HHS Public Access

Author manuscript

Lipids. Author manuscript; available in PMC 2016 January 23.

Published in final edited form as:

Lipids. 2014 June ; 49(6): 505–515. doi:10.1007/s11745-014-3907-6.

Lipidomic analyses of female mice lacking hepatic lipase and endothelial lipase indicate selective modulation of plasma lipid species

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Abstract

Hepatic lipase (HL) and endothelial lipase (EL) share overlapping and complementary roles in lipoprotein metabolism. The deletion of HL and EL alleles in mice raises plasma total cholesterol and phospholipid concentrations. However, the influence of HL and EL *in vivo* on individual molecular species from each class of lipid is not known. We hypothesized that the loss of HL, EL or both *in vivo* may affect select molecular species from each class of lipids. To test this

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hypothesis, we performed lipidomic analyses on plasma and livers from fasted female wild-type, HL-knockout, EL-knockout, and HL/EL-double knockout mice. Overall, the loss of HL, EL, or both, resulted in minimal changes to hepatic lipids; however, select species of CE were surprisingly reduced in the livers of mice only lacking EL. The loss of HL, EL, or both, reduced the plasma concentrations for select molecular species of triacylglycerol, diacylglycerol, and free fatty acid. On the other hand, the loss of HL, EL, or both, raised the plasma concentrations for select molecular species of phosphatidylcholine, cholesteryl ester, diacylglycerol, sphingomyelin, ceramide, plasmanylcholine, and plasmenylcholine. The increased plasma concentration of select ether phospholipids was evident in the absence of EL, thus suggesting that EL might exhibit a phospholipase A_2 activity. Using recombinant EL, we showed that it could hydrolyse the artificial phospholipase A_2 substrate 4-nitro-3-(octanoyloxy)benzoic acid. In summary, our study shows for the first time the influence of HL and EL on individual molecular species of several classes of lipids *in vivo* using lipidomic methods.

Keywords

lipoproteins; hepatic lipase; endothelial lipase; knockout mice; lipidomics; mass spectrometry; phospholipase A_2

Introduction

Hepatic lipase (HL) and endothelial lipase (EL) are members of an extracellular lipase family that hydrolyse triacylglycerols (TAG) and phospholipids (PL) within circulating lipoproteins [1]. The expression of HL and EL are mainly distinct from each other, whereby HL is expressed primarily by hepatocytes [2, 3], and EL is expressed in vascular endothelial cells [4, 5]. However, both HL and EL are commonly expressed in macrophages [6, 7]. The expressed lipases are exposed to the bloodstream, where they can hydrolyse TAG and PL from all classes of lipoproteins [1]; however, EL preferentially hydrolyses PL from high density lipoproteins (HDL) [8]. In addition to their catalytic functions, HL and EL also have a non-catalytic “bridging” function: HL and EL associated with cell surfaces can capture lipoproteins independent of hydrolytic activity [9–12], thus bringing lipoproteins in close proximity to various cell surface molecules associated with lipoprotein metabolism.

Previous studies using HL-knockout (ko), EL-ko, and HL/EL-double ko (dko) mice have shown that HL and EL exhibit complementary and redundant roles in lipoprotein metabolism. The genetic ablation of alleles for both HL and EL lead to increased levels of plasma total cholesterol, HDL cholesterol (HDL-C), non-HDL-C, and PL, while interestingly causing no changes to levels of total TAG [13]. Both HL and EL also protect against the generation of small-dense low-density lipoproteins (sdLDL): the plasma of HL/EL-dko mice have sdLDL likely because of impaired PL hydrolysis and increased plasma TAG lipase activity, whereas the plasma from HL-ko and EL-ko mice does not contain sdLDL [13]. However, mouse models of HL and EL deficiency have not clearly defined these lipases as being pro- or anti-atherogenic. Conflicting studies exist that show the absence of HL or EL in mice has either no effect [13] or an improvement [14] on the anti-atherogenic process of reverse cholesterol transport. In addition, conflicting studies on

plaque formation in the absence of HL or EL exist that are dependent on the genetic background [1].

Details of the molecular species for each class of lipid in HL and EL deficiency are unknown. Information about the lipidome from models of HL and EL deficiency can be rapidly obtained through mass spectrometry approaches [15]. It is conceivable that the lipidome from models of HL and EL deficiency could contribute in defining how HL and EL may be detrimental or non-influential on atherosclerosis. Information of the lipidome from these models could also provide molecular details about the substrate specificities of HL and EL *in vivo*.

Materials and Methods

Reagents and materials

High-performance liquid chromatography grade methanol and chloroform were purchased from Burdick and Jackson (Morristown, NJ, USA). Arachidic acid (20:0 free fatty acid (FFA)), Δ10-*cis*-triheptadecenoin (tri-17:1 TAG), diarachidin (di-20:0 diacylglycerol (DAG)), and cholesteryl heptadecanoate (17:0 cholesteryl ester (CE)) were purchased from Nu-Chek Prep (Elysian, MN, USA). 1,2-Diarachidoyl-*sn*-glycero-3-phosphocholine (di-20:0 phosphatidylcholine (PtdCho)), 1-heptadecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (17:0 lysoPtdCho), N-heptadecanoyl-D-*erythro*-sphingosine (17:0 ceramide (Cer)), and N-heptadecanoyl-D-*erythro*-sphingosylphosphorylcholine (17:0 sphingomyelin (CerPCho)) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). 4-Nitro-3-(octanoyloxy)benzoic acid (NOB) was purchased from Enzo Life Sciences (Farmingdale, NY, USA). Honey bee venom phospholipase A_2 (PLA_2), pentafluorobenzyl bromide, and heparin were purchased from Sigma-Aldrich (St. Louis, MO, USA). HEK293 cells were obtained from ATCC (Manassas, VA, USA). Lipofectamine™ was purchased from Invitrogen (Burlington, ON, Canada). Anti-EL polyclonal antibody (#NB400-111) was purchased from Novus Biologicals (Littleton, CO, USA). Horseradish peroxidase-conjugated anti-rabbit IgG (#SA1-200) was purchased from Pierce Biotechnology (Rockford, IL, USA). ECL™ Prime was purchased from GE Healthcare (Baie d'Ufre, QC, Canada).

Animals

C57BL/6 (or wild-type (WT)) mice and HL-ko mice on a C57BL/6 background were obtained from Jackson Laboratories – (stock numbers 000664 and 002056, respectively). EL-ko mice [13, 16] and HL/EL-dko mice [13] were previously described. All mice were maintained on a normal chow diet with unlimited access to water and a 12 h light/12 h dark cycle. Plasma was obtained from blood collected from 8–12 week old female mice that were fasted for 4 h via the retroorbital plexus while under anaesthesia using isofluorane. Liver samples were collected from mice that were fasted for 4 h following cervical dislocation while under isoflurane anaesthesia. Animals were housed at the University of Pennsylvania, and all studies were approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

Extraction of Plasma and Hepatic Lipids

Lipids from 10 μl plasma or 10 mg liver were extracted using the Bligh-Dyer method [17], in the presence of internal standards for each lipid assessed. Internal standard lipids added to each plasma sample were 1.0 μg 20:0 FFA, 0.5 μg tri-17:1 TAG, 0.2 μg di-20:0 DAG, 5 μg di-20:0 PtdCho, 1.2 μg 17:0 lysoPtdCho, 20 ng 17:0 Cer, 0.6 μg 17:0 CerPCho, and 6.0 μg 17:0 CE. Internal standard lipids added to each liver sample were 1.0 μg 20:0 FFA, 40 μg tri-17:1 TAG, 6.0 μg di-20:0 DAG, 30 μg di-20:0 PtdCho, 2.0 μg 17:0 lysoPtdCho, 100 ng 17:0 Cer, 1.0 μg 17:0 CerPCho, and 6.0 μg 17:0 CE. Extracts were resuspended in 500 μl chloroform and stored at −20°C under $N_{2(g)}$ until needed.

Mass Spectrometry

For electrospray ionization-mass spectrometry (ESI-MS) analyses, 50 μl of lipid extract was mixed with 200 μl methanol and 2 μl of 10 mM NaOH (in methanol); the mixture was directly injected into a Thermo TSQ Ultra tandem ESI-MS system at a flow rate of 3.5 μl/min. In positive ion mode, the electrospray needle voltage was 3,500 V with a capillary temperature of 270°C; in negative ion mode, the electrospray needle voltage was 2,500 V with a capillary temperature of 270°C. Shotgun lipidomic analyses were performed as previously described [15, 18, 19]. Briefly, sodiated adducts of select PtdCho, lysoPtdCho, plasmanylcholines (PakCho), plasmenylcholines (PlsCho), and CerPCho were quantified following their identification in positive ion mode by scanning for the neutral loss (NL) of choline (*m/z* 59.1) using a collision energy of −28 eV. Sodiated adducts of TAG were quantified following their identification in positive ion mode by survey scanning for $[M+Na]^+$ between *m/z* 800 and 950. Sodiated adducts of CE were quantified following their identification in positive ion mode by scanning for the NL of cholestane (*m/z* 368.5) using a collision energy of −25 eV; all CE data except for 20:5 CE were corrected using our previously described response factors for CE [20]. The 20:5 species of CE was semi-quantified as intensity of the NL of cholestane from sodiated 20:5 CE (*m/z* 693) per intensity of the NL of cholestane from sodiated 17:0 CE (*m/z* 661). Sodiated species of DAG were identified in positive ion mode by selective reaction monitoring (SRM) for the NL of fatty acyl groups using a collision energy of −35 eV; data were semi-quantified as intensity versus the intensity quantified from the SRM transition for di-20:0 DAG. (See Supplementary Table 1 for the DAG assessed and their associated SRM transitions). Cer were quantified following identification in negative ion mode by scanning for the NL of *m/z* 256.2 using a collision energy of −32eV. All ESI-MS data were corrected for isotopic contributions, as previously described [15]. FFAs were esterified into pentafluorobenzyl esters and quantified using gas chromatography-mass spectrometry by selective ion monitoring by a similar method to that previously described [21]. For these analyses, we used a DB-1 column (12 m length, 0.2 mm diameter, 0.33 μm film) with He as carrier gas and an Agilent 6890N gas chromatographer with an Agilent 5973 mass spectrometer equipped with a chemical ion source. Methane was used for chemical ionization. The inlet temperature was 250°C and auxiliary temperature was 280°C. The ion source and quadrupole temperatures were set at 150°C and 106°C, respectively. Initial oven conditions were at 150°C for the first 3.5 min, followed by a gradient of 25°C/min to 310°C, followed by 4 min at 310°C. Fatty acids were detected by selective ion monitoring of their negative

ion fragments with the loss of the pentafluorobenzyl group. All FFA data were corrected using response factors for FFAs, relative to the internal standard of arachidic acid (Supplementary Table 2).

Recombinant EL expression and phospholipase A_2 activity

HEK293 cells were transiently transfected using Lipofectamine™ with an empty pcDNA3 mammalian expression vector, pcDNA3 containing the cDNA for human EL (GenBank: NM_006033), or with no DNA (mock), exactly as we previously described [22]. Heparinized media from transfected cells were collected at 48h post-transfection, centrifuged at 1,200 rpm for 10 min to remove cell debris, and aliquots of the supernatant were stored at −80°C also as we previously described [22]. Proteins in heparinized media samples from transfected cells were denatured and separated on 10 % SDS-PAGE gels, then transferred to nitrocellulose membranes. Nitrocellulose membranes were subjected to immunoblot analysis for EL using a 1:500 dilution of the anti-human EL polyclonal antibody. Detection was by chemiluminescence using a 1:1,000 dilution of the horseradish peroxidase-conjugated anti-rabbit IgG and ECL™ Prime, according to manufacturer's instructions. To assess *sn*-2 hydrolytic activity in heparinized media, 100 μl of media was mixed with 100 μl of an assay buffer (150 mM KCl, 10 mM $CaCl_2$, 50 mM Tris-HCl, pH 7.5). The mixture was tested for the hydrolysis of NOB to liberate 4-nitro-3-benzoic acid, exactly as previously described [23]. As a positive control, 100 μl of a stock solution with 1775 U/ml of honey bee venom PLA_2 in phosphate-buffered saline (pH 7.4) was mixed with 100 μl of assay buffer. Assay data were calculated as nmol 4-nitro-3-benzoic acid produced per ml media (or per ml PLA_2 stock) per hour.

Statistical analyses

Data were analyzed using a one-way analysis of variance with a 95% confidence interval, followed by a comparison of groups using a Tukey's Test.

Results

We hypothesized that mice with a deficiency in HL and EL will exhibit changes to select molecular species of lipid to account for reported plasma increases in total cholesterol and PL [13]. To test this hypothesis, we quantified molecular species of plasma and hepatic CE and PtdCho levels using ESI-MS; in addition, we quantified select molecular species of TAG, DAG, FFA, lysoPtdCho, CerPCho, Cer, PlsCho, and PakCho.

As previously reported, an increased trend for plasma total cholesterol and for PL was observed, in order, from WT < HL-ko < EL-ko < HL/EL-dko [13]. We observed this trend for CE in our tandem ESI-MS analyses with the 18:2, 20:4, and 22:6 species of CE (Fig. 1a and supplementary Table 3), as well as the 20:5 species of CE relative to the internal standard (Supplementary Fig. 1a). Interestingly, this trend was not observed with other species of CE: only the loss of EL in both the single knockout and dko mice lead to significantly raised levels of the 14:0, 16:0, and 18:1 species of CE in plasma. We also observed this trend in our tandem ESI-MS analyses with all of the assessed species of plasma PtdCho with the exception of the 16:0–20:4 species (Fig. 2a and supplementary

Table 5), which had comparable levels in both the single HL- and EL-ko plasma that were lower than those for the HL/EL-dko mice. The hepatic levels of CE molecular species for HL-ko and HL/EL-dko mice were comparable to WT levels, but interestingly the hepatic levels of 14:0, 18:2, 18:0, 20:4, and 22:6 CE were significantly lower in the EL-ko mice versus WT mice (Fig. 1b and supplementary Table 4). The hepatic levels of all of the PtdCho species assessed were not different between groups (Fig. 2b and supplementary Table 6).

LysoPtdCho is a key product of HL and EL hydrolysis of PtdCho. No distinct trends were observed for the plasma levels of lysoPtdCho; amongst the species assessed, only a modest but significant elevation was observed for the 18:0 species of lysoPtdCho in the HL/EL-dko mice versus WT mice (Fig. 3a and supplementary Table 7). Similar to what was observed for PtdCho, no differences were observed between all groups for the levels of lysoPtdCho molecular species within the liver (Fig. 3b and supplementary Table 8).

The concentrations of total TAG between WT, HL-ko, EL-ko, and HL/EL-dko mice were previously reported to not be different [13]. However, the dissection of the molecular species of TAG revealed unexpected significant reductions of select species of plasma TAG between lipase knockout and WT mice (Fig. 4a and supplementary Table 9). Notably, our data show that the absence of EL in the single knockout and dko mice led to a significant reduction of the 52:4 species of TAG, the absence of HL in the single knockout or dko mice exhibited a significant reduction of the 54:5 species of TAG, and the absence of HL and/or EL led to a significant reduction of the 54:4 species of TAG. No differences were observed for the molecular species of TAG assessed from the livers of all groups of mice (Fig. 4b and supplementary Table 10).

Compared to WT mice, mice lacking HL, EL, or both had significantly reduced plasma levels of 16:0-16:0 DAG (Fig. 5a and supplementary Table 11). Very interestingly, our assessment of plasma DAG species revealed two unique trends. The DAG species containing 18:1 or 18:2 tended to be lower than WT when HL or EL was absent. Compared to WT levels, significant differences were observed for the 16:0–18:2, 16:0–18:1, 18:1–18:2, 18:1–18:1, 18:0–18:2, 18:0–18:1, and 18:1–22:6 species of plasma DAG in the absence of HL; significant differences were observed for the 16:0–18:2, 16:0–18:1, 18:1–18:2, 18:1–18:1, 18:0–18:2, and 18:0–18:1 species of DAG in the absence of EL. Significance was only observed for the 18:1–18:2 and 18:1–18:1 in the absence of both HL and EL, as the levels of other species of plasma DAG with 18:1 or 18:2 were elevated compared to the individual lipase knockout mice. We also uniquely observed that the DAG species containing a saturated fatty acyl group together with either 20:4 or 22:6 tended to be elevated specifically in the plasma of HL/EL-dko mice. Compared to WT levels, significance was observed for the 18:0–20:4 and 18:0–22:6 species of DAG. The hepatic levels of DAG all species assessed in the HL-ko and EL-ko were comparable to the levels observed in WT mice (Fig. 5b and supplementary Table 12). Similar to what was observed in plasma, the hepatic levels for both the 18:0–20:4 and 18:0–22:6 species of DAG were significantly higher in the HL/EL-dko mice versus WT mice; in addition, 18:1–20:4 DAG was also significantly raised in the HL/EL-dko mice.

Gas chromatography-mass spectrometry analyses of plasma FFA species revealed that HL-ko ad HL/EL-dko mice have significantly reduced concentrations of 16:0 and 20:5 versus WT mice (Fig. 6a and supplementary Table 13). While the plasma from HL-ko and EL-ko mice exhibited an insignificant reduction of 18:2 and 18:1 FFA species, significantly lower levels were only observed in the plasma from HL/EL-dko mice for these species versus WT mice. Compared to the plasma of WT mice, significantly lower levels of the 20:4 and 22:6 FFA species were observed in the plasma of all lipase-ko mice. With the exception of the 22:6 FFA species in the livers of HL/EL-dko mice, no significant differences were observed between all groups of mice for all species of FFA (Fig. 6b and supplementary Table 14).

We assessed two species of CerPCho and six Cer species. We observed a significant increase of 16:0 CerPCho in the plasma of EL-ko and HL/EL-dko mice versus WT, and a significant increase of plasma 18:0 CerPCho in only the HL/EL-dko mice versus WT (Fig. 7a and supplementary Table 15). Analyses of livers only showed a modest but significant increase of 16:0 CerPCho levels in HL/EL-dko mice versus WT mice (Fig. 7b and supplementary Table 16). On the other hand, no differences were observed for the Cer species assessed in the plasma of all groups of mice (Fig. 8a and supplementary Table 17), and a modestly significant increase was observed for only hepatic 16:0 Cer in the HL/EL-dko mice (Fig. 8b and supplementary Table 18).

Lastly, we quantified select species of PlsCho and PakCho in the plasma of the lipase-ko mice. Our data interestingly show that the compared to WT mice, the concentrations of the 16:0–18:2, 16:0–18:1, 18:0–18:2, and 18:0–18:1 species of PakCho were significantly increased in the EL-ko and HL/EL-dko mice (Fig. 9 and Supplementary Table 19). Compared to WT mice, the plasma concentrations of the 16:0–16:1, 16:0–20:4, and 18:0–20:4 species of PakCho were raised in only HL/EL-dko mice. No differences were observed between groups for the plasma concentration of 18:0–20:4 PlsCho, while the concentrations of the 16:0–20:4 and 18:1–20:4 species of PlsCho were significantly elevated in HL/EL-dko mice versus WT mice.

The increased levels of select PakCho species from the plasma of mice lacking EL suggested that EL might in fact exhibit an *sn*-2 activity. To test this possibility, we used heparinized media from HEK293 cells transiently expressing human EL in an enzyme assay using the *sn*-2 substrate NOB. From triplicate experiments, we show that EL significantly hydrolyses NOB versus control heparinized media from HEK293 cells transiently transfected with the empty expression vector pcDNA3 (Fig. 10). Of note, the hydrolysis of NOB by heparinized media from mock transfected cells was comparable to heparinized media from cells transfected with empty vector (Supplementary Fig. 2).

Discussion

Our study is the first comprehensive report addressing the lipidome for animal models of HL and EL deficiency, and it provides additional insight into their activities at the molecular level *in vivo*. Plasma PL concentrations in mice were previously shown to be increased in order of WT < HL-ko < EL-ko < HL/EL-dko [13]. As expected, we show that this trend exists for almost all species of PtdCho assessed. Our data at the molecular species level

clearly reflects the fact that EL exhibits predominantly a phospholipase activity compared to HL [8]. In addition, we show that both HL and EL are complementary in PL metabolism. The observed increases in plasma PtdCho due to the loss of HL and EL *in vivo* is tied with increases in plasma CE; this is likely because of an increased availability of PtdCho that can be used by lecithin:cholesterol acyltransferase toward esterifying plasma cholesterol.

At this point in time, we can only speculate about our interesting finding of the significant reduction of five molecular species of CE in the livers of EL-ko mice. It is possible that EL might have an intracellular function associated with providing select fatty acids for the esterification of cholesterol, but it still remains to be determined if EL is catalytically active within cells. On the other hand, HL is known to become catalytically active early within the secretory pathway of hepatocytes [24, 25] and it appears to play a role in very low-density lipoprotein assembly [26, 27]. Thus, it is also possible that in the absence of EL, intracellular HL might utilize species of fatty acids that are not made available for CE. We also cannot explain our finding that in the absence of EL we observe a significant increase in the plasma concentration of 16:0 CerPCho, and that in the absence of both HL and EL we observe an increase in the plasma concentration of 18:0 CerPCho, plus an increase in hepatic 16:0 CerPCho and 16:0 Cer. Future studies to identify the pool of these species of CerPCho that is/are responsible for the increased plasma concentrations in the absence of HL and EL, plus the identity of the lipoproteins that are responsible for carrying these CerPCho species in the absence of HL and EL, would provide a valuable insight into the connection between HL and EL, and the 16:0 and 18:0 species of CerPCho.

A confusing observation was previously reported for total TAG in mouse plasma, such that concentrations did not change between WT, HL-ko, EL-ko, and HL/EL-dko mice [13]. It would be expected that a deletion of at least HL in mice would lead to raised plasma TAG levels, as human subjects with HL deficiency exhibit hypertriglyceridemia [28]. We did not observe an increase to any of the individual TAG species assessed from plasma, but rather we unexpectedly observed a decrease to select species in the absence of HL, EL, or both. Both HL and EL appear to have compensatory roles in mouse models that prevent the accumulation of sdLDL in plasma [13]. It is likely that additional compensatory roles exist. EL might compensate for the loss of HL by hydrolysing select species of TAG – notably 52:3, 52:2, 54:5, 54:4, 54:3, 56:7, and 58:8; and HL might compensate for the loss of EL by selectively hydrolysing the 52:4 and 54:4 species of TAG. Lipoprotein lipase (LPL), a family member of HL and EL that exhibits predominantly a TAG lipase activity, was previously shown to be elevated in post-heparin plasma from HL/EL-dko mice [13]. Thus, we suspect that an increase of LPL activity would also contribute to the reduction of select species of TAG in plasma.

Two interesting trends were observed through our analyses of DAG: species containing 18:1 or 18:2 tended to be lower in the plasma of mice with an absence of HL and/or EL, and species containing a saturated fatty acyl group together with either 20:4 or 22:6 tended to be elevated specifically in the plasma of HL/EL-dko mice. It would be anticipated that plasma DAG levels would be lower in the absence of HL or EL, and that this would be tied to an increase of plasma TAG levels. Since select species of TAG actually decrease, possibly from the influence of a compensating lipase activity, it is likely that the observed reduction

of DAG species with 18:1 or 18:2 fatty acyl chains lipase-ko mouse plasma is in part due also to a compensating lipase activity. More intriguing is the trend showing an increase in the HL/EL-dko plasma levels of DAG species containing a saturated fatty acyl group together with either 20:4 or 22:6. These species of DAG may be derived from the hydrolysis of TAG by LPL, but it is likely that they cannot be processed any further by LPL. In support of this idea, the TAG from plasma intermediate- and low-density lipoproteins was previously shown to be enriched with C_{20} and C_{22} fatty acyl chains in euthyroid and hypothyroid rats, plus the hydrolysis of TAG-rich lipoproteins from rats using heart perfusates containing LPL also led to the accumulation of 20:5 and C_{22} fatty acyl chains in intermediate-density lipoproteins [29]. In addition, LPL was shown to exhibit a low efficiency for hydrolysing TAG, DAG, and PtdCho containing 20:4 fatty acyl chains [30]. The levels of 20:4 and 22:6 FFA are interestingly also reduced in mice lacking HL, EL, or both. Thus, our observations indicate that HL and EL can effectively hydrolyse acylglycerides with these fatty acyl groups *in vivo*. To date, no *in vitro* studies have been carried out to address the fatty acyl species specificity on the hydrolysis of acylglycerides by EL. However, our observations are in agreement with *in vitro* data that show HL can effectively hydrolyse DAG containing 20:4 fatty acyl chains [30].

Our observation of increased plasma concentrations for select PakCho species in the absence of EL, plus the increased plasma concentrations for two species of PlsCho in the absence of both HL and EL, might simply reflect a potentially delayed clearance of lipoprotein associated ether PLs, since the clearance of plasma HDL is impaired in the absence of both HL and EL [13]. However, because the plasma concentration of the 18:0–20:4 species of PlsCho was not different between groups, we speculated that the raised plasma concentrations of select ether PL species in the absence of EL might be due to a reduced *sn*-2 hydrolytic activity that potentially could come from EL. A previous report showed that both *sn*-1 and *sn*-2 fatty acids could be liberated from reconstituted HDL particles containing PtdCho by EL, with the rate of the release of *sn*-1 fatty acids being greater than the rate of fatty acid release from the *sn*-2 position [31]. The authors of the latter study concluded that the hydrolysis of PtdCho occurs initially at the *sn*-1 position, followed by a slower release of the fatty acyl chain from the *sn*-2 position. However, in the same study the authors found that EL could not hydrolyse the 16:0–20:4 species of PlsCho. Consistent with the latter study, we found that in the absence of EL, there was no change versus WT mice to the plasma concentration of the 16:0–20:4 species of PlsCho. Thus, we believe that EL simply cannot hydrolyse this specific PlsCho. Our data show that EL can in fact hydrolyse the artificial *sn*-2 substrate NOB. Thus, we suspect it is possible that EL may exhibit an *sn*-2 hydrolytic activity specifically toward the PlsCho and PakCho species that we identified as being increased in the plasma of mice lacking EL, or both EL and HL. While the use of the aqueous substrate NOB to assess PLA_2 activity eliminates the difficulty of preparing lipid emulsions with reproducible physical properties, the hydrolysis of the NOB ester by EL may not reflect how EL might hydrolyse the *sn*-2 acyl groups of ether PLs in a lipid environment. Future work to assess the hydrolysis by EL (as well as HL and LPL) of emulsions containing the PlsCho and PakCho species that we identified as being increased in the plasma of mice lacking EL, or both EL and HL, would be necessary to conclusively determine if EL or other *sn*-1 lipases can in fact exhibit an *sn*-2 hydrolytic activity toward

select molecular species of ether PLs. Such future studies would be important toward the understanding of how EL (and potentially also HL) and the ether PLs we identified could influence atherosclerosis, especially as PLA_2 activity in plasma has a detrimental impact on coronary artery disease [32, 33].

Overall, our study shows for the first time the influence of HL and EL on individual molecular species of several classes of lipids *in vivo* using lipidomic methods. Our wealth of data show that there are several differing effects on plasma and hepatic lipids that are unique to HL and EL, but other effects that are also complementary to both HL and EL. Our study also supports the idea that EL might exhibit an *sn*-2 hydrolytic activity *in vivo*. Our data provides a reference that could be used to compare the lipidome of human subjects with HL or EL deficiency; any commonly modulated lipids between the two lipidomes would be of great interest for future studies from a lipid standpoint toward addressing their possible associations with atherosclerosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by an IgniteR&D grant from the Research & Development Corporation of Newfoundland and Labrador (R.J.B.), a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada (R.J.B.), a National Scientist Development Grant (#11SDG7210077) from the American Heart Association (W.R.L.), National Institutes of Health Grants HL-022633 and HL-55323 (D.J.R.), and National Institutes of Health Grants HL-074214 and HL-111906 (D.A.F.). The authors with to thank Ms. Catherine Wright (University of Washington, Seattle, WA, USA) for advice with statistical analyses.

Abbreviations

CE	Cholesteryl ester
Cer	Ceramide
CerPCho	Sphingomyelin
DAG	Diacylglycerol
dko	Double knockout
EL	Endothelial lipase
ESI-MS	Electrospray ionization-mass spectrometry
FFA	Free fatty acid
HDL	High-density lipoprotein
HDL-C	High-density lipoprotein cholesterol
HL	Hepatic lipase
ko	Knockout
LPL	Lipoprotein lipase

LysoPtdCho	Lysophosphatidylcholine
NL	Neutral loss
NOB	4-Nitro-3-(octanoyloxy)benzoic acid
PakCho	Plasmanylcholine
PLA_2	Phospholipase A_2
PlsCho	Plasmenylcholine
PtdCho	Phosphatidylcholine
PL	Phospholipid
sdLDL	Small-dense low-density lipoprotein
SRM	Selective reaction monitoring
TAG	Triacylglycerol
WT	Wild-type

References

1. Brown RJ, Rader DJ. Lipases as modulators of atherosclerosis in murine models. Curr Drug Targets. 2007; 8:1307–1319. [PubMed: 18220707]
2. Ehnholm C, Greten H, Brown WV. A comparative study of post-heparin lipolytic activity and a purified human plasma triacylglycerol lipase. Biochim Biophys Acta. 1974; 360:68–77. [PubMed: 4859189]
3. Sanan DA, Fan J, Bensadoun A, Taylor JM. Hepatic lipase is abundant on both hepatocyte and endothelial cell surfaces in the liver. J Lipid Res. 1997; 38:1002–1013. [PubMed: 9186917]
4. Jaye M, Lynch KJ, Krawiec J, Marchadier D, Maugeais C, Doan K, South V, Amin D, Perrone M, Rader DJ. A novel endothelial-derived lipase that modulates HDL metabolism. Nat Genet. 1999; 21:424–428. [PubMed: 10192396]
5. Hirata K, Dichek HL, Cioffi JA, Choi SY, Leeper NJ, Quintana L, Kronmal GS, Cooper AD, Quertermous T. Cloning of a unique lipase from endothelial cells extends the lipase gene family. J Biol Chem. 1999; 274:14170–14175. [PubMed: 10318835]
6. Gonzalez-Navarro H, Nong Z, Freeman L, Bensadoun A, Peterson K, Santamarina-Fojo S. Identification of mouse and human macrophages as a site of synthesis of hepatic lipase. J Lipid Res. 2002; 43:671–675. [PubMed: 11971936]
7. Qiu G, Hill JS. Endothelial lipase enhances low density lipoprotein binding and cell association in THP-1 macrophages. Cardiovasc Res. 2007; 76:528–538. [PubMed: 17822686]
8. McCoy MG, Sun G-S, Marchadier D, Maugeais C, Glick JM, Rader DJ. Characterization of the lipolytic activity of endothelial lipase. J Lipid Res. 2002; 43:921–929. [PubMed: 12032167]
9. Fuki IV, Blanchard N, Jin W, Marchadier DH, Millar JS, Glick JM, Rader DJ. Endogenously produced endothelial lipase enhances binding and cellular processing of plasma lipoproteins via heparan sulfate proteoglycan-mediated pathway. J Biol Chem. 2003; 278:34331–34338. [PubMed: 12810721]
10. Dichek HL, Brecht W, Fan J, Ji Z-S, McCormick SPA, Akeefe H, Conzo L, Sanan DA, Weisgraber KH, Young SG, Taylor JM, Mahley RW. Overexpression of hepatic lipase in transgenic mice decreases apolipoprotein B-containing and high density lipoproteins: evidence that hepatic lipase acts as a ligand for lipoprotein uptake. J Biol Chem. 1998; 273:1896–1903. [PubMed: 9442022]

11. Ji Z-S, Dichek HL, Miranda RD, Mahley RW. Heparan sulfate proteoglycans participate in hepatic lipase and apolipoprotein E-mediated binding and uptake of plasma lipoproteins, including high density lipoproteins. J Biol Chem. 1997; 272:31285–31292. [PubMed: 9395455]
12. Zambon A, Deeb SS, Bensadoun A, Foster KE, Brunzell JD. In vivo evidence of a role for hepatic lipase in human apoB-containing lipoprotein metabolism, independent of its lipolytic activity. J Lipid Res. 2000; 41:2094–2099. [PubMed: 11108744]
13. Brown RJ, Lagor WR, Sankaranaravanan S, Yasuda T, Quertermous T, Rothblat GH, Rader DJ. Impact of combined deficiency of hepatic lipase and endothelial lipase on the metabolism of both high-density lipoproteins and apolipoprotein B-containing lipoproteins. Circ Res. 2010; 107:357–364. [PubMed: 20558822]
14. Escolà-Gil JC, Chen X, Julve J, Quesada H, Santos D, Metso J, Tous M, Jauhiainen M, Blanco-Vaca F. Hepatic lipase- and endothelial lipase-deficiency in mice promotes macrophage-to-feces RCT and HDL antioxidant properties. Biochim Biophys Acta. 2013; 1831:691–697. [PubMed: 23328279]
15. Han X, Gross RW. Shotgun lipidomics: electrospray ionization mass spectrometric analysis and quantitation of cellular lipidomes directly from crude extracts of biological samples. Mass Spectrom Rev. 2005; 24:367–412. [PubMed: 15389848]
16. Ishida T, Choi S, Kundu RK, Hirata K, Rubin EM, Cooper AD, Quertermous T. Endothelial lipase is a major determinant of HDL level. J Clin Invest. 2003; 111:347–355. [PubMed: 12569160]
17. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol. 1959; 37:911–917. [PubMed: 13671378]
18. Han X, Gross RW. Quantitative analysis and molecular species fingerprinting of triacylglyceride molecular species directly from lipid extracts of biological samples by electrospray ionization tandem mass spectrometry. Anal Biochem. 2001; 295:88–100. [PubMed: 11476549]
19. Ford DA, Monda JK, Brush RS, Anderson RE, Richards MJ, Fliesler SJ. Lipidomic analysis of the retina in a rat model of Smith–Lemli–Opitz syndrome: alterations in docosahexaenoic acid content of phospholipid molecular species. J Neurochem. 2008; 105:1032–1047. [PubMed: 18182048]
20. Bowden JA, Shao F, Albert CJ, Lally JW, Brown RJ, Procknow JD, Stephenson AH, Ford DA. Electrospray ionization tandem mass spectrometry of sodiated adducts of cholesteryl esters. Lipids. 2011; 46:1169–1179. [PubMed: 21904795]
21. Quehenberger O, Armando A, Dumlao D, Stephens DL, Dennis EA. Lipidomics analysis of essential fatty acids in macrophages. Prostaglandins Leukot Essent Fatty Acids. 2008; 79:123–129. [PubMed: 18996688]
22. Essaji Y, Yang Y, Albert CJ, Ford DA, Brown RJ. Hydrolysis products generated by lipoprotein lipase and endothelial lipase differentially impact THP-1 macrophage cell signalling pathways. Lipids. 2013; 48:769–778. [PubMed: 23794138]
23. Petrovic N, Grove C, Langton PE, Misso NLA, Thompson PJ. A simple assay for a human serum phospholipase A2 that is associated with high-density lipoproteins. J Lipid Res. 2001; 42:1706–1713. [PubMed: 11590228]
24. Verhoeven AJM, Neve BP, Jansen H. Intracellular activation of rat hepatic lipase requires transport to the Golgi compartment and is associated with a decrease in sedimentation velocity. J Biol Chem. 2000; 275:9332–9339. [PubMed: 10734075]
25. Ben-Zeev O, Doolittle MH. Maturation of hepatic lipase: formation of functional enzyme in the endoplasmic reticulum is the rate-limiting step in its secretion. J Biol Chem. 2004; 279:6171–6181. [PubMed: 14630921]
26. Bamji-Mirza M, Sundaram M, Zhong S, Yao EF, Parks RJ, Yao Z. Secretion of triacylglycerol-poor VLDL particles from McA-RH7777 cells expressing human hepatic lipase. J Lipid Res. 2011; 52:540–548. [PubMed: 21189265]
27. Erickson B, Selvan SP, Ko KWS, Kelly K, Quiroga AD, Li L, Nelson R, King-Jones K, Jacobs RL, Lehner R. Endoplasmic reticulum-localized hepatic lipase decreases triacylglycerol storage and VLDL secretion. Biochim Biophys Acta. 2013; 1831:1113–1123. [PubMed: 23376223]
28. Hegele RA, Little JA, Vezina C, Maguire GF, Tu L, Wolever TS, Jenkins DJ, Connelly PW. Hepatic lipase deficiency. Clinical, biochemical, and molecular genetic characteristics. Arterioscler Thromb. 1993; 13:720–728. [PubMed: 8485124]

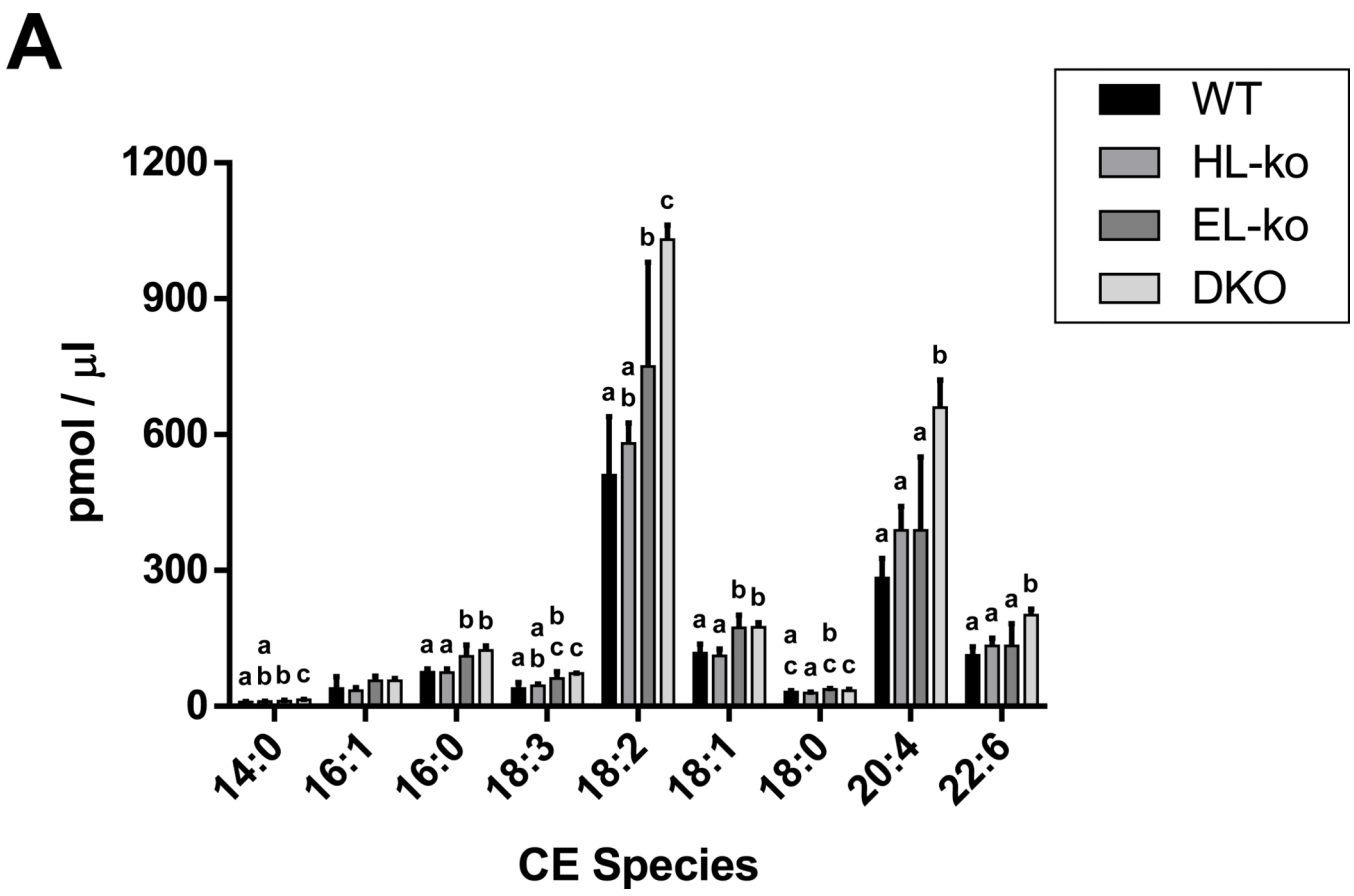
29. Ridgway N, Dolphin PJ. Lipoprotein lipase-mediated sequestration of longchain polyunsaturated triacylglycerols in serum LDL from normal and hypothyroid rats. Biochim Biophys Acta. 1984; 796:64–71. [PubMed: 6487646]

30. Nilsson A, Landin B, Schotz MC. Hydrolysis of chylomicron arachidonate and linoleate ester bonds by lipoprotein lipase and hepatic lipase. J Lipid Res. 1987; 28:510–517. [PubMed: 3598396]

31. Gauster M, Rechberger G, Sovic A, Hörl G, Steyrer E, Sattler W, Frank S. Endothelial lipase releases saturated and unsaturated fatty acids of high density lipoprotein phosphatidylcholine. J Lipid Res. 2005; 46:1517–1525. [PubMed: 15834125]

32. Rosengren B, Jönsson-Rylander A-C, Peilot H, Camejo G, Hurt-Camejo E. Distinctiveness of secretory phospholipase A2 group IIA and V suggesting unique roles in atherosclerosis. Biochim Biophys Acta. 2006; 1761:1301–1308. [PubMed: 17070102]

33. Stewart RH, White H. The role of lipoprotein-associated phospholipase A2 as a marker and potential therapeutic target in atherosclerosis. Curr Atheroscler Rep. 2011; 13:132–137. [PubMed: 21207201]



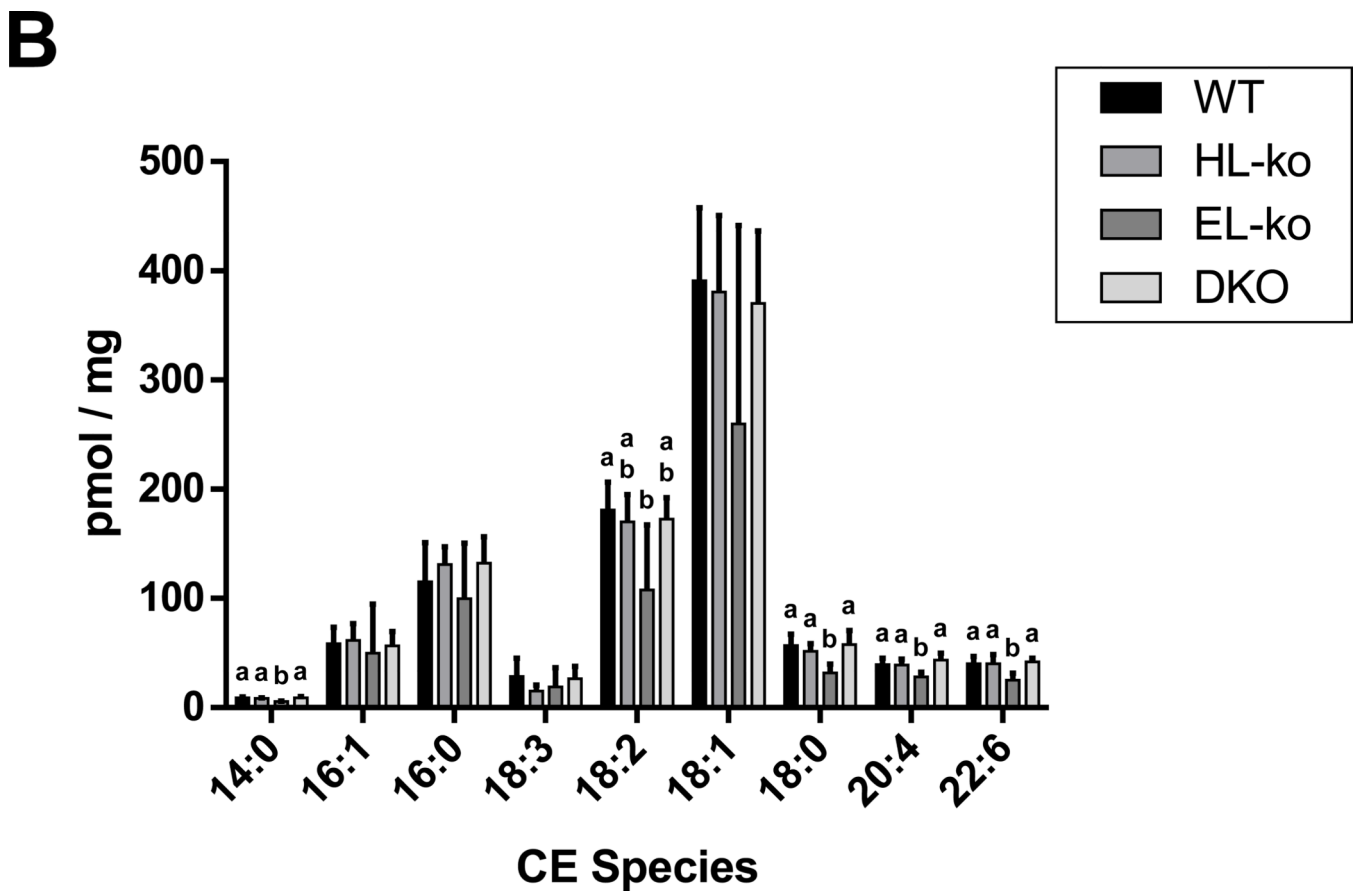
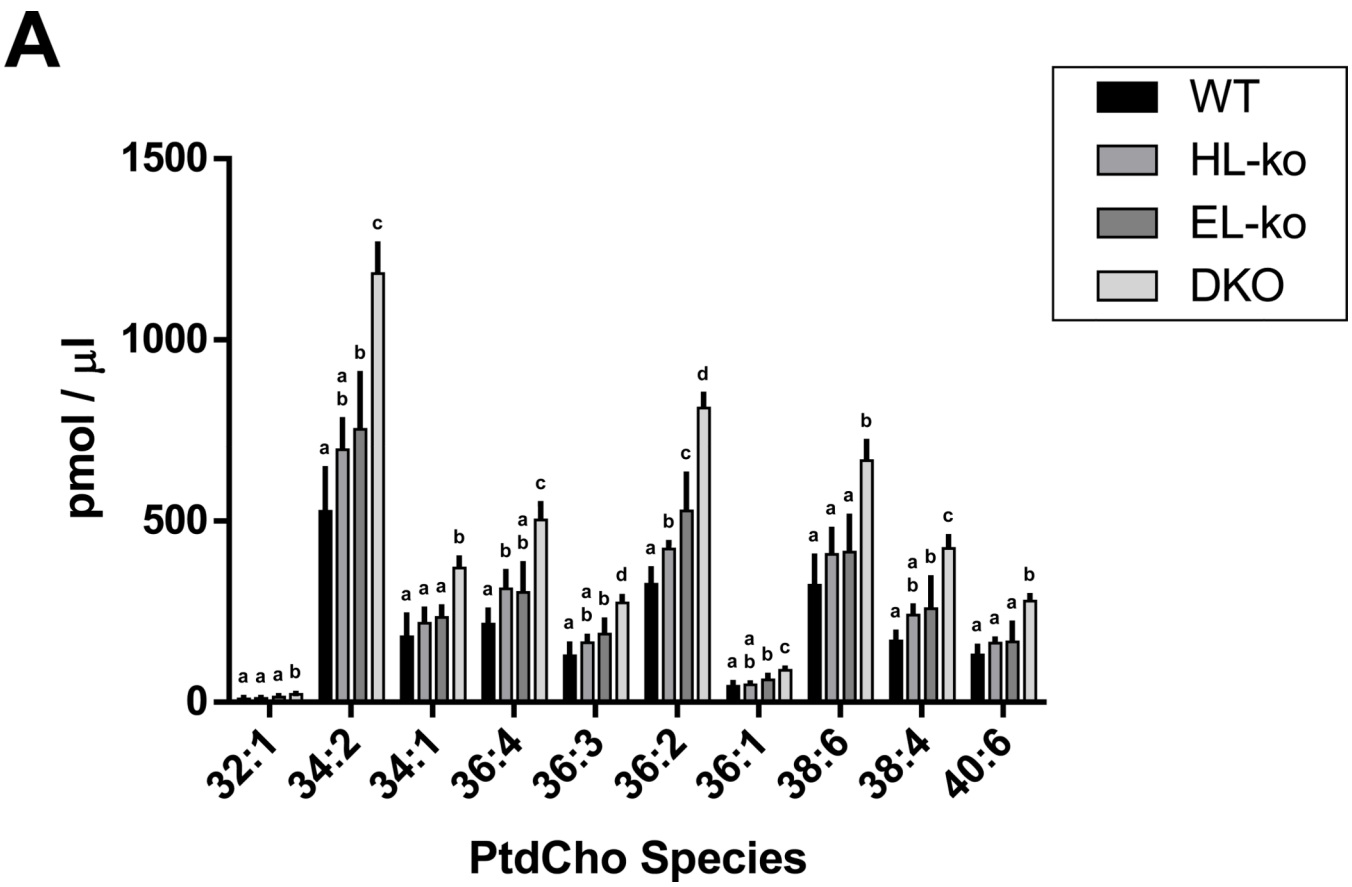


Fig. 1.

Plasma and hepatic levels of cholesteryl ester species in lipase deficient mice. (A) Plasma lipids were extracted from fasted 8–12 week old female wild-type (WT, *n*=7), hepatic lipase-knockout (HL-ko, *n*=5), endothelial lipase-ko (EL-ko, *n*=4), and HL/EL-double ko (DKO, *n*=5) mice. The pmol cholesteryl ester (CE) species per µl plasma were quantified with cholesteryl heptadecanoate as an internal control, as described under "Materials and Methods". The average means of data ±SD are shown. (B) Hepatic lipids were extracted from fasted 8–12 week old female WT (*n*=4), HL-ko (*n*=5), EL-ko (*n*=4), and DKO (*n*=5) mice. The pmol CE species per mg tissue were quantified as above. The average means of data ±SD are shown. For all statistically significant values, $p<0.05$.



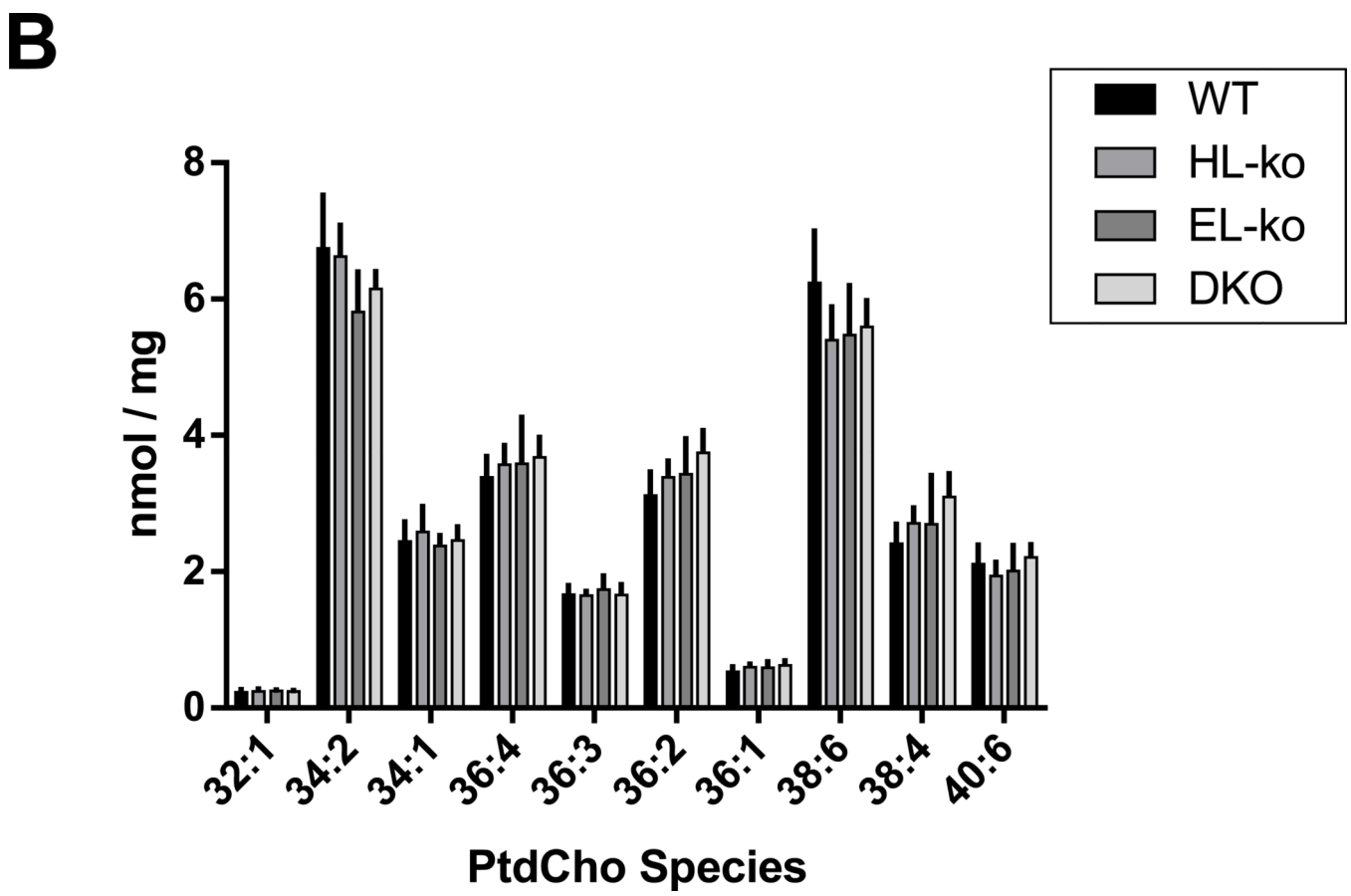
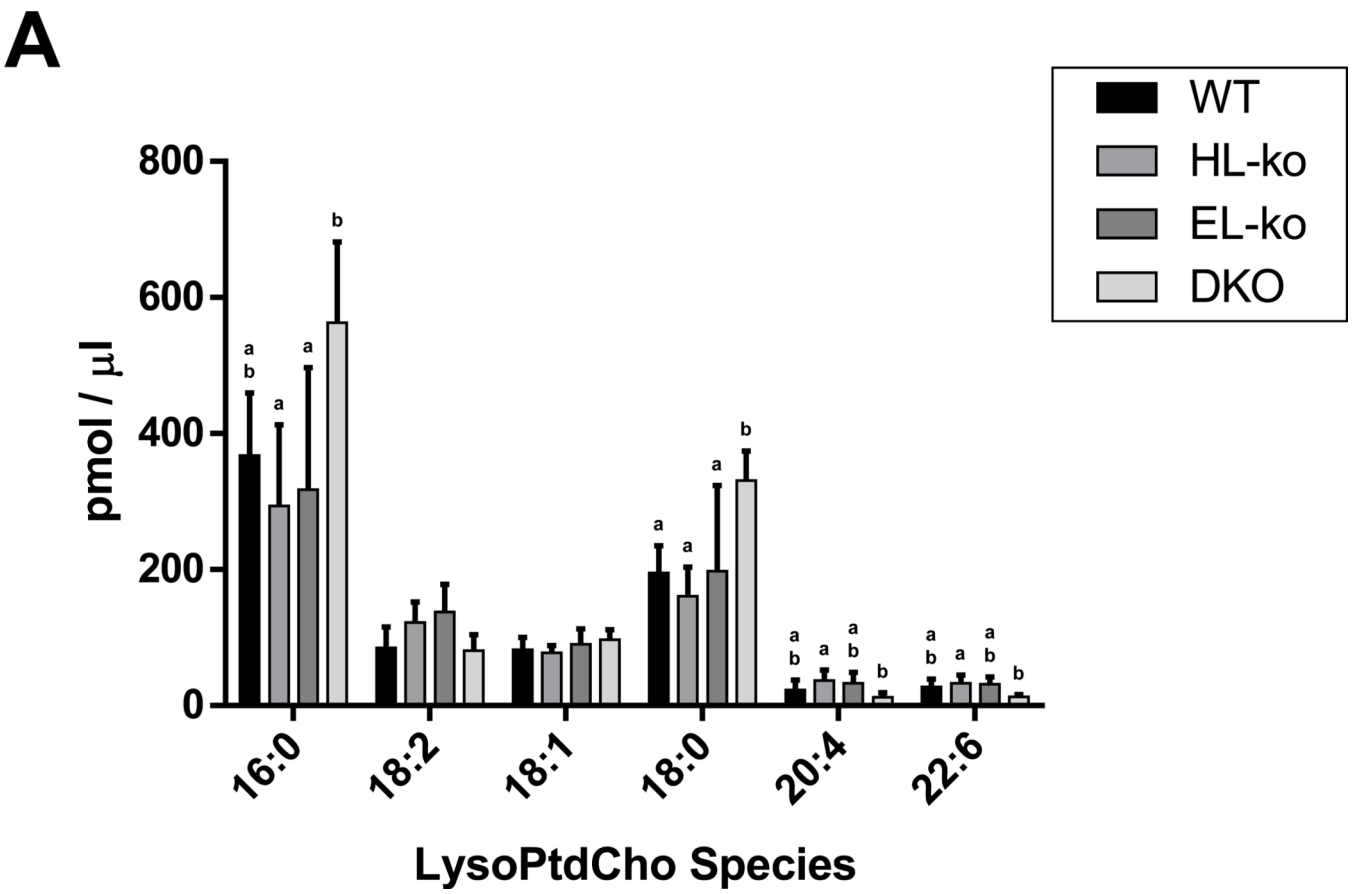


Fig. 2.

Plasma and hepatic levels of phosphatidylcholine species in lipase deficient mice. (A) Plasma lipids were extracted from fasted 8–12 week old female wild-type (WT, *n*=7), hepatic lipase-knockout (HL-ko, *n*=5), endothelial lipase-ko (EL-ko, *n*=4), and HL/EL-double ko (DKO, *n*=5) mice. The pmol phosphatidylcholine (PtdCho) species per μl plasma were quantified with 1,2-diarachidoyl-*sn*-glycero-3-phosphocholine as an internal control, as described under "Materials and Methods". The average means of data ±SD are shown. (B) Hepatic lipids were extracted from fasted 8–12 week old female WT (*n*=4), HL-ko (*n*=5), EL-ko (*n*=4), and DKO (*n*=5) mice. The nmol PtdCho species per mg tissue were quantified as above. The average means of data ±SD are shown. For all statistically significant values, $p<0.05$.



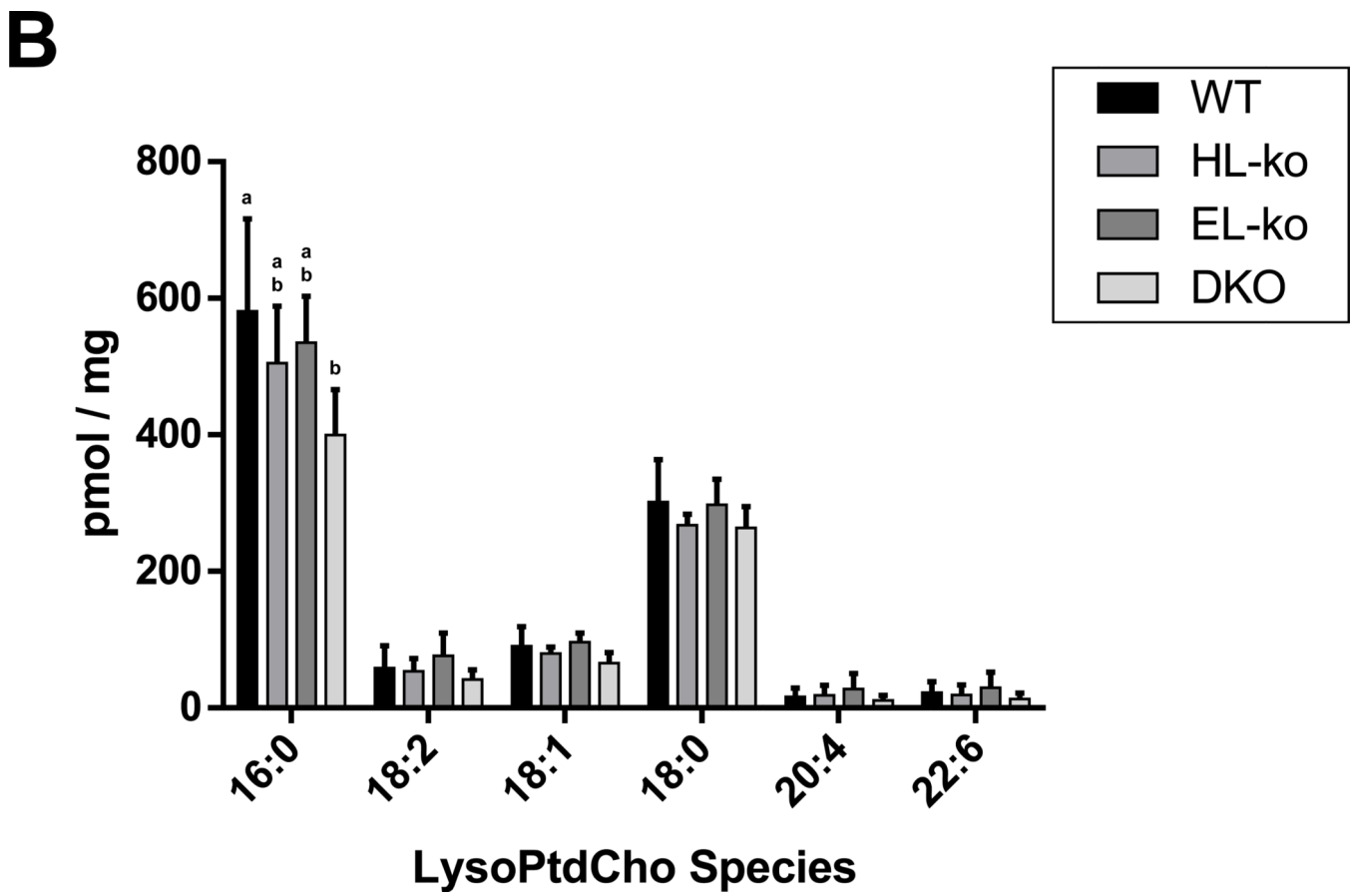
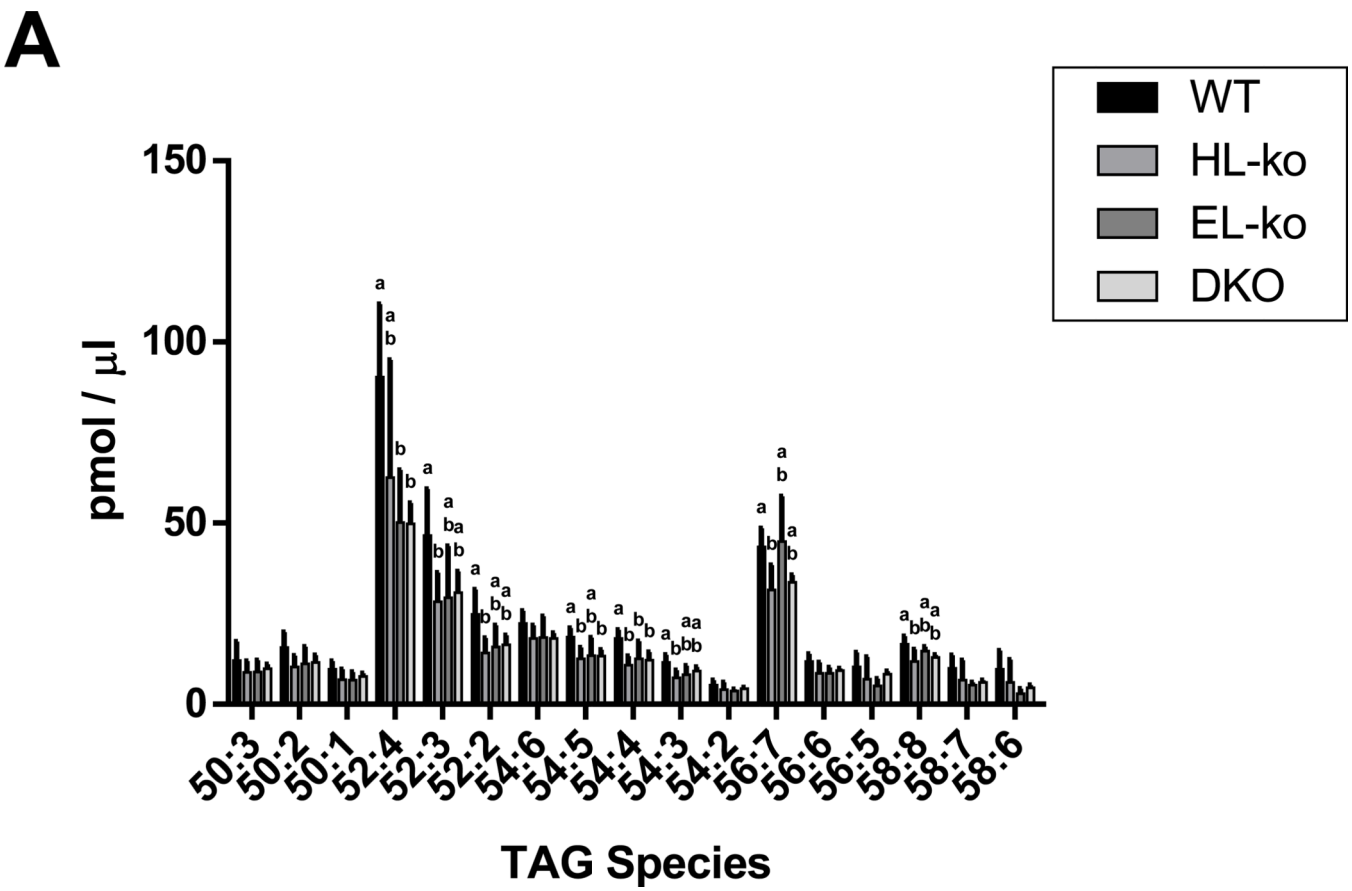


Fig. 3.

Plasma and hepatic levels of lysophosphatidylcholine species in lipase deficient mice. (A) Plasma lipids were extracted from fasted 8–12 week old female wild-type (WT, *n*=7), hepatic lipase-knockout (HL-ko, *n*=5), endothelial lipase-ko (EL-ko, *n*=4), and HL/EL-double ko (DKO, *n*=5) mice. The pmol lysophosphatidylcholine (LysoPtdCho) species per μl plasma were quantified with 1-heptadecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine as an internal control, as described under “Materials and Methods”. The average means of data ±SD are shown. (B) Hepatic lipids were extracted from fasted 8–12 week old female WT (*n*=4), HL-ko (*n*=5), EL-ko (*n*=4), and DKO (*n*=5) mice. The pmol LysoPtdCho species per mg tissue were quantified as above. The average means of data ±SD are shown. For all statistically significant values, $p<0.05$.



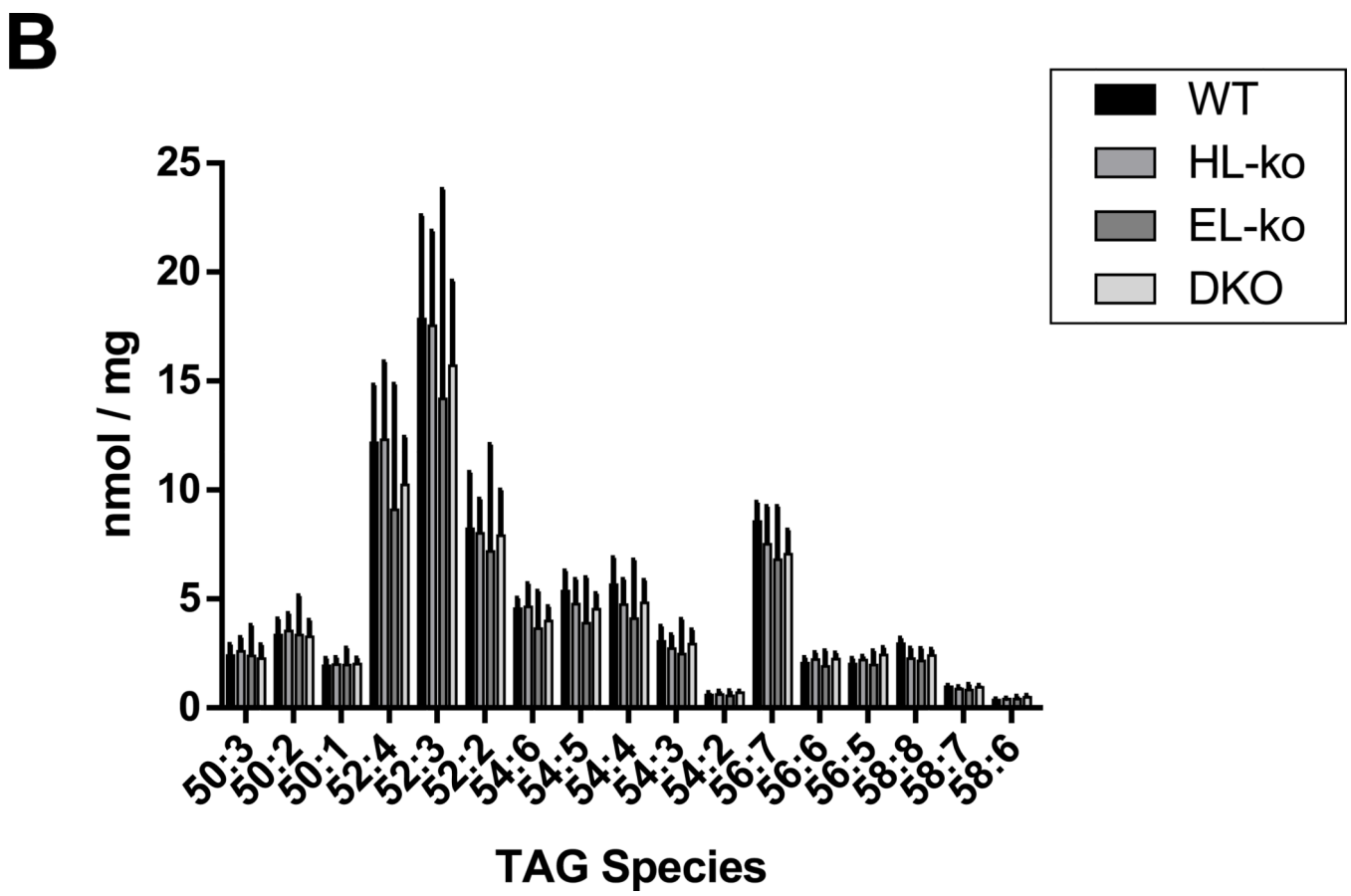
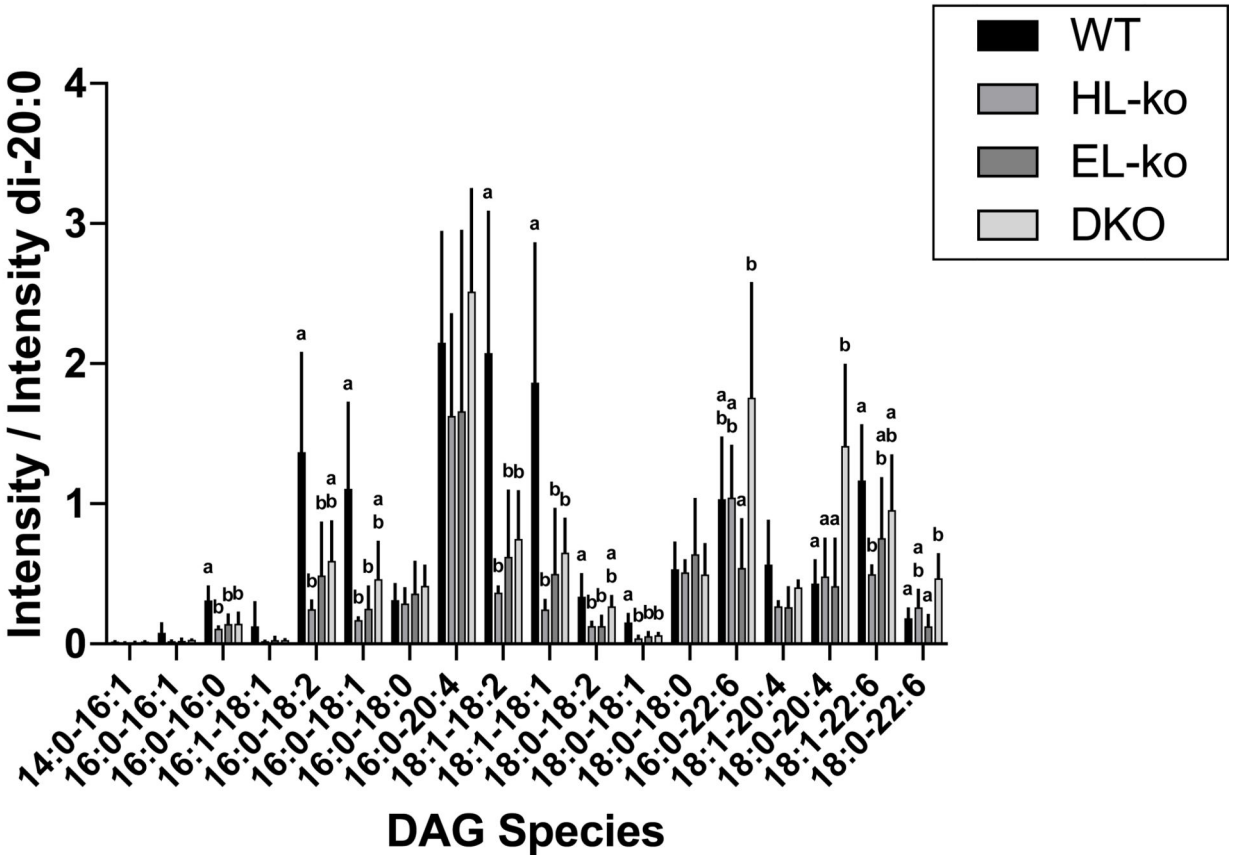


Fig. 4.

Plasma and hepatic levels of triacylglycerol species in lipase deficient mice. (A) Plasma lipids were extracted from fasted 8–12 week old female wild-type (WT, *n*=7), hepatic lipase-knockout (HL-ko, *n*=5), endothelial lipase-ko (EL-ko, *n*=4), and HL/EL-double ko (DKO, *n*=5) mice. The pmol triacylglycerol (TAG) species per μl plasma were quantified with Δ9-*trans*-triheptadecenoin as an internal control, as described under "Materials and Methods". The average means of data ±SD are shown. (B) Hepatic lipids were extracted from fasted 8–12 week old female WT (*n*=4), HL-ko (*n*=5), EL-ko (*n*=4), and DKO (*n*=5) mice. The nmol TAG species per mg tissue were quantified as above. The average means of data ±SD are shown. For all statistically significant values, $p<0.05$.

A



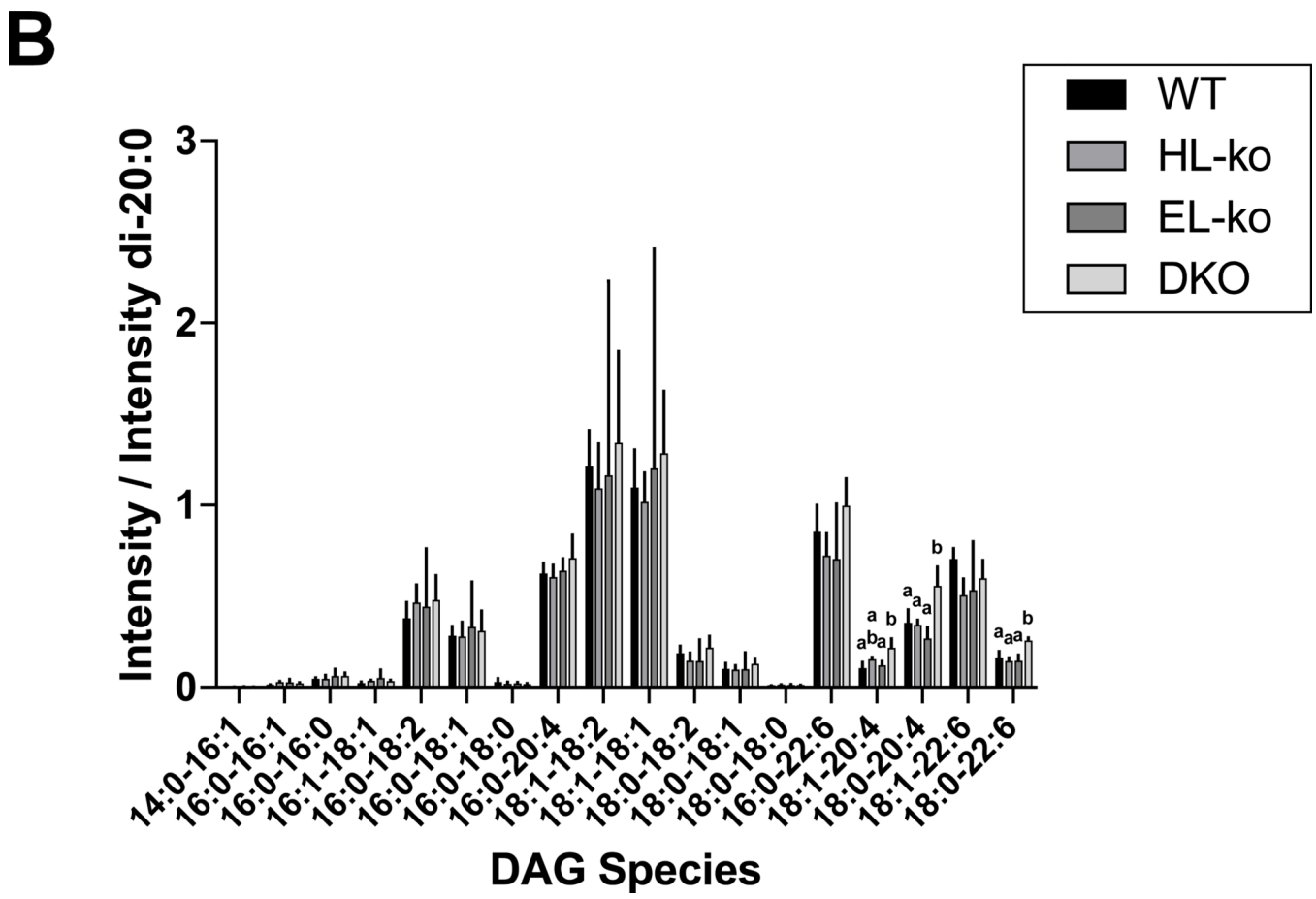
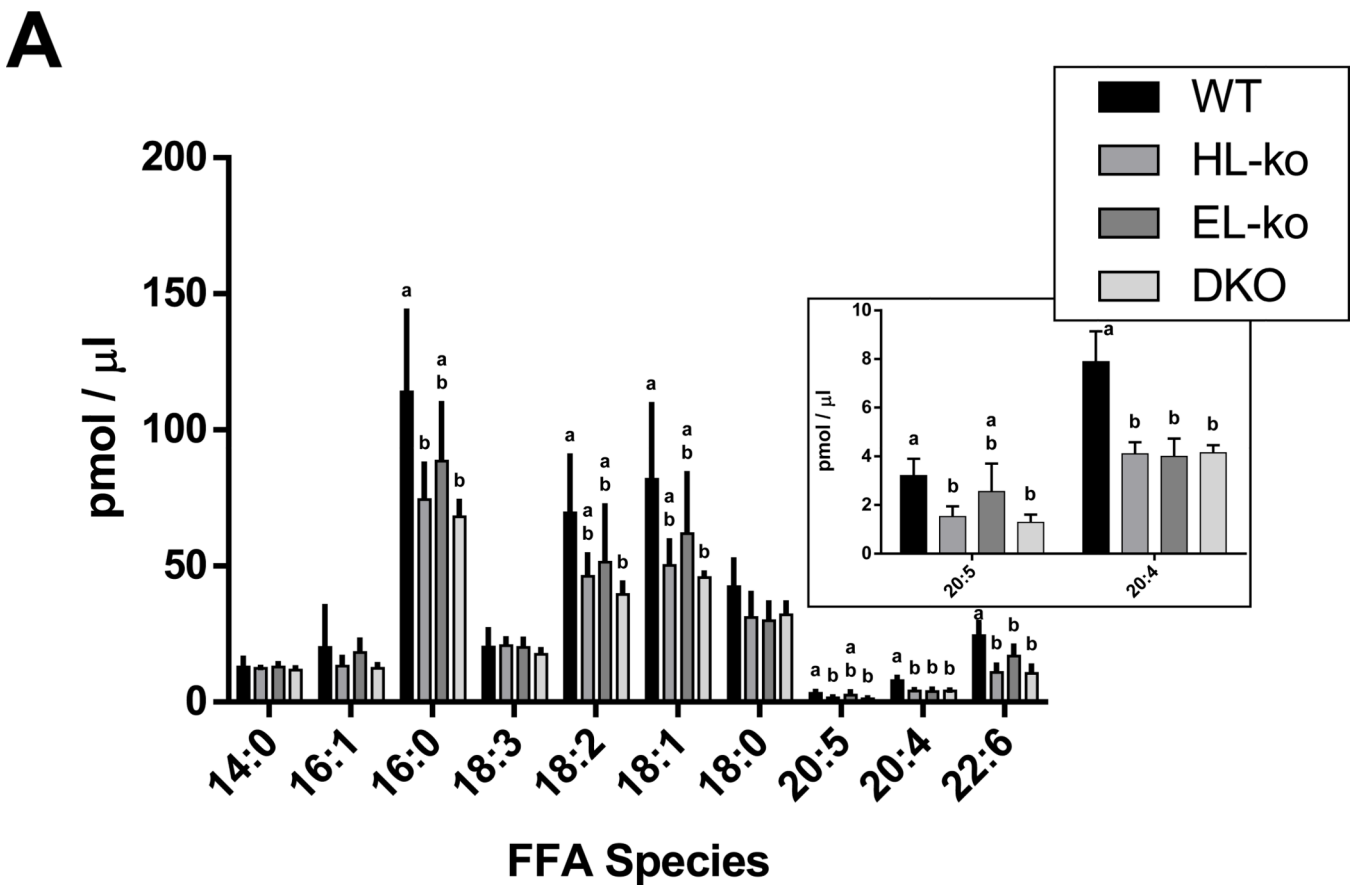


Fig. 5.

Plasma and hepatic levels of diacylglycerol species in lipase deficient mice. (A) Plasma lipids were extracted from fasted 8–12 week old female wild-type (WT, *n*=7), hepatic lipase-knockout (HL-ko, *n*=5), endothelial lipase-ko (EL-ko, *n*=4), and HL/EL-double ko (DKO, *n*=5) mice. The diacylglycerol (DAG) species were semi-quantified as a ratio of intensity relative to the intensity of the internal control diarachidin (di-20:0), as described under "Materials and Methods". The average means of data ±SD are shown. (B) Hepatic lipids were extracted from fasted 8–12 week old female WT (*n*=4), HL-ko (*n*=5), EL-ko (*n*=4), and DKO (*n*=5) mice. The DAG species were semi-quantified as above. The average means of data ±SD are shown. For all statistically significant values, $p<0.05$.



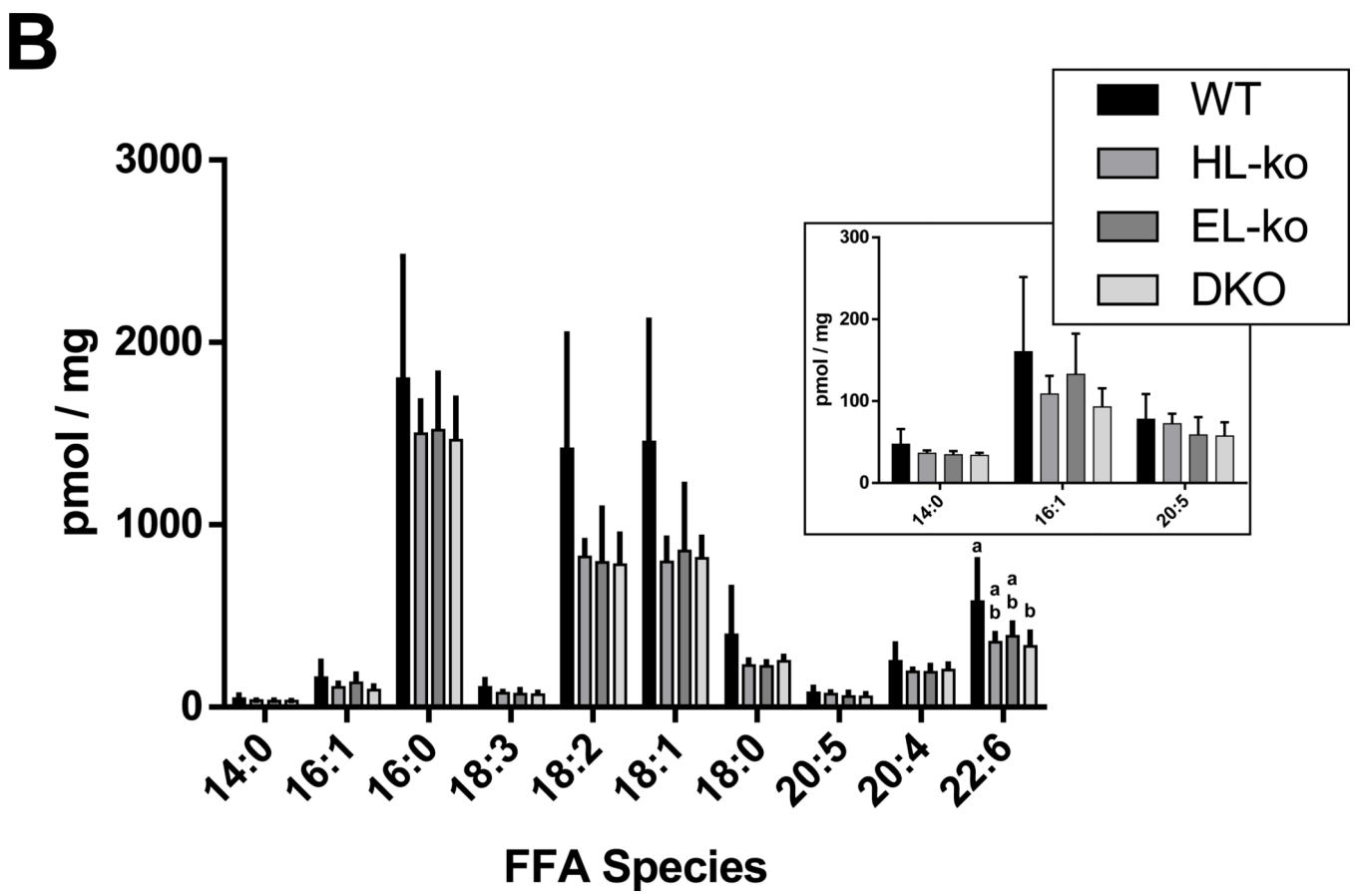
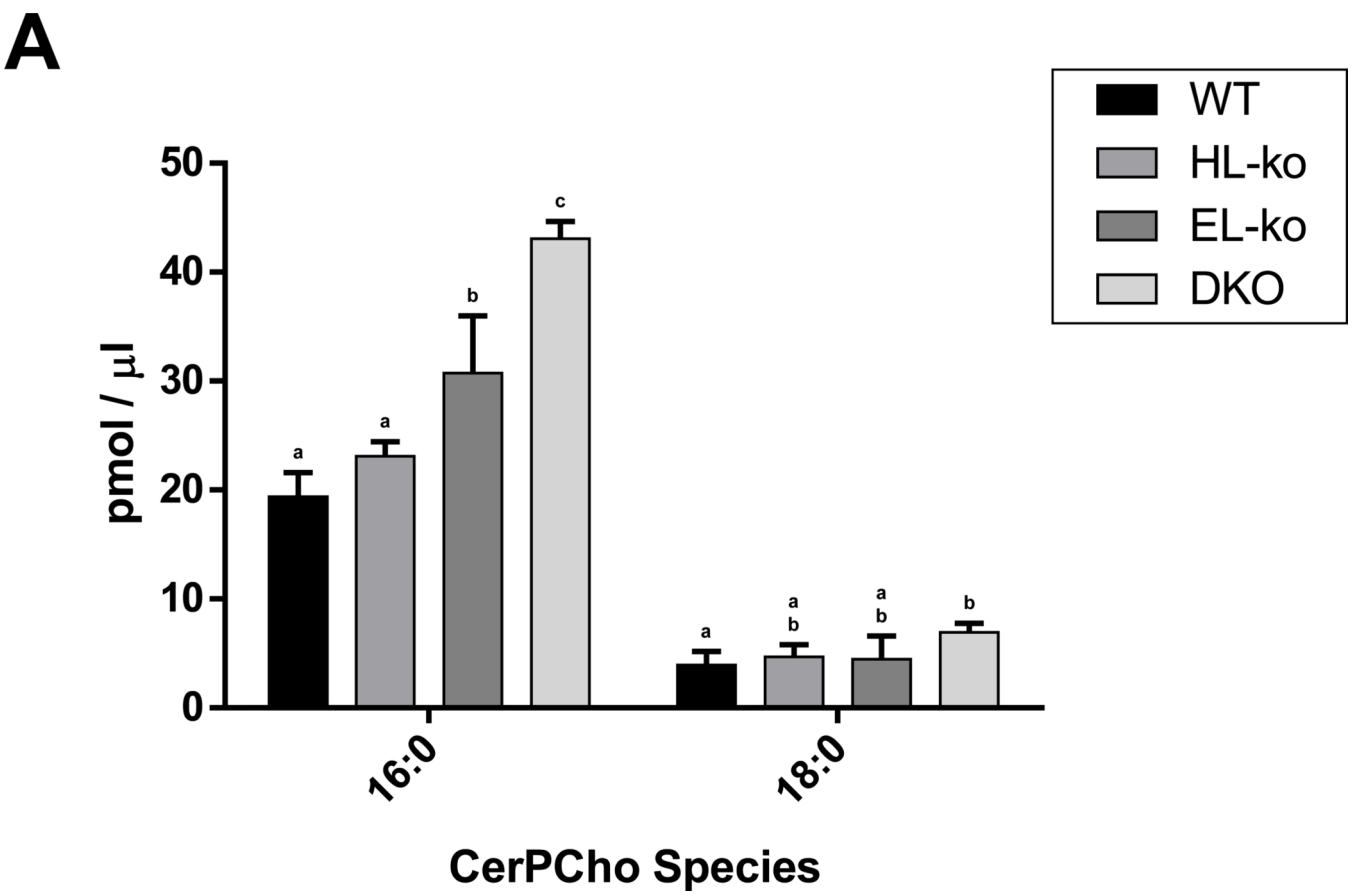


Fig. 6.

Plasma and hepatic levels of free fatty acid species in lipase deficient mice. (A) Plasma lipids were extracted from fasted 8–12 week old female wild-type (WT, *n*=7), hepatic lipase-knockout (HL-ko, *n*=5), endothelial lipase-ko (EL-ko, *n*=4), and HL/EL-double ko (DKO, *n*=5) mice. The pmol free fatty acid (FFA) species per μl plasma were quantified with arachidic acid as an internal control, as described under "Materials and Methods". The average means of data ±SD are shown. *Inset:* 20:5 and 20:4 FFA. (B) Hepatic lipids were extracted from fasted 8–12 week old female WT (*n*=4), HL-ko (*n*=5), EL-ko (*n*=4), and DKO (*n*=5) mice. The pmol FFA species per mg tissue were quantified as above. The average means of data ±SD are shown. *Inset:* 14:0, 16:1, and 20:5 FFA. For all statistically significant values, $p<0.05$.



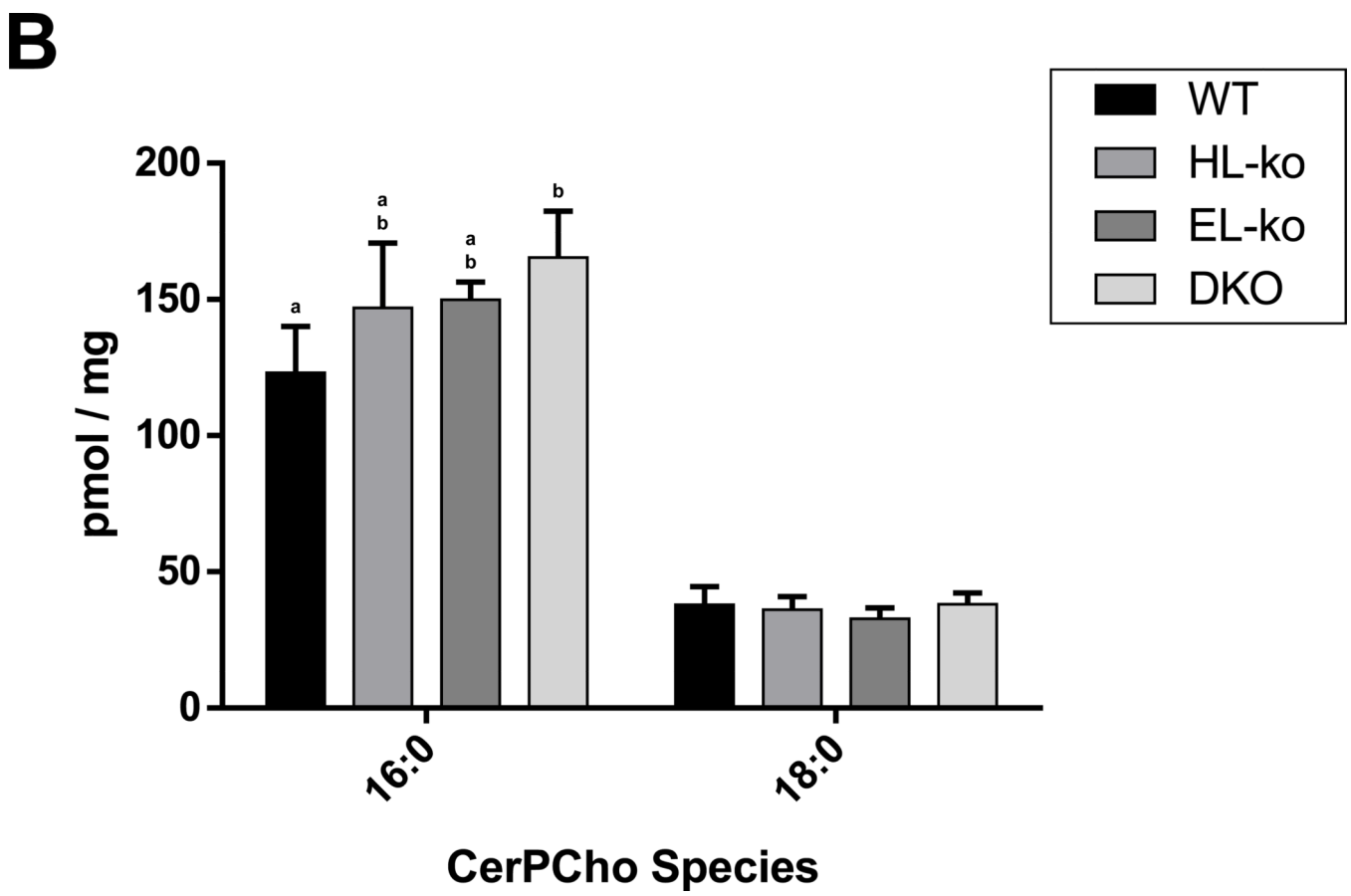
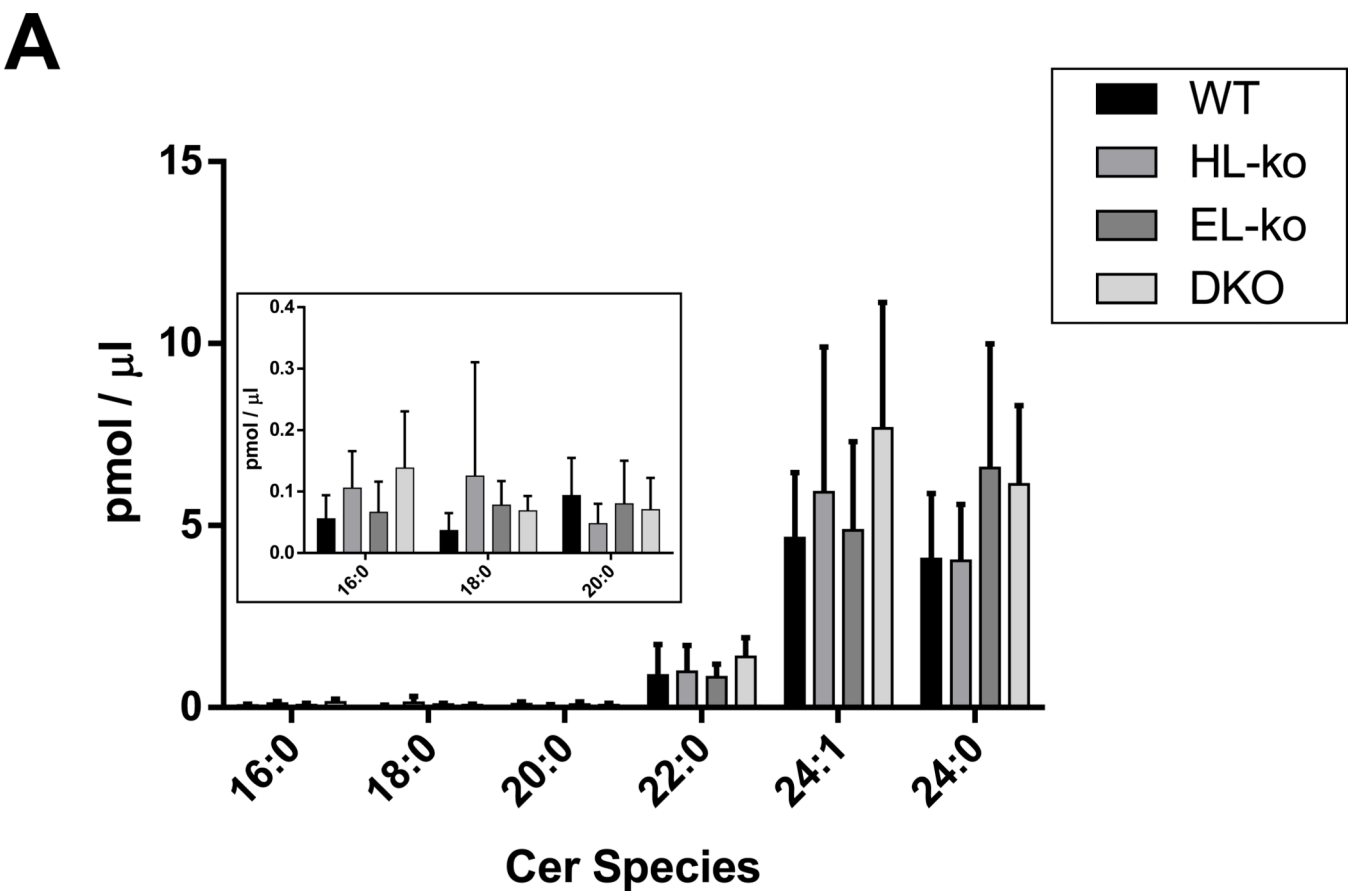


Fig. 7.

Plasma and hepatic levels of sphingomyelin species in lipase deficient mice. (A) Plasma lipids were extracted from fasted 8–12 week old female wild-type (WT, *n*=7), hepatic lipase-knockout (HL-ko, *n*=5), endothelial lipase-ko (EL-ko, *n*=4), and HL/EL-double ko (DKO, *n*=5) mice. The pmol sphingomyelin (CerPCho) species per μl plasma were quantified with N-heptadecanoyl-D-*erythro*-sphingosylphosphorylcholine as an internal control, as described under “Materials and Methods”. The average means of data ±SD are shown. (B) Hepatic lipids were extracted from fasted 8–12 week old female WT (*n*=4), HL-ko (*n*=5), EL-ko (*n*=4), and DKO (*n*=5) mice. The pmol CerPCho species per mg tissue were quantified as above. The average means of data ±SD are shown. For all statistically significant values, $p<0.05$.



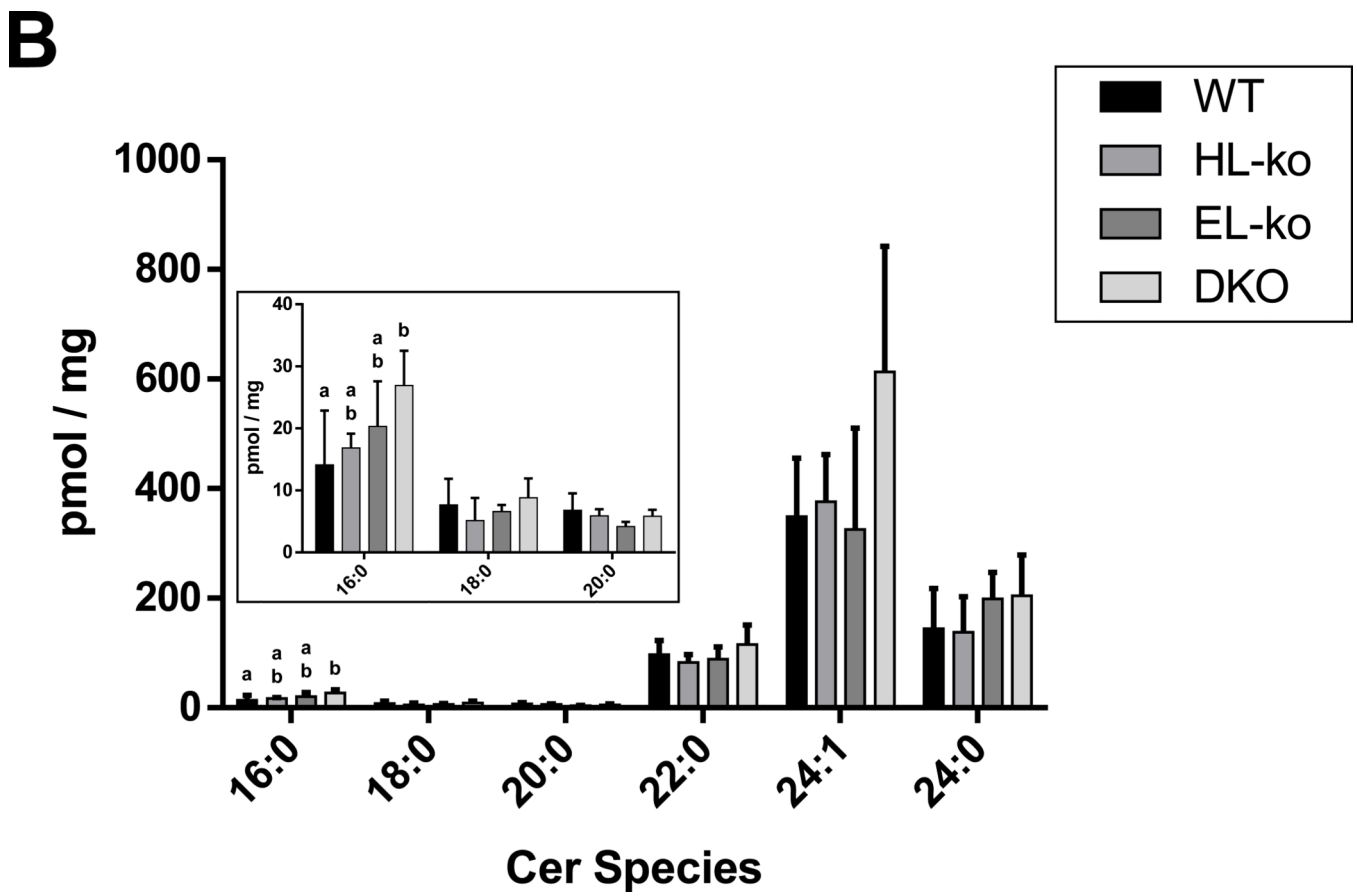


Fig. 8.

Plasma and hepatic levels of ceramide species in lipase deficient mice. (A) Plasma lipids were extracted from fasted 8–12 week old female wild-type (WT, *n*=7), hepatic lipase-knockout (HL-ko, *n*=5), endothelial lipase-ko (EL-ko, *n*=4), and HL/EL-double ko (DKO, *n*=5) mice. The pmol ceramide (Cer) species per μl plasma were quantified with N-hepadecanoyl-D-*erythro*-sphingosine as an internal control, as described under "Materials and Methods". The average means of data ±SD are shown. *Inset:* 16:0, 18:0, and 20:0 Cer. (B) Hepatic lipids were extracted from fasted 8–12 week old female WT (*n*=4), HL-ko (*n*=5), EL-ko (*n*=4), and DKO (*n*=5) mice. The pmol CerPCho species per mg tissue were quantified as above. *Inset:* 16:0, 18:0, and 20:0 Cer. The average means of data ±SD are shown. For all statistically significant values, $p<0.05$.

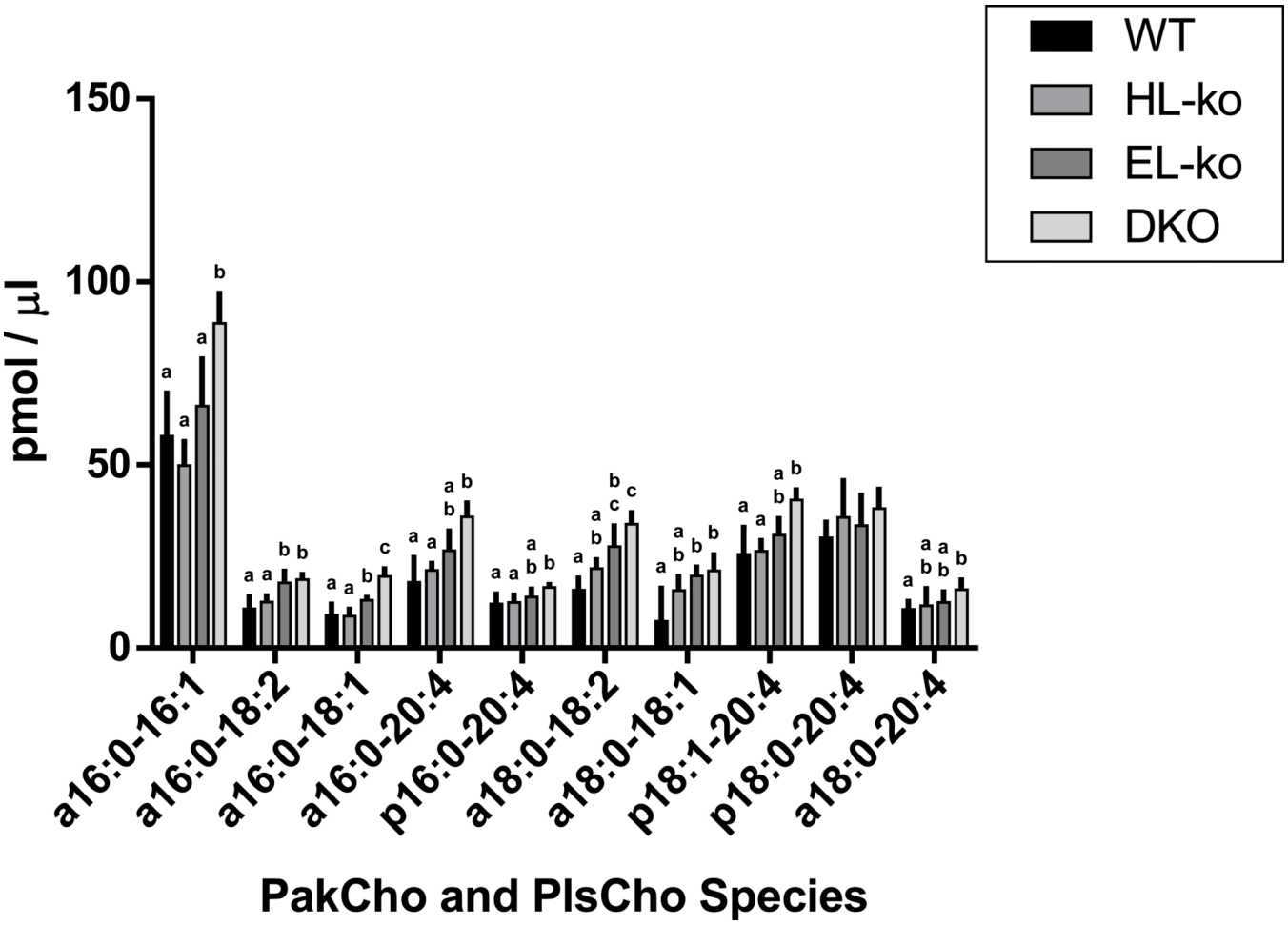


Fig. 9.

Plasma levels of plasmenylcholine and plasmanylcholine species in lipase deficient mice. Plasma lipids were extracted from fasted 8–12 week old female wild-type (WT, *n*=7), hepatic lipase-knockout (HL-ko, *n*=5), endothelial lipase-ko (EL-ko, *n*=4), and HL/EL-double ko (DKO, *n*=5) mice. The pmol of plasmenylcholine (PlsCho, indicated by “p”) and plasmanylcholine (PakCho, indicated by “a”) species per µl plasma were quantified with 1,2-diarachidoyl-*sn*-glycero-3-phosphocholine as an internal control, as described under “Materials and Methods”. The average means of data ±SD are shown. For all statistically significant values, $p<0.05$.

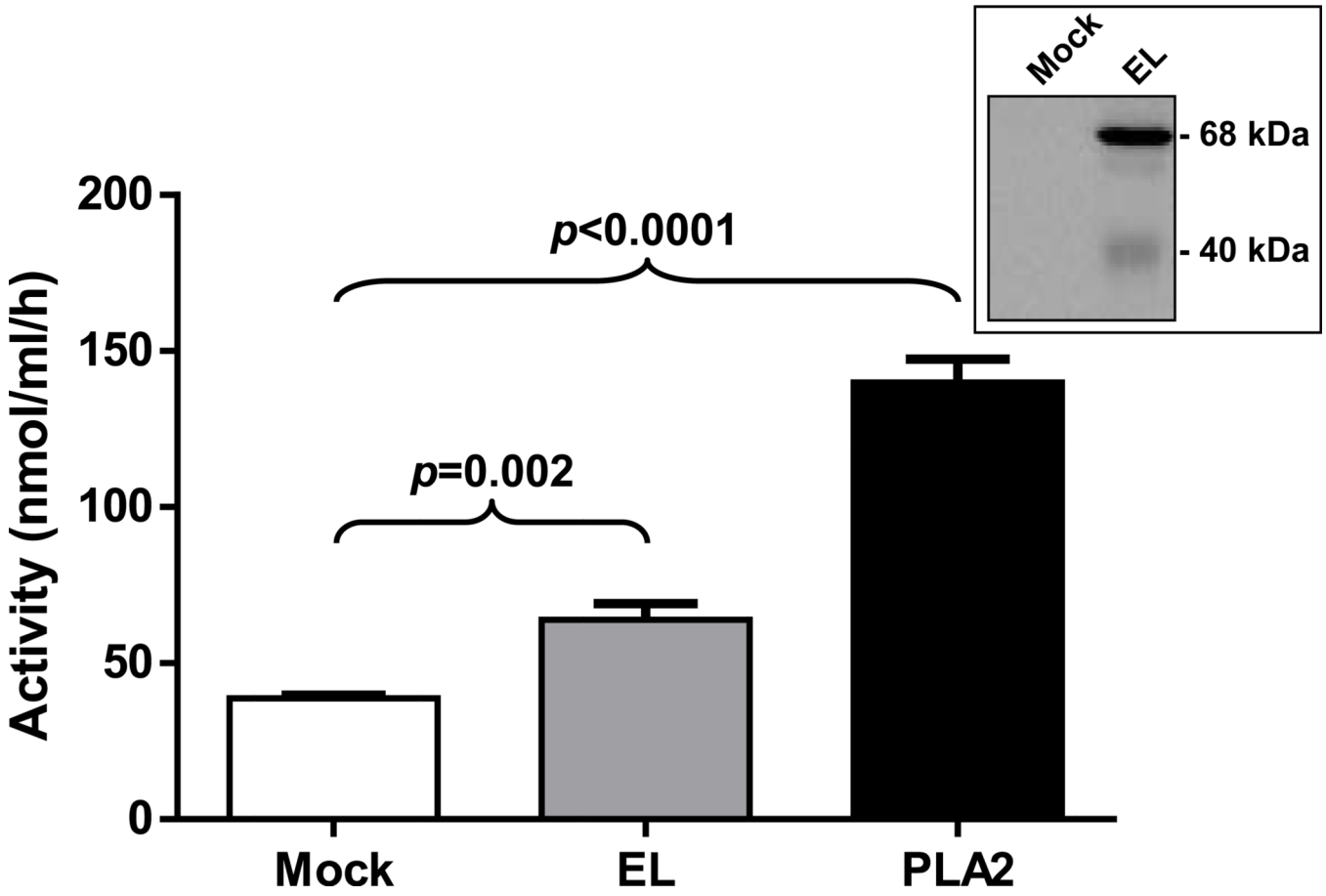


Fig. 10.

Phospholipase A_2 activity of recombinant endothelial lipase. Heparinized media from mock transfected (Mock) HEK293 cells, heparinized media from HEK293 cells expressing endothelial lipase (EL), and honey bee venom phospholipase A_2 (PLA2, as a positive control) were tested for their ability to hydrolyse the phospholipase A_2 substrate 4-nitro-3-(octanoyloxy)benzoic acid. Data are expressed as the nmol of 4-nitro-3-benzoic acid generated per ml sample per hour. The average means of data ±SD from triplicate experiments are shown. *Inset:* immunoblot of heparinized media from mock transfected and EL expressing HEK293 cells; full-length 68 kDa EL and 40 kDa proprotein convertase cleaved EL products are indicated.