and calculation.

Statistical Analysis

Statistical significance between groups was determined by Mann-Whitney U test. A probability of error less than 5% was considered significant (i.e., $P < 0.05$).

Results

Total lipids and lipid classes in liver and plasma from WT and TKO mice

Synuclein deficiency resulted in a significant reduction of total lipids in liver compared to those in WT animals, whereas the concentrations of total lipids in plasma were unchanged brain, the similar amounts of total lipids were found (data not shown).

The profiles of lipid classes found in liver and plasma from WT and TKO mice are shown in Fig. 2. In liver, total polar lipids (TPL) and TAG were the major fractions with SE being a minor class in both, WT and TKO mice. The relative amounts of TPL increased in liver of TKO animals (from 34–48.8%), whereas those of TAG and SE decreased (from 63.7–50% and from 2.3–1.2%, consequently) (Fig. 2a). In plasma, free fatty acids (FFA, or non-esterified FA) were also found, and the level of this fraction significantly decreased in TKO samples (4.8%) in comparison to WT mice (11.1%) (Fig. 2b). TPL and TAG were unaltered in plasma of TKO mice; the relative amount of SE was much higher than in liver and increased in TKO mutants up to 21.3% compared to WT animals (16.4%).

Fatty acid composition of total lipids in liver and plasma from WT and TKO mice

Palmitic (C16:0), stearic (C18:0), oleic (C18:1n9), linoleic (C18:2n6, LA) and arachidonic (C20:4n6, ARA) and docosahexaenoic (C22:6n3, DHA) acids were the major FA with moderate amounts of dihomo-γ-linolenic (C20:3n6, DGLA), vaccenic (C18:1n7) and two isomers of C16:1, palmitoleic (n7) and n9-hexadecenoic acids in plasma and liver of WT and TKO mice (supplemental Table 1). The percentages of four major PUFA, namely LA, ARA, DHA and DGLA, were lower in plasma of TKO mice compared to WT, whereas the level of palmitic acid was increased in this mice model. In liver of TKO animals, the percentages of oleic acid were lower and those of palmitic and DGLA were higher when compared to WT (supplemental Table 1).

Fatty acid composition of lipid classes in liver and plasma from WT and TKO mice

In liver and plasma, all separated lipid classes were analysed for their fatty acid composition. FA profiles of TAG isolated from liver and plasma are quite similar with the domination of palmitic and oleic acids followed by LA, stearic and isomers of C16:1. Only minor amounts of PUFA (non-exceeding 2%) were present in plasma and liver with higher percentages of α-linolenic acid (C18:3n3, ALA) in TAG of plasma of both, WT and TKO animals (supplemental Table 2).

In TAG of both, plasma and liver, only subtle differences in the FA percentages were found when compared WT and TKO mice. In plasma, only the level of stearic acid was affected by synuclein deficiency: its relative amount was lower in plasma TAG from TKO mice. In liver TAG, the percentages of both isomers of C16:1 were higher whereas the level of other monoenoic FA, oleic acid, which is predominant in TAG, was lower and accounted for 52.5% and 47.3% in liver from WT and TKO mice, relatively (supplemental Table 2).

FA of SE from plasma are characterised by high levels of ARA (about 30% in both, WT and TKO mice) and LA accounted for 34% in plasma SE from WT and for a higher level (42.4%) in TKO mice (supplemental Table 3). Other PUFA, including ALA, DGLA, EPA and DHA were detected in small amounts (from about 1% – 4%) in WT and TKO mice. Saturated palmitic and stearic acids were found in the relative amounts of non-exceeding 6% and 4%, respectively, in WT mice. In TKO mice model, the level of palmitic acid was not changed comparing to WT, whereas that of oleic acid decreased from 3.7–1.9% under synuclein deficiency. In SE fraction isolated from liver, oleic acid was dominated in both WT and TKO mice with a significant decrease in the latter (from 61.1–43.2%). Two other major FA in SE of liver were LA (no difference in its relative amounts were found between WT and TKO mice) and palmitic acid. The percentages of this acid were significantly higher in SE of liver from TKO animals. The level of other saturated FA, oleic acid, was also increased in TKO mice up to 9.8% in comparison to 4.7% in WT. Also, higher percentages of C16:1n9 and ALA in SE were detected in liver of TKO mice. No long-chain PUFA, except ARA (1.9% in both WT and mutant mice), were detected in liver of the studied animals (supplemental Table 3).

The FA profiles of the TPL fraction isolated from plasma and liver are present in Table 1. In general, FA compositions of this lipid fraction in both samples from WT were characterised by large relative amounts of saturated FA: palmitic and oleic acids, mono- and dienoic C18 FA: oleic acid and LA, and ARA followed by DHA, DGLA, nervonic acid (C24:1n9) and minor amounts of ALA and palmitoleic acid. These parameters were remarkable affected by synuclein deficiency especially in plasma. In TPL of plasma from TKO mice, significant reduction in the level of LA (from 19.2% in WT to 2.7% in mutants) and only trace (< 0.5%) amounts of ARA, DHA and DGLA in TPL of plasma from these animals. These changes were accompanied by a significant increase in the level of saturated palmitic acid and monoenic oleic acid (Table 1). In liver of studied animals, analysis of FA in TPL fraction also revealed an increase in palmitic and oleic acids, and a decline in the percentages of ARA and DHA. The relative amounts of LA and DGLA were not altered in TPL fraction in liver from TKO mice.

Composition of free fatty acids in plasma

A comparison of profiles of free fatty acids isolated from plasma of WT and TKO animals is illustrated by Fig. 3. In consonance with the data (described above) on FA profiles of total lipids, as well as in SE and in TPL, the analysis of FFA showed a reduction of percentages of LA (from 16.6% in WT to 12.4% in mutants), ARA (from 6.2–1.5%) and DHA (from 2.5–0.3%) together with an increase in C16:1n9, oleic acid and ALA. No differences were noted for saturated FA and palmitoleic acid (Fig. 3).

Fatty acid composition of individual phospholipids in liver from WT and TKO mice

Figure 4 shows the data on fatty acid composition of the individual polar lipids (PL) in liver from WT and TKO mice. PL composition was in keeping with generally known lipids found in mammalian livers, particularly: phosphatidylcholine (PC) and phosphatidylethanolamine (PE) as the predominant

phosphoglycerolipids, followed by phosphatidylserine (PS), phosphatidylinositol (PI), cardiolipin (CL) and sphingomyelin (SM) (data not shown).

Analysis of FA in PC revealed six dominant FA, namely palmitic, stearic, LA, ARA, oleic and DHA varying in their percentages from 18.9% (palmitate) to 8.8% (DHA) in PC from WT mice. Vaccenic, palmitoleic and ALA were found in low or trace amounts in PC of liver from studied animals. In synuclein-free mice, a level of ARA was significantly reduced from 14.5–8.9% with a reciprocal increase in relative amounts of LA (from 15–18.6%); the reduced percentage of stearate was also noted in PC of liver of TKO mice (Fig. 4). Liver PE was dominated by the same FA as found in PC, although their relative concentrations were different (Fig. 4). Thus, ARA, stearate and DHA showed the highest percentages accounting for 22%, 19.5% and 17.5%, respectively, then palmitate (15.2%), oleate (11.1%) and LA (9.2%), in WT animals. Palmitoleic acid was present only in trace amounts; vaccenic, eicosapentaenoic (C20:5n3, EPA) and docosapentaenoic (C22:5n3, DPA) acids were the minor ones in PE of liver from both, WT and TKO mice. Synuclein-deficiency resulted in a decline of DHA percentages and in an appearance of small (0.6–0.8% of total FA) amounts of ALA and gondoic acid in liver PE.

In PS, stearate, ARA and DHA were dominant acyl chains (43.7%, 25.1% and 12.8%, respectively) with palmitic and oleic acid accounted for 6.2% and 4.5% of total FA in liver of WT (Fig. 4). Minor percentages of vaccenate, LA, ALA, adrenic acid (C22:4n6) and DPA were identified in PS of liver from studied animals. A notable decrease in the level of ARA (from 25.1–17.3%) alongside with an increase in palmitate (from 6.2–11.1%) percentage was found in PS of liver in TKO mutants. Two FA, namely stearate and DHA were prevalent reaching 46.3% and 41.2% in PI of liver from the studied animals (Fig. 4). Moderate amounts of palmitate (3.1%), oleate (2.4%), DGLA (2%), eicosadienoic acid (C20:2n6; 1.4%) and LA (1.3%) as well as the trace and low amounts of vaccenate, ALA and DHA were detected in WT mice. In PI of liver from TKO mutants, a significant reduction in the percentage of DHA (from 41.2–29.9%) was noted that is similar to the tendency showed for PC and PS (Fig. 4). This decline was accompanied by an enlargement in the amounts of palmitate (from 3.1–6.6%), oleate (from 2.4–4.4%) and LA (from 1.3–3.1%) in liver of synuclein-free mice.

In cardiolipin, more than 50% of the total fatty acid chains was accounted for LA. Other FA with the relative amounts in the range between 4% and 10% were: DHA, DGLA, vaccenate, palmitate, stearate and oleate. Modest levels of eicosadienoic, arachidic and trace amounts of ARA and eruric acid (C20:1n9) were also determined as other acyl chains in cardiolipin from mouse liver. No differences in cardiolipin FA profiles in liver form WT and TKO mutants were revealed during this study (Fig. 4). Sphingomyelin (SM) (sphingophospholipid) was characterised by appreciable amounts of saturated fatty acids including palmitic (20.2%), stearic (15.7%), behenic (11.1%) and lignoceric (11.2%) acids as well as a long-chain monoenoic acid, nervonic (C24:1n9) acid (20.7%) in liver from WT. Other shorter chain monoenoic acids, namely eruric and oleic acids were found in the relative amounts of 4.8% and 4.6%, respectively, in WT mice. Small percentages of DHA, ARA, DGLA, isomers of C16:1 and vaccenic acid were also determined in these samples. Under synuclein deficiency, a significant increase in behenic acid (from 11.1–19.1%) was

recorded in liver SM with no statistically significant changes in other acyl chains of this sphingophospholipid (Fig. 4).

Fatty acid composition of total lipids in midbrain and cortex from WT and TKO mice

Midbrain FA were characterised by high levels of palmitate and oleate (about 20% of total FA), followed by palmitate (17% in WT and 18% in TKO mice) and DHA (12% and 14% in two groups of studied animals) (supplemental Table 4). The percentages of ARA were 8% in WT animals and 9% in TKO mutants; the levels of adrenic, gondoic and vaccenic acids were in the range of 3–5% of the total FA; moderate/small amounts of lignoceric, nervonic, hexacosenoic acid (C26:1n9) and palmitoleic acids were also detected. In this brain region, synuclein deficiency resulted in a slight increase of ARA from 8.3 ± 0.5% in WT to 9.3 ± 0.4% in TKO mice in parallel to a little decrease in the percentages of hexacosenoic (from 2.8 ± 0.2% to 1.8 ± 0.1%), nervonic (from 2.7 ± 0.3% to 1.8 ± 0.2%) and lignoceric (from 0.9 ± 0.1% to 0.6 ± 0.1%) acids (supplemental Table 6).

Two major saturated FA, namely palmitate and stearate, were determined at 21–22% of the total FA in cortex from WT and TKO animals, followed by DHA (18%), oleate (16%) and ARA (11%). In comparison to FA composition in midbrain, in cortex, the lower levels of oleate, vaccenate, gondoic, adrenic, lignoceric, nervonic and hexacosenoic acids alongside with the higher percentages of palmitate, ARA and DHA were found (supplemental Table 4). No changes in cortex FA were noted in TKO mice when compared to WT animals.

Composition of individual phospholipids in midbrain and cortex from WT and TKO mice

Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were most abundant in both brain regions (around 30–40% of TPL), followed by phosphatidylserine (PS), accounted for about 15% of PL, phosphatidylinositol (PI), sphingomyelin (SM), cardiolipin (CL), sulfatide (SL) and cerebroside (CER): the latter five lipids were found in the percentages non-exciding 10% of TPL (Fig. 5). In midbrain, the amounts of PC and SM were lower, while the levels of SL, CER and CL were higher than the concentrations of these polar lipids in cortex. An effect of synuclein ablation was seen only in midbrain, where the concentration of PC was elevated in TKO mice and the amount of CER was reduced when compared to WT animals (Fig. 5).

Fatty acid composition of individual phospholipids in midbrain and cortex from WT and TKO mice

Figure 6 shows the data on fatty acid composition of the individual polar lipids in midbrain and cortex from WT and TKO mice. The FA distribution in these lipids is typical of that which has been established in murine brain tissues including our previous study (63). Analysis of FA in PI showed a domination of stearic acid and ARA in both brain regions from WT mice and TKO mutants with a higher level of ARA in

cortex in comparison to midbrain (34% vs. 42%) (Fig. 6). In cortex, the lower relative amounts of oleic acid and DHA were also noted. Appreciable amounts of palmitic acid and two isomers of C18:1 acid together with rather low (about 0.5% of total FA in PI) level of palmitoleic acid were also determined in PI from both brain regions of studied mice. In midbrain, in addition to described FA, gondoic acid (C20:1n9 and adrenic acid (C22:4n6) were found in the relative amounts of about 1% of FA, whereas in cortex these acids were present only in trace amounts (less than 0.5% of total FA). There were no differences in FA composition in PI in midbrain and cortex between WT and TKO animals.

In PS, stearic acid, combined isomers of C18:1 (n7 + n9) and DHA were the major acyl chains with the higher levels of stearate (43% vs. 40–41%) and DHA (33% vs 19%) in cortex of both, WT and synuclein-free mice, and significantly lower (nearly halved) percentages of C18:1 (n7 + n9) (14% vs. 25–26%) in this brain region. In addition, the lower relative amounts of gondoic acid and ARA were found in cortex. Synuclein-deficiency did not course any changes in FA composition of PS in midbrain and cortex from the studied animals (Fig. 6).

Analysis of FA in PC showed the domination of palmitate in midbrain (34% in WT and 37% in TKO mice) and in cortex (42% in WT and 44% in TKO mice) of studied animals. In both brain regions, palmitate and oleate were also determined in considerable amounts in PC with slightly lower levels in cortex (13% vs. 17%, and 6% vs. 8%, respectively, in WT mice). ARA, DHA and vaccenic acid were present in the range of 4–8% of the total FA in midbrain and cortex of the studied animals. In minor amounts, adrenic, gondoic and isomers of C16:1 were determined in this phospholipid from both brain regions. In midbrain PC of synuclein-free mice, the proportions of DHA, gondoic and stearic were slightly reduced in comparison to WT animals. No effect of synuclein ablation was seen in the studied mouse model.

In other major phospholipid of brain, PE, the dominated FA in midbrain were C18:1 (22% as two combined isomers), DHA (18%), stearate (16% in WT) and ARA (10–11%). In addition, a significant amount of plasmalogen form of PE, estimated as percentages of dimethyl acetals (DMA), were detected in the proportion of 2.1% of C16:0 DMA and 14% C18:0 + C18:1 DMA in midbrain with lower amounts of the latter in cortex (8% in WT mice). Brain PE also contains moderate amounts of palmitic, gondoic and adrenic acids as well as a minor quantity of two isomers of C16:1 in studied animals (Fig. 6). A reduction in C18 DMA (from 14.2 ± 1.0% to 11.4 ± 1.3%) in midbrain as a result of synuclein-deficiency was revealed when comparing PE from WT mice with TKO mutants. This decline was accompanied by a little rise (1–2%) in the relative concentrations of saturated palmitic and stearic acids. In cortex, FA composition of this lipid was unaffected by synuclein ablation in studied mouse model (Fig. 6).

Cardiolipin isolated from brain tissues contains oleate as a dominant FA (39% in midbrain and 34% in cortex of WT) followed by ARA, DHA and stearate in the range from 17–9% in midbrain and from 18% and 11% in cortex of WT mice (Fig. 6). In moderate amounts (2–7%), palmitate, isomers of C16:1, vaccenate, and gondoic acid were determined in this lipid from both brain regions. Adrenic acid was present in relatively small concentrations with 1% and 2% in midbrain and cortex, respectively. It should be noted, that LA, which is a predominant FA in cardiolipin from liver, was not detected in cardiolipin from

brain samples in this work (as well as in other analysed polar lipids). These findings are in accordance with the previous studies (see Discussion). Synuclein-deficiency affected only FA composition in cardiolipin from cortex with no changes found in midbrain. The relative amount of oleic acids was increased in TKO in comparison to WT animals (37.6 ± 0.9% vs. 33.5 ± 0.5%) for the expenses of the levels of vaccenic acid that was slightly reduced from 5.6 ± 0.1% in cardiolipin WT to 3.9 ± 0.2% in TKO mice model.

FA profile in SM was characterised by dominated amount of saturated FA, namely stearate (63% in midbrain and 80% in cortex from WT mice), and moderate percentages of palmitic, lignoceric, behenic and arachidic acids (4–7% in midbrain from WT animals). The relative concentrations of three latter FA were lower in in SM of cortex from these animals. Monoenoic acids, including nervonic acid and isomers of C16:1 and C18:1 acids, were another group of acyl chains detected in this lipid with nervonic acid being dominated and counted for 13% in midbrain and for 9% in cortex from WT mice. The relative concentration of this acid was much lower (9% vs. 13%) in cortex, whereas the level of stearic acid was significantly higher (80% vs. 63%) in cortex when compared to midbrain from WT animals. In midbrain of synuclein-free mice, subtle reductions in the levels of C18:1 isomers (from 1.6 ± 0.4% to 0.7 ± 0.2%) and arachidic acid (from 3.7 ± 0.5% to 2.2 ± 0.4%) were noted. No changes in FA composition of SM were found in cortex in TKO mutants.

The composition of the conventional (non-hydroxylated) FA of two major brain glycosphidgolipids, e.g. sulfatides and cerebrosides, is shown in Fig. 6. As indicative for these lipid classes, SL and CER contain saturated and monounsaturated (n9) FA in the range of carbon chains from C16 to C26. Stearic and nervonic acids were the major FA in SL from midbrain and cortex ranging from 22–28%. Substantial proportions of oleic, hexacosenoic and palmitic acids were found in this sphingolipid from both brain regions. Some lower but appreciable amounts of 9-hexadecenoic, arachidic, gondoic, behenic, eruric and lignoceric acids were detected in SL in brain of studied animals. In TKO mutants, the relative concentrations of lignoceric (7.5 ± 1.1%), nervonic (21.8 ± 2.0%) and hexacosenoic (5.7 ± 0.1%) acids were reduced in comparison to those in WT mice (10.4 ± 1.1%; 25.4 ± 1.7%; 7.5 ± 0.4%, respectively). Analysis of FA in SL of cortex revealed only a small decline in the proportion of hexacosenoic acid from 7.0 ± 0.2% in WT mice to 6.2 ± 0.3% in synuclein-free mice.

Three very-long-chain FA, namely lignoceric (9–13%), nervonic (30–33%) and hexacosenoic (8–16%) acids were predominant in CER isolated from both brain regions of the studied animals (Fig. 6). When comparing the FA profiles of this lipid class between midbrain and cortex, the latter was characterised by higher levels of 9-hexadecenoicstearic, stearic, lignoceric and tricosanoic (C23:0) acids and the lower percentages of hexacosenoic acid. Synuclein-deficiency resulted in the reduced amounts of hexacosenoic acid in CER isolated from midbrain from 15.6 ± 1.2% in WT to 12.7 ± 1.1% in TKO mice. No changes in CER acyl chains from cortex between WT and synuclein-free animals were noted in our study (Fig. 6).

Discussion

Mammalian cells produce a remarkable diversity of fatty acids and lipids, but deeper understanding of molecular complicity of lipids in cells is arising from the technological advances offered by lipidomics [70, 71]. Such complicity is the result of many networking processes including the synthesis, trafficking and turnover of lipid compounds which are strongly regulated in different ways in different cell types and tissues [72].

Involvement of synucleins in the modulation of lipid metabolism has been shown by numerous studies [59, 73]. Special attention has been paid to the complex interactions between lipids (fatty acids and membrane lipids) and synucleins, which have been briefly outlined in Introduction. Indeed, previous studies on synuclein-lipid binding have provided pivotal information on structural properties of these protein and membrane interactions, but studies of membrane lipids/proteins, using simplified mixture of common lipids and *in vitro* approaches, may not adequately reveal the role of synucleins in membrane binding, reorganisation or signalling. In particular, a link between dysregulation of lipid metabolism and the pathology of Parkinson's disease has been widely recognised [30, 73], and more complex research on the involvement of synucleins in modulation of lipids on the tissue level is needed on the role of synucleins in PD, and in the cell in general. Based on this suggestion, we analysed and compared lipid and fatty acid profiles of plasma, liver, and two brain regions in triple-synuclein knockout mice with these parameters in WT animals.

Liver plays a key role in lipid metabolism and, importantly, a special role in supplying FA to the central nervous system. Lipid metabolism in the liver affects the concentration and composition of secreted lipoproteins (LP) in plasma and, eventually, the lipids and FA in peripheral tissues.

Our study showed that lipid content in liver from TKO mice was significantly lower than that in WT animals (Fig. 1). This reduction was on the account of the decrease in TAG and SE accumulation with no changes (only relative, as %) of TPL (Fig. 2). TAG in hepatocytes, depending on energy demand, are stored in LD and also packed into lipoprotein which are secreted and hydrolyzed by lipoprotein lipases in the vascular system. PL synthesis supplies lipid substrates for VLDL as well as contributes to the biogenesis and maintenance of ER/Golgi membranes. This has an impact on both, the cargo and trafficking machinery for VLDL assembly and secretion (74). PC, cholesteryl ester, FA, and TAG affect VLDL assembly [75]. In our study, no changes in circulated PL and TAG were found suggesting that synuclein ablation did not affect the amount of secreted lipids as VLDL compounds in TKO mice in spite of the reduced TAG accumulation in the liver, while the FA compositions of circulating lipids were significantly altered. In our opinion, one of the main findings of this work is a remarkable modification of PUFA synthesis, and esterification of the major polar lipids by PUFA acyl chains in the liver that was accompanied by enormous reduction of these FA in plasma polar lipids. The observed changes will affect the structure/fluidity of surface monolayer of VLDL, their density, lipases access to TAG and SE during hydrolysis of VLDL, and, consequently and more importantly, the supply of PUFA to the peripheral tissues.

The first step of VLDL assembly takes place in the ER with the Golgi complex has been implicated as a second site of VLDL maturation for their ultimate secretion from hepatocytes. Proteins are important

regulators of lipid transport and play a role in lipoprotein assembly, lipid transport and lipid metabolism by mediating interactions with receptors, enzymes and lipid transport proteins. The following components of the SNARE play a role in docking and fusion of the VLDL transport vesicles: Sec22b (vesicle SNARE), syntaxin 5, rBet1 and Gos28 (target membrane SNARE) [76]. Several mechanisms of synuclein involvement in modulation of FA and lipid metabolism have been suggested. This protein has structural similarities to class A2 lipoproteins and some sequence similarity to FABP. In addition, it is present in large quantities in microsomes where the synthesis of complex lipids takes place. Since in the brain, but not in the liver, a specific role of α-synuclein in FA uptake and trafficking has been demonstrated, it was hypothesized that α-synuclein may function as FABP in the CNS (77). For instance, its deletion decreased palmitic acid uptake and ARA, and altered the incorporation of these FA into individual PL classes in mice brain [77].

Some direct evidence for this hypothesis was provided by a study demonstrated that in α-synuclein-deficient astrocytes, the cellular lipid pool has been altered by reducing the uptake of palmitic acid similarly to the effect of FABP3 in cell cultures [78, 79]. However, the binding affinity of α-synuclein to PUFA was two orders of magnitude much less than that for FABP; thereby, the role of α-synuclein in modulation of ER-located long chain-acyl-CoA synthetases (ACSL) has been suggested [61, 77]. ACSLs are essential in complex lipid biosynthesis and in FA targeting for incorporation into specific lipid pools.

ACSL catalyze the conversion of LCPUFA to fatty acyl-CoA. Five isoforms of ACSL (ACSL1, ACSL3, ACSL4, ACSL5, and ACSL6) have been identified in humans and rodents. Although ACSL are ubiquitously expressed, they have individual functions in FA metabolism, depending on substrate preferences and tissue specificity as well as on physiological conditions and hormonal signaling [80]. ACSL1 shows a broad substrate specificity expressing the liver, heart, and adipose tissue where various FA are used for energy production and storage. ACSL3 and ACSL6 are abundantly expressed in brain. ACSL4 is a ubiquitous peripheral membrane protein, but highly expressed in brain and adrenal glands, being selective for LCPUFA. ACSL5, the major intestinal ACSL isoform, targets a wide range of FA, mainly dietary, for TAG esterification that are secreted into the circulation [80].

The metabolic fates of various acyl-CoA are determined by a network of proteins that channel them toward or away from specific pathways to promote FA partitioning [81]. Hepatic FA partitioning requires the interaction of ACSL1 with other specific proteins, such as peroxisomal and lipid droplet proteins, tethering proteins, and vesicle proteins, that uncovers a dynamic role for ACSL1 in organelle and LD interactions. Proteins involved in lipid metabolism were also identified, including acyl-CoA-binding proteins (ACBP) and ceramide synthase (CerS) isoforms 2 and 5 [80]. Protein complexes composed of several ACSL isoforms have been found in mitochondria. They include ACSL3 and several SNARE proteins such as SNAP23 (synaptosomal-associated protein 23), VAMP (vesicle-associated membrane protein) and syntaxin 17, suggesting a role for ACSL at membrane contact sites between mitochondria and other organelles or vesicles. In liver, syntaxin 17 interacts with ACSL3, but not with LD formation-unrelated ACSL1 or ACSL4, through its SNARE domain promoting ACSL3 translocation to the ER to the surface of LD [82]. It should be stressed that more research needed to reveal a role of synuclein deficiency

on mitochondrial PUFA synthesis and turnover since mitochondrial dysfunction is a critical component in nigral degeneration in PD.

We suggest that in the absence of all synucleins, ACSL4 activity is reduced impairing the synthesis of PUFA-CoA and their incorporation into hepatic phospholipids, namely ARA to PC, PS and PI, and DHA in PE. As to the possible role of synucleins in TAG storage, VLDL synthesis and secretion in blood, it may be connected to the well-known interactions of synucleins with SNARE proteins (which is, in turn, also interact with ACSL isoforms) that may modulate the formation and trafficking of TAG/LD and phospholipids to/from the ER/Golgi/mitochondria/peroxisomes. Lipoprotein assembly is regulated by proteins by mediating interactions with receptors, enzymes and lipid transport proteins. The following components of the SNARE play a role in docking and fusion of the VLDL transport vesicles: Sec22b (vesicle SNARE), syntaxin 5, rBet1 and Gos28 (target membrane SNARE) [76]. Thus, it is tenable to suggest that synucleins can be involved in VLDL synthesis via several anticipated mechanisms. The assembling of VLDL with only trace amounts of PUFA in their polar lipids what ensured by the trace levels of PUFA in plasma TPL, implies the mechanisms of specific binding and/or preferential transport of phospholipids esterified mainly with palmitic and palmitoleic acids but not with PUFA to nascent VLDL. It is noteworthy, that the degree of PUFA esterification of steryl esters in plasma lipoproteins is substantially higher than that in liver, and it was not altered by synuclein ablation (supplemental Table 3). In additional to the *de novo* synthesis of SE, the mechanism of SE formation via transferring 2-acyl groups from phospholipids (this *sn*-2 position of glycerol backbones predominantly esterified by PUFA) to cholesterol also exists. The further (onward lipoprotein secretion pathway) remarkable reduction of PUFA levels in plasma phospholipids can hereby be explained in TKO mice where PUFA incorporation into liver total polar lipids is already impaired. The circulation of highly unsaturated SE in blood stream of synuclein mutants may ensure supply to peripheral tissues with levels of PUFA required for maintaining lipid synthesis and homeostasis, in spite of the significantly reduced total pool of circulating PUFA. The increased plasma concentration of SE also contributes to this plasma PUFA balancing.

Although plasma lipoproteins are the major transport medium for esterified FA, appreciable amounts of FA are transported in non-esterified form as bound to albumin. In our study, the total pool of these free FA was significantly reduced in synuclein-ablated animals with a remarkable reduction in circulating free ARA and DHA (Fig. 2, 3). Passive diffusion across the plasma membrane is well-known to be modulated by FABPs which possess acyl-CoA synthetase activities that in turn may be regulated by synucleins (as discussed above). Thus, synuclein deficiency markedly affects the hepatic lipid homeostasis as well as the diffusion and transport of PUFA to peripheral tissues. The elucidation of precise mechanisms of these actions deserves further investigations.

The brain is highly enriched with PUFAs, especially with ARA and DHA, and their uptake from circulating lipid pool was previously believed to be essential to maintain homeostatic pool of LCPUFA in nervous tissues. Further studies using rodents demonstrated that synthesis from the essential precursors, namely LA and ALA, could provide sufficient ARA and DHA for the estimated adult brain requirement for these LCPUFAs [83, 84]. The synthesis of these LCPUFA from their precursors takes place primarily in

hepatocytes and then transported, as mentioned, in both, esterified and non-esterified forms to the nervous system.

Besides uptake of preformed PUFA from the circulating blood, some appreciable amounts of ARA and DHA can be synthesized from their uptaken precursors (LA, DGLA and ALA) in the microvascular endothelium, oligodendrocytes and astrocytes [87, 88]. Neurons themselves are inefficient in lipid synthesis, although they showed a limited capacity to produce DHA from omega-3 precursors under conditions of its low dietary supply as well as some elongation of shorter chain precursors to produce DHA and ARA [87].

PUFA play numerous physiological roles, including generally known structural, energy storage and production, inflammation, and cell signaling in the cells of various organ systems [91]. They regulate gene expression by affecting transcription factors such as hepatic peroxisome proliferatoractivated receptor alpha (PPAR-α), sterol regulatory element-binding protein 1 (SREBP-1), carbohydrate-responsive element-binding protein (ChREBP), and max-like protein X (MLX) that regulate proteins which are involved in lipid synthesis and oxidation, and lipoprotein secretion [92]. At the subcellular level, PUFAs modulate membrane functioning via influencing lipid rafts, where they alter the raft structural and dynamic characteristics such as domain size and membrane order, most likely by the lateral segregation of LCPUFA-rich domains from cholesterol-rich membrane regions [91]. The neurophysiological outcomes are substantial because lipid rafts participate in neurotransmission, including regulation of dopamine transporters, dopamine uptake and dopamine D_2 receptor oligomerization [93].

Polar lipids in the brain are characterised by very high structural complexity due to a substantial number of different molecular species of both, glycerophospholipids and sphingolipids. These two main lipid classes play an important structural and functional role in cell membranes in general and in lipid In midbrain of TKO mice, the levels of C24-C26 sulfatides and cerebrosides were reduced indicating the altered activity of CerS2 that catalyses the acylation of sphingolipids with this group of long acyl chains. The mechanism of the modulation of CerS2 activity may be connected to similarity of synucleins to fatty acid binding proteins or ACSLs, discussed above, since the regulation of very-long acyl chain ceramide synthesis by acyl-CoA binding protein has been demonstrated in mice model work [94].

In midbrain of TKO mice, a significant increase in PC with a concomitant reduction of cerebrosides was noted as results of synuclein deficiency. Not only the accumulation of these lipid classes was altered, but remarkable alterations of acyl chain, e.g. decreased DHA in PC and C26:1n9 in ceramides, were also demonstrated. Since the level of this fatty acid was not altered in midbrain in TKO mutants, we suggest that the channelling of DHA to PC, not its synthesis in the brain, was specifically affected decreasing the unsaturation level of this major brain phospholipid by synuclein deficiency. It is generally accepted, that changes in membrane fluidity can alter the equilibrium of free *versus* membrane bound α-synuclein, and therefore impact the aggregation of α-synuclein into amyloid fibrils. Recently, the importance of unsaturation level of zwitterionic PC (in addition to previously studied anionic phospholipids) for conformation and aggregation of N-terminally acetylated α-synuclein has been evidenced [38]. We

showed, that in brain, the relative amount of DHA-enriched PS and its fatty acids were not affected by synuclein deficiency. The importance of this phospholipid in interaction with the N-terminal of α-synuclein has been found to facilitate SNARE complex formation that was critical for vesicle docking using single-vesicle docking/fusion assays [95]. Moreover, DHA-enriched PC and PS exhibited different improvements on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mice with PD by conducting behavioural experiments: DHA-PS showed more pronounced effects on PD symptoms and increased the number of dopaminergic neurons in comparison to DHA-PC [96]. As to the additional role of DHA in the brain, its endogenously synthesised endocannabinoid-like metabolite, N-docosahexaenoylethanolamine (synaptamide) has emerged as a potent mediator of neurotrophic and neuroprotective effects of DHA [83]. It is interesting that N-arachidonylethanolamine (anandamide) synthesised from ARA, being a structural analogue of synaptamide, possesses distinctive neuro-bioactivity modulating the brain reward circuitry and neuroinflammation [97].

In the liver, PS was enriched with ARA (not with DHA) that markedly reduced in TKO mice. This fatty acid is known to stimulate SNARE complex formation and exocytosis, and α-synuclein, which is, in turn, can be also modified by ARA, efficiently blocks ARA-induced SNARE interactions [98].

In spite of remarkable PUFA modifications in individual lipid classes in liver and plasma of TKO mice, we can conclude that the total supply of PUFA (both, LCPUFA and their short chain essential precursors) was sufficient to maintain characteristically high level of LCPUFA in midbrain and cortex of studied mutants. More research on the synthesis of PUFA in liver and brain cells/tissue is required to understand the mechanisms of its metabolic regulation in the synuclein-free mice models. It is also worth elucidating these mechanisms since some findings, linking α-synuclein scavenging activity towards harmful PUFA oxidation and its involvement in the maintenance of the correct levels of PUFA, have been revealed [57].

In midbrain, a marked reduction in ethanolamine plasmalogens in synuclein free mice can be considered as an important finding, since abnormal ether lipid levels has been previously implicated in neurological disorders. Plasmalogens is a class of ether lipids with an ether bond attached to *sn-1* position of the glycerol backbone as opposite to an ester bond of the more common diacyl phospholipids [99]. Plasmalogens are present in mammalian plasma and intracellular membranes. Nervous, immune and cardiovascular systems are enriched in these lipids where plasmalogens have been proposed to influence membrane dynamics and intracellular signalling being an important component of lipid rafts, and also act as membrane antioxidants and reservoir of polyunsaturated fatty acids [99]. Reduced brain plasmalogen level has been demonstrated in various neurodege-nerative and metabolic deregulated diseases, which were associated with increased lipid oxidation. Regarding PD, patients with this disease showed a significant decrease in post-mortem frontal cortex lipid raft plasmalogens level [99, 100]. The results of other studies also showed an importance of plasmalogens for brain functions, albeit contrary to the hypothesis that synthesis of these lipids predominantly occurred in the brain and less likely supplied by circulation from peripheral tissues [101]. In our study, the level of plasma DMA among fatty acids of total polar lipids was non-detectable because of the small sample volumes. Lipidomics approach, which is under our consideration for the future work in this direction, will allow to estimate the

level of circulation plasmalogens in TKO mice. From the results obtained in the present study, and based on the notion, that plasmalogen may function as potential endogenous antioxidants, we suggest that the reduced level of plasmalogen PE in midbrain of TKO mice makes the cells more susceptible to oxidative stress. In support, there are some evidence of involving of plasmalogens in scavenging a variety of reactive oxygen species and in protecting of unsaturated membrane lipids from oxidation by singlet oxygen [99]. Moreover, plasmalogen supplementation protected striatal dopamine neurons that degenerated in response to MPTP-treatment in mice, a PD model [102].

Our results one more time demonstrated that the ablation of synucleins is equally important as their accumulation at the level of lipid metabolism. The present study underlined a number of lipid biosynthetic pathways that were modified by synuclein deficiency in TKO mice model. The future research in this direction by studying the highlighted “targets” of synuclein actions as modulators of lipid synthesis will reveal the mechanisms and the physiological relevance of the crosstalk between synucleins and lipids not only in the brain but at the level of organism in general. Using lipidomics approach for studying lipid rafts, plasmalogen and sphingolipid synthesis, lipoprotein formation and circulating lipids and fatty acids, as well as for the better understanding of lipid synthesis in astrocytes, will undoubtedly integrate the knowledge on functions of synucleins in the cells.

Table 1
Fatty acid composition of total polar lipids (% of TPL fatty acids) in plasma and liver from triple-synuclein null mutant (TKO) and wild-type (WT) mice

Fatty acid	PLASMA		LIVER	
	WT	TKO	WT	TKO
C16:0	23.6 ± 1.2	44.2 ± 5.5*	21.6 ± 1.5	27.7 ± 3.4*
C16:1n7	0.7 ± 0.4	1.8 ± 0.6	0.8 ± 0.4	1.2 ± 0.8
C18:0	24.1 ± 1.0	28.4 ± 4.7	22.4 ± 1.9	21.2 ± 2.8
C18:1n9	12.7 ± 0.9	18.1 ± 1.0*	13.0 ± 1.0	15.2 ± 0.8*
C18:2n6	19.2 ± 1.4	2.7 ± 0.8*	13.5 ± 1.1	14.4 ± 0.7
C18:3n3	0.9 ± 0.2	0.8 ± 0.1	tr.	tr.
C20:3n6	1.6 ± 0.2	tr.	1.7 ± 0.3	1.8 ± 0.3
C20:4n6	10.0 ± 1.3	tr.	15.7 ± 1.7	10.4 ± 2.7*
C22:6n3	4.0 ± 0.6	tr.	7.6 ± 1.4	4.2 ± 2.2
C24:1n9	1.4 ± 0.2	1.3 ± 0.4	0.9 ± 0.1	0.7 ± 0.1

Data as means ± SD ($n = 6$)

Fatty acids are indicated with the number before colon showing the number of carbon atoms, the figure afterwards denoting the number of double bonds. The position of the first double bond is shown after “n”. Only the major fatty acids (≥ 0.5%) are listed; tr. < 0.5%

The asterisk (*) indicates a significant effect of triple-synuclein deficiency when compared with WT ($p < 0.05$)

Abbreviations

ACSL, long chain-acyl-CoA synthetases; ALA, α-linolenic acid; ARA, arachidonic; CER, cerebrosides; ChREBP, carbohydrate-responsive element-binding protein; CL, cardiolipin; DGLA, dihomo-γ-linolenic; DHA, docosahexaenoic acid; DMA, dimethyl acetals; EPA, eicosapentaenoic acid; FA, fatty acids; FABS, fatty acid binding protein; FAME, fatty acid methyl esters; GC, gas chromatography; HPLC-MS-MS, high-performance liquid chromatography-mass spectrometry; LA, linoleic acid; LCPUFA, long-chain polyunsaturated fatty acids; LP, lipoproteins; MLX max-like protein X; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NAC, non-Aβ component; PC, phosphatidylcholine; PD, Parkinson’s disease; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PPAR-α, peroxisome proliferator-activated receptor alpha; PS, phosphatidylserine; SE, steryl esters; SL, sulfatide; SM, sphingomyelin; SNAP23, synaptosomal-associated protein 23; SNARE, *N*-ethylmaleimide-sensitive factor attachment protein receptors; SREBP-1, sterol regulatory element-binding protein 1; SV, synaptic vesicle; TAG, triacylglycerol; TKO, triple synuclein-knockout; TPL, total polar lipids; VLDL, very low-density lipoproteins; WT, wild type.

Declarations

Ethics approval and consent to participate

All animal work was carried out in accordance with the United Kingdom (Scientific Procedures) Act (1986) and European Directive EC 86/609, and has been approved by the Cardiff University Ethical Review Committee and the Home Office (Project Licences 30/2844 and 30/3412).

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Author’s contributions

IAG: data collection and analysis, writing original draft; NN: study design, data collection, manuscript preparation; AYD: statistical analysis; MVP: knockout mice supply and analysis; VLB: conceptuality, original draft writing - review and editing.

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Consent for publication

All authors agree to publish in the current form.

Conflict of Interest

The authors declared none to disclose regarding conflict of interest.

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Figures

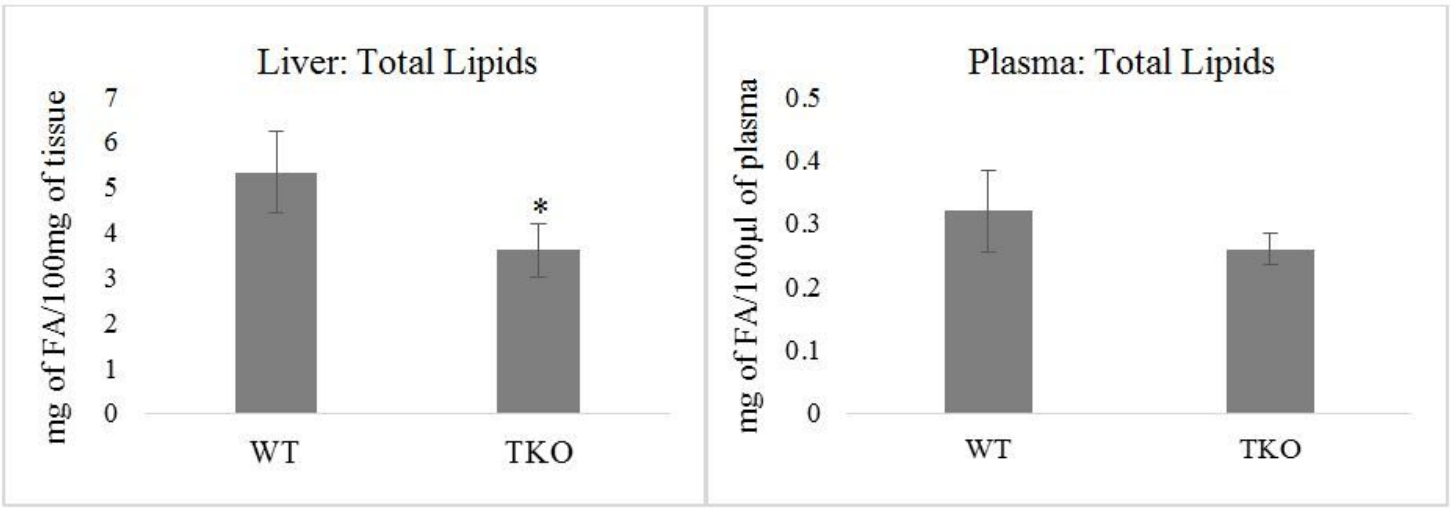

Figure 1

Total lipid content in liver (mg of fatty acids in 100 mg of tissue) and plasma (mg of fatty acids in 100 µl of plasma) from wild-type (WT) and triple-synuclein null mutant (TKO) mice. Means ± s.d. (n = 5) are shown. *P <0.05.

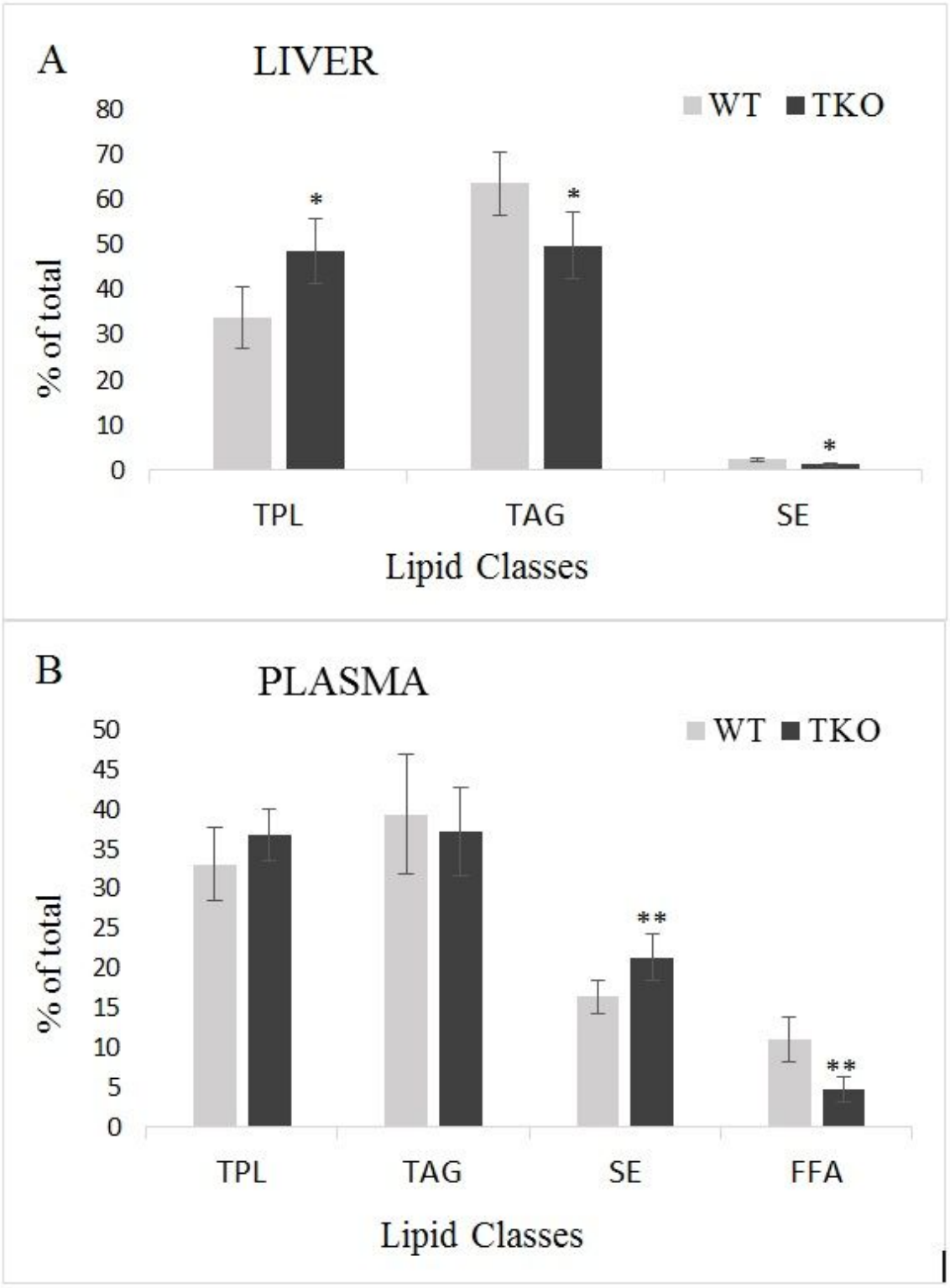

Figure 2

Relative concentrations (% of total) of the major lipid classes in liver (A) and plasma (B) from wild-type (WT) and triple-synuclein knockout mice: total polar lipids (TPL), triacylglycerols (TAG), steryl esters (SE) and free (non-esterified) fatty acids (FFA). Values represent means ± s.d. (n = 6). *P < 0.05, **P < 0.01.

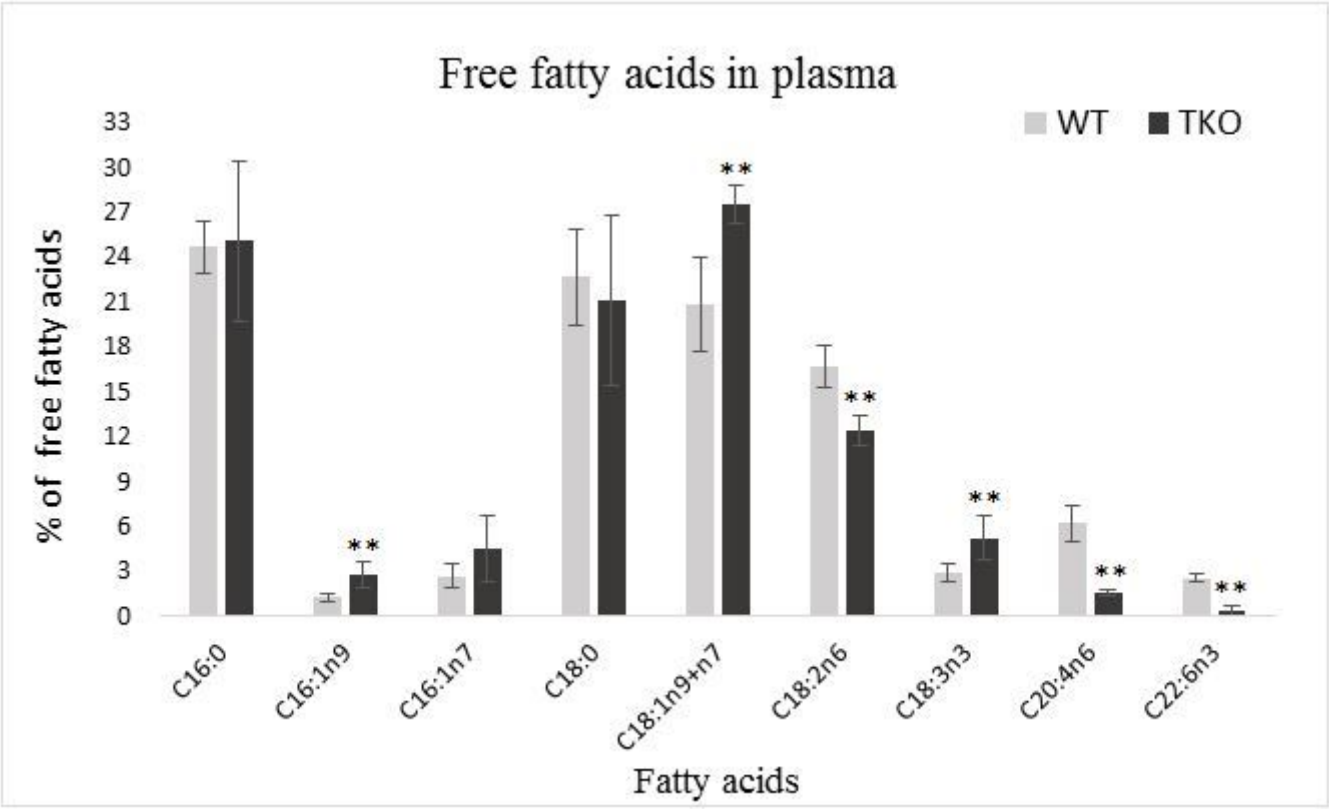

Figure 3

Comparison of free (non-esterified) fatty acids (FFA) in plasma from wild-type (WT) and triple-synuclein knockout mice (% of total FFA). Values represent means ± s.d. (n = 6). **P < 0.01.

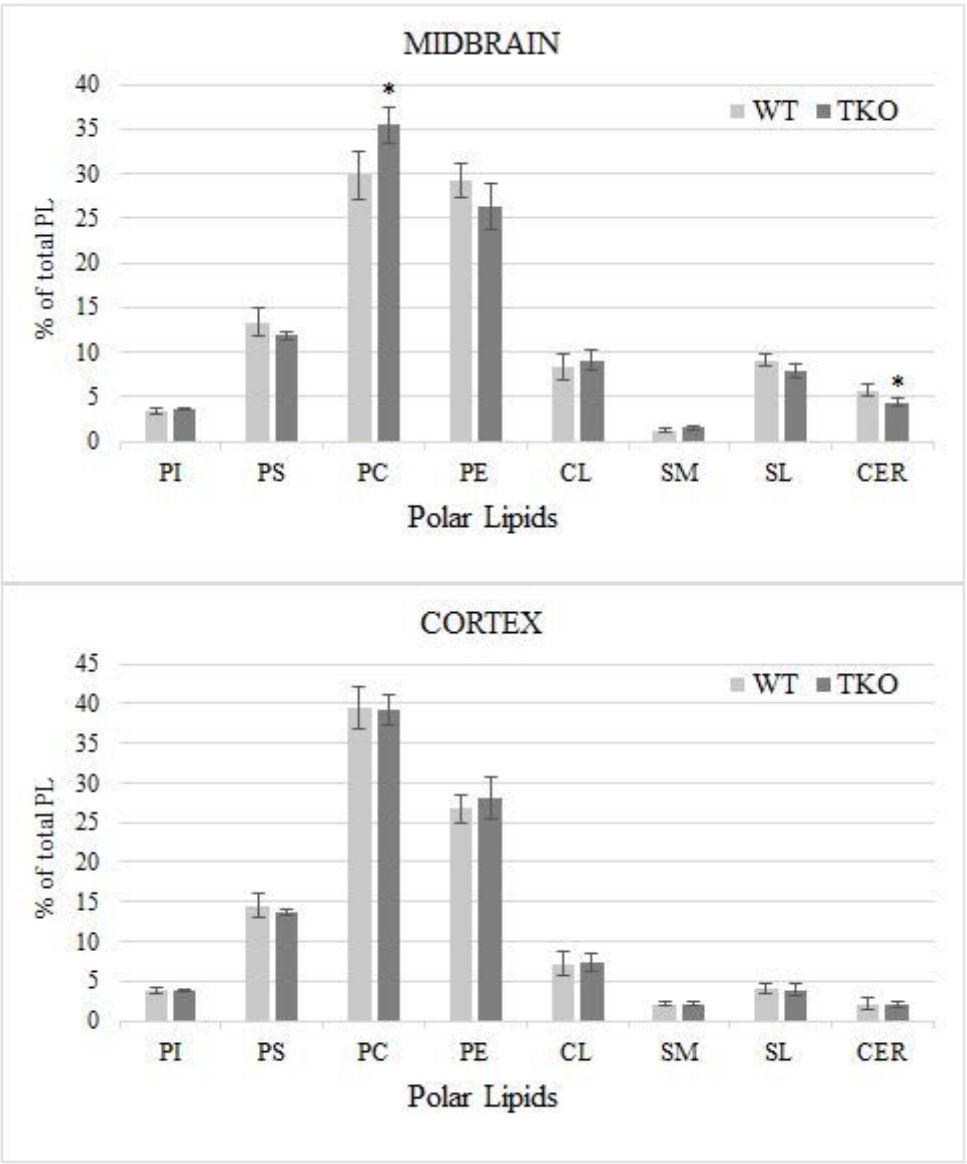

Figure 4

Midbrain and cortex polar lipid composition (% of total polar lipids) from wild-type (WT) and triple-synuclein null mutant (TKO) mice. Means ± s.d. (n = 5) are shown. *P <0.05. Abbreviations: PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; CL, cardiolipin; SM, sphingomyelin; SL, sulfatide; CER, cerebroside.

Supplementary Files

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