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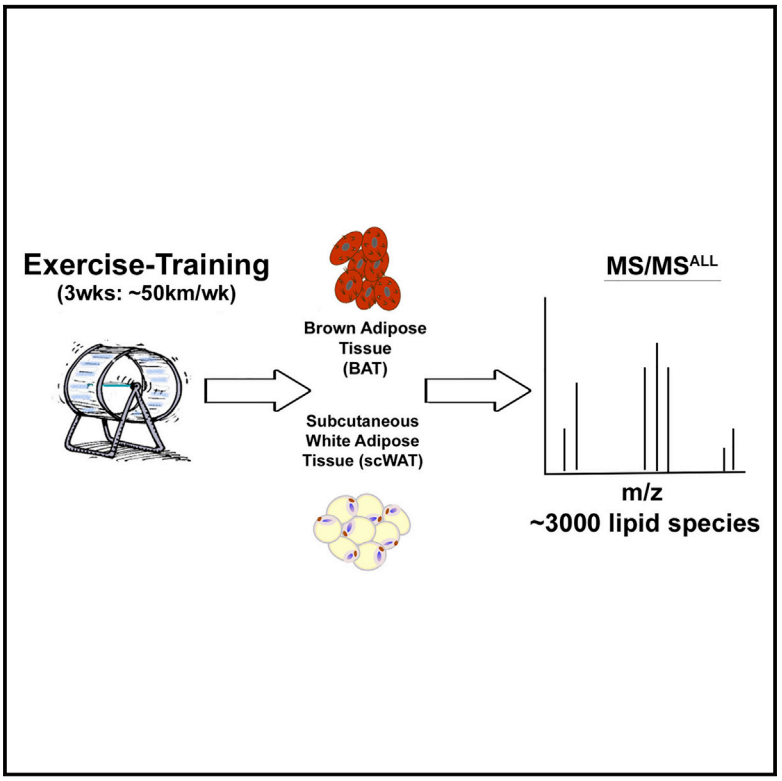
Lipidomic Adaptations in White and Brown Adipose Tissue in Response to Exercise Demonstrate Molecular Species-Specific Remodeling

Graphical Abstract



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In Brief

Using an MS/MS^{ALL} shotgun lipidomics approach, May et al. demonstrate that exercise causes a molecular species-specific remodeling of subcutaneous white adipose tissue (scWAT) and brown adipose tissue (BAT). These species-specific changes are depot dependent (divergent changes in scWAT versus BAT) and specific to the stimulus (exercise-induced adaptations to the BAT lipidome are distinct from cold-induced changes to the BAT lipidome).

Highlights

- This study examines the effects of exercise on the lipidome of scWAT and BAT
- Exercise results in distinct phospholipid species-specific remodeling of scWAT and BAT
- Exercise decreases TAGs in both scWAT and BAT

May et al., 2017, Cell Reports *18*, 1558–1572
February 7, 2017
http://dx.doi.org/10.1016/j.celrep.2017.01.038

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Lipidomic Adaptations in White and Brown Adipose Tissue in Response to Exercise Demonstrate Molecular Species-Specific Remodeling

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http://dx.doi.org/10.1016/j.celrep.2017.01.038

SUMMARY

Exercise improves whole-body metabolic health through adaptations to various tissues, including adipose tissue, but the effects of exercise training on the lipidome of white adipose tissue (WAT) and brown adipose tissue (BAT) are unknown. Here, we utilize MS/MS^{ALL} shotgun lipidomics to determine the molecular signatures of exercise-induced adaptations to subcutaneous WAT (scWAT) and BAT. Three weeks of exercise training decrease specific molecular species of phosphatidic acid (PA), phosphatidylcholines (PC), phosphatidylethanolamines (PE), and phosphatidylserines (PS) in scWAT and increase specific molecular species of PC and PE in BAT. Exercise also decreases most triacylglycerols (TAGs) in scWAT and BAT. In summary, exercise-induced changes to the scWAT and BAT lipidome are highly specific to certain molecular lipid species, indicating that changes in tissue lipid content reflect selective remodeling in scWAT and BAT of both phospholipids and glycerol lipids in response to exercise training, thus providing a comprehensive resource for future studies of lipid metabolism pathways.

INTRODUCTION

White adipose tissue (WAT) and brown adipose tissue (BAT) are critical modulators of energy metabolism. The adipocytes within WAT store large amounts of triglycerides as chemical energy in unilocular droplets and are also involved in hormone production, immune function, and local tissue architecture (Tran and Kahn, 2010). WAT acts as a reserve of lipid energy and releases lipids into circulation as needed. Increases in WAT mass (adiposity) are directly associated with increased rates of metabolic diseases such as type 2 diabetes and obesity (Wang et al., 2005). In contrast to WAT, brown adipose tissue (BAT) is made up of multilocular brown adipocytes that contain numerous mitochondria, which function to mediate adaptive thermogenesis and protect against hypothermia and obesity (Cannon and Nedergaard, 2004). While the lipid in WAT is released for use as a substrate by other tissues, the lipid in BAT releases energy as heat and also stores energy as lipids for use in non-shivering thermogenesis. BAT is characterized by high levels of expression of uncoupling protein 1 (UCP1), the protein responsible for non-shivering thermogenesis (Lowell and Spiegelman, 2000; Ouellet et al., 2012).

Exercise improves metabolic health by increasing whole-body glucose homeostasis, insulin sensitivity, and fatty acid oxidation (Bonadonna et al., 1993; Jeukendrup, 2002). The effects of endurance exercise training to increase lipolysis and free fatty acid mobilization from WAT during acute bouts of exercise (Craig et al., 1981; Gollisch et al., 2009), to reduce adiposity (Craig et al., 1981; Gollisch et al., 2009), and to increase the expression of several metabolic proteins including GLUT4 and PGC1α in WAT (Craig et al., 1981; Gollisch et al., 2009; Hirshman et al., 1989; Stallknecht et al., 1991; Stanford et al., 2015b; Sutherland et al., 2009) have been well established. The effects of exercise on BAT, however, are less clear. BAT is a highly innervated tissue, and exercise stimulates sympathetic nervous system (SNS) activity (Nedergaard and Cannon, 2014; Ranallo and Rhodes, 1998). From a biological perspective, it is possible that exercise-induced β-adrenergic receptor stimulation results in activation of BAT and stimulation of lipolysis, although this has not been clearly established. In fact, the effects of exercise on gene expression and metabolic activity in BAT have resulted in conflicting observations; some studies have demonstrated increased BAT activity with exercise (Hirata, 1982; Hirata and Nagasaka, 1981; Ignacio et al., 2012; Xu et al., 2011a, 2011b; Yoshioka et al., 1989), others studies showed no exercise-induced changes in BAT activity (Leblanc et al., 1982; Richard et al., 1986, 1987; Wickler et al., 1987), and another set of studies

reported a decrease in BAT activity with exercise (Boss et al., 1998; Larue-Achagiotis et al., 1994; Stanford and Goodyear, 2016; Sullo et al., 2004; Vosselman et al., 2015; Wu et al., 2014). Although the effects of exercise on BAT are unclear, both WAT and BAT can regulate fatty acid homeostasis in addition to glucose metabolism (Chondronikola et al., 2014; Stanford et al., 2013, 2015b).

Recent studies have investigated the role of diet or cold exposure on the lipidomic profile of WAT and BAT (Caesar et al., 2010; Chondronikola et al., 2016; Duarte et al., 2014; Marcher et al., 2015). One recent study performed lipidomics analysis on BAT after acute cold exposure and demonstrated significant and species-specific remodeling of phospholipids and triacylglycerols (TAGs) in BAT (Marcher et al., 2015). The effect of exercise on the lipid metabolic signature of scWAT and BAT, however, has not been investigated. Given the increasing interest in exercise as a regulator of adipose tissue, and its possible role in regulating the beneficial effects of exercise in metabolism (Stanford and Goodyear, 2016; Stanford et al., 2015a, 2015b; Trevellin et al., 2014) it is critical to determine how exercise alters the lipidome of adipose tissue. Here, we report a comprehensive analysis of lipid composition and metabolic pathways in mouse subcutaneous WAT (scWAT) and BAT in response to chronic exercise training using a highly sensitive MS/MS^{ALL} mass spectrometry approach (Simons et al., 2012). We found species-specific changes in diacylglycerols (DAG), triglycerides (TAG), phosphatidic acids (PA), phosphatidylcholines (PC), phosphatidylethanolamines (PE), and phosphatidylserines (PS) in scWAT and BAT. We determined the activation and repression of genes in pathways corresponding to changes in lipid subclasses identified by lipidomics and bioinformatics analyses. Rather than large shifts in the total amounts of lipid subtypes, these changes were highly specific to certain molecular lipid species, indicating that changes in tissue lipid content reflect selective remodeling in both scWAT and BAT in response to exercise training. These changes likely have significant functional implications. These data represent a comprehensive investigation of the effects of exercise on the lipid signature of scWAT and BAT and will provide a valuable resource for future investigations of the exercise-induced changes in scWAT and BAT lipid composition and gene expression.

RESULTS

Exercise Training Has Differential Effects on the Overall Composition of Lipid Classes in scWAT and BAT

Exercise training can result in dramatic changes in the gene expression profile and overall lipid content of adipose tissue, yet little is known about the effects of exercise training on the lipidomic profile of adipose tissue. We applied an MS/MS^{ALL} shotgun lipidomics approach to comprehensively characterize the effects of exercise training on the content and composition of structural lipids in scWAT and BAT. Three weeks of exercise training significantly decreased body mass and reduced scWAT mass but had no effect on BAT mass (Figure S1). In scWAT, exercise training resulted in a significant decrease in the abundance of three phospholipids, phosphatidylserines (PS), lysophosphatidylglycerols (LPG), and lysophosphatidylinositols (LPI) (Figures 1A and 1B; Table S1). There was also a significant decrease in triacylglycerols (TAGs) in scWAT (Figure 1C; Table S1). There was no change in CE, Cer, CoQ, Glycolipids, or fatty acid hydroxyl fatty acids (FAHFA) (Figure 1D; Table S1). Although there were only significant changes in overall concentration of four lipid classes, significant differences in the abundance of specific molecular species of each lipid class were identified (Figure 1E). Given the dramatic response to exercise training on overall lipid content, gene expression, and alterations to the metabolic profile of scWAT (Hirshman et al., 1989; Stanford et al., 2015a, 2015b; Sutherland et al., 2009; Trevellin et al., 2014), it is interesting to note that only four out of 24 classes of lipids are significantly altered with exercise.

Examination of BAT after 3 weeks of exercise training revealed a significant increase in the abundance of phosphatidylcholine (PC) (Figure 2A) and cholesterol esters (CE) (Figure 2C; Table S1), and decreases in overall abundance of cardiolipins (CL), LPG, and TAG (Figures 2A and 2B; Table S1). Similar to scWAT, while significant changes were only observed in overall concentration of five lipid classes, there were significant differences in the abundance of several specific molecular species of each lipid class (Figure 2D).

The overall abundance of TAGs, which are the most abundant lipid class in WAT and BAT, was significantly decreased in both scWAT and BAT in response to exercise (Figures 1C and 2B). Thus, while exercise training significantly decreases TAGs in both scWAT and BAT, exercise training regulates different lipid classes in scWAT and BAT. Interestingly, these changes in overall abundance of major lipid classes in BAT in response to exercise are greater than previously reported changes to major lipid classes in BAT after short-term cold exposure (Marcher et al., 2015).

Exercise Training Decreases Abundance of Individual Phospholipids in scWAT

To determine the specific chain lengths that are altered in abundance after to chronic exercise, we examined each individual PA, PC, PE, and PS species in scWAT of sedentary and trained mice. Individual lipid species were determined by unique acyl chain length and saturation. Lipidomic analysis revealed an overall decrease in abundance of several phospholipid species in scWAT in response to chronic exercise. This included significant decreases in major phospholipid molecular species of PA, PC, PE, and PS (species that are significantly changed are displayed in Figure 3A, species that are non-significantly altered are in Table S1), even though the only change in total concentration of phospholipids was in PS (Figures 1A–1C). Highly abundant PA species with side chains of 16:0/20:4 and 18:1/20:2 were significantly decreased in scWAT after chronic exercise. There were significant decreases of several PC species of high abundance in trained scWAT, including PC 36:4 (Figure 3A; Table S1). Many PCs of lesser abundance, notably several with even chain length (32–36 carbons), were also significantly decreased in trained scWAT (Figure 3A; Table S1). PE species of minor abundance containing 34:0, 36:1, 36:6, 42:4, or PE-42:0/PE_O-42:7 chains, and PS species containing 16:0 and 18:0, 18:1, or 18:2 chains were significantly decreased in scWAT after chronic exercise (Figure 3A; Table S1). These molecular

A

Lipid Class	Abbreviation
TAG	Triacylglycerols
DAG	Diacylglycerols
SM	Sphingomyelins
FAHFA	Fatty acid hydroxy fatty acids
CL	Cardiolipins
PS	Phosphatidylserines
PI	Phosphatidylinositols
PG	Phosphatidylglycerol
PE	Phosphatidylethanolamines
PC	Phosphatidylcholines
AC	Acylcarnitines
PA	Phosphatidic acid
FFA	Free fatty acids
MAG	Monoacylglycerols
CE	Cholesterol esters
Cer	Ceramides
CoQ	Coenzyme Q
LPA	Lysophosphatidic acids
LPC	Lysophosphatidylcholines
LPE	Lysophosphatidylethanolamines
LPS	Lysophosphatidylserines
LPG	Lysophosphatidylglycerols
LPI	Lysophosphatidylinositols

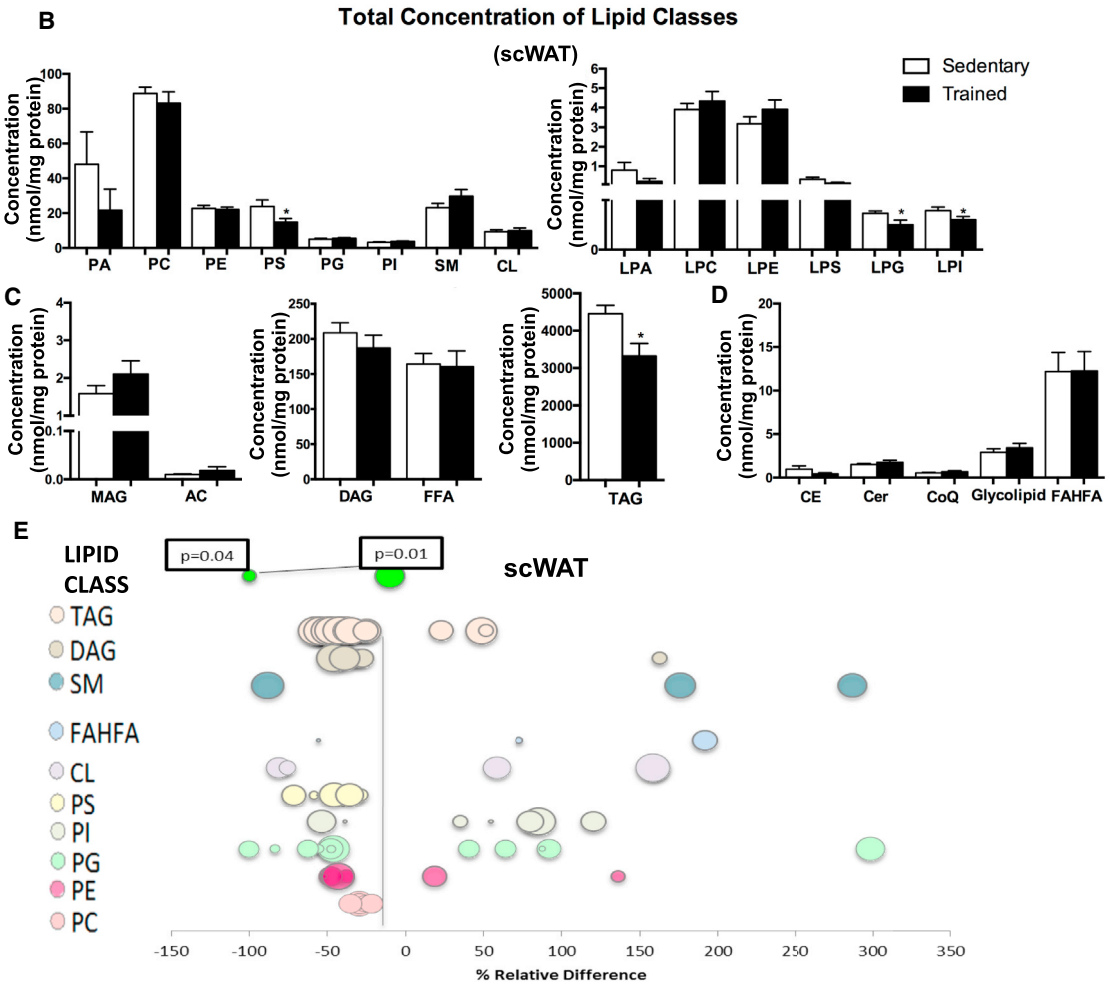


Figure 1. Changes in Lipid Composition of scWAT after Chronic Exercise

(A) Quantified lipid classes and their abbreviations.

(B–D) Concentration of quantified lipid classes in scWAT of sedentary versus exercise-trained mice. Data are presented as means ± SEM (n = 6/group; *p < 0.05).

(E) The relative percentage difference in concentration of quantified lipid species between sedentary and exercise-trained mice. Each circle represents a significantly changed particular species of lipid within the indicated lipid class. Size of circle indicates level of significance (n = 6/group; p < 0.05, increasing size with increasing significance).

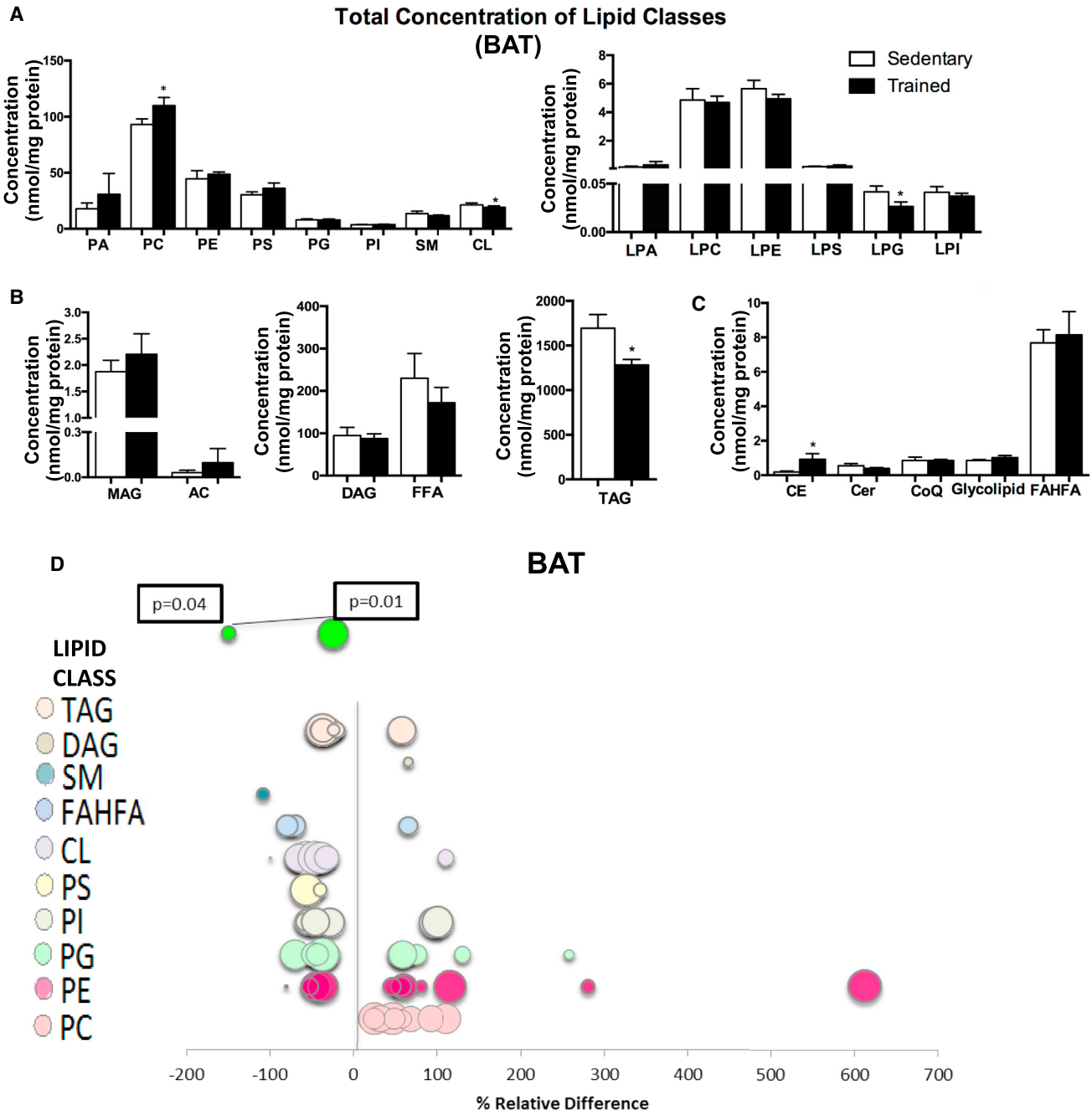
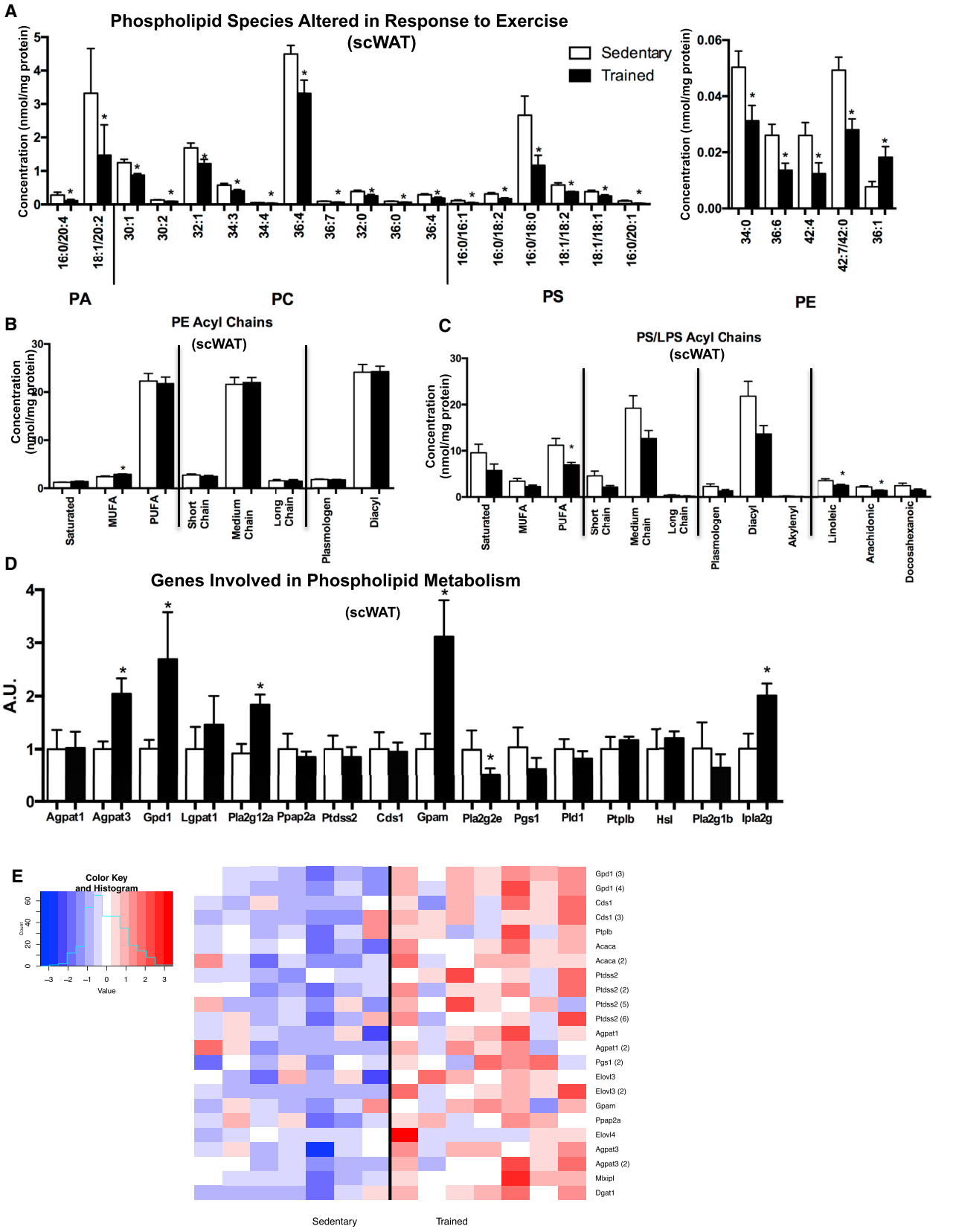


Figure 2. Changes in Lipid Composition of BAT after Chronic Exercise

(A–C) Concentration of quantified lipid classes in scWAT of sedentary versus exercise-trained mice. Data are presented as means ± SEM (n = 6/group; *p < 0.05). (D) The relative percentage difference in concentration of quantified lipid species between sedentary and exercise-trained mice. Each circle represents a significantly changed particular species of lipid within the indicated lipid class. Size of circle indicates level of significance (n = 6/group; p < 0.05, increasing size with increasing significance).

species-specific changes indicate marked phospholipid remodeling in scWAT in response to chronic exercise training.

To determine the effects of exercise on each phospholipid species, we grouped individual phospholipid species based on saturation and length. Species were placed into unsaturated, monounsaturated (MUFA), polyunsaturated (PUFA), and total saturated groups based solely on number of acyl chain double bonds, without accounting for bond location. Based on chain length, lipid species were placed into groups of short, medium, or long chain length. Plasmologens (phospholipids with an



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aldehyde replacing an acyl group) and diacyl (phosphopholipids with an acyl group at the sn-1 and sn-2 position) groups were also identified. PA and PS species were also further subdivided into groups if they contained a linoleic acid 18:2, arachidonic acid 20:4, or docosahexaenoic acid 22:6 acyl chain.

Chronic exercise training did not alter chain length or saturation of PA or PC species (Figures S2A and S2B), but there was a significant effect of exercise training on the chain length and saturation of PE, PS, and LPS species. Exercise significantly increased the total monounsaturated PEs, driven mainly by the increase of PE_O-36:1 (Figures 3A and 3B). In PS/LPS species, polyunsaturated species were significantly decreased, as were 18:2- and 20:4-containing species. Other chain-length categories in the PS/LPS species also trend to be decreased with chronic exercise, including diacyl and short- and medium-length chains (Figure 3C). While these data indicate changes in phospholipid content in scWAT after exercise, previous studies in humans and rodents have demonstrated that low intensity exercise decreased the phospholipid content in skeletal muscle and that this decrease in phospholipid content improved skeletal muscle insulin sensitivity (Andersson et al., 1998; Goto-Inoue et al., 2013; Helge et al., 1999). Exercise improves insulin sensitivity in scWAT (Burstein et al., 1992; Koivisto and Yki-Jarvinen, 1987; Stanford et al., 2015b), and while it is has not been established that the reduction in phospholipids or phospholipid remodeling is a mechanism for the exercise-induced increase in insulin sensitivity in scWAT, it is possible that this decrease in specific phospholipid species contributes to WAT insulin sensitivity after exercise.

Phospholipid Metabolism Is Altered in scWAT with Exercise

These lipidomics data indicate that several phospholipid species are significantly decreased in scWAT, indicating a remodeling of phospholipids after chronic exercise. To determine the cause of these changes, we measured expression of key genes in the phospholipid pathway that likely contribute to the remodeling of lipid species. Surprisingly, there were significant increases in several genes known to regulate phospholipid metabolism.

Expression of *Gpam*, the enzyme that regulates the first step in the phospholipid pathway, as well as expression of *Agpat3*, *Gpd1*, and *Pla2g12a*, were significantly increased after exercise in scWAT, indicating an upregulation of genes coding for major enzymes involved in phospholipid remodeling (Figure 3D). Interestingly, *Pla2g2e*, a phospholipase that is upregulated in obesity and plays a role in insulin resistance (Sato et al., 2014), is significantly decreased in exercise-trained scWAT. An increase in *Gpam,* which prefers saturated substrates for synthesis of PA, should indicate an increase in monounsaturated species of PA, but the increase in monounsaturated species is limited to PE. In contrast, polyunsaturated PS/LPS species are significantly decreased, suggesting that remodeling occurs downstream of PA synthesis in the glycerolipid metabolic pathway. *Agpat3*, *Gpd1*, and *Pla2g12a* are all involved in the synthesis and remodeling of PC, PE, PS, and LPS. Since phospholipid fatty acid composition influences cell permeability and receptor stability at the cell membrane, it is possible that expression of these genes are increased in order to counterbalance the decrease in specific lipid species and maintain membrane structure (Body, 1988).

Using our previous microarray analysis of scWAT from sedentary and trained mice (Stanford et al., 2015b), in the current study we performed in silico comparison of gene pathways specifically involved in both phospholipid and fatty acid metabolism and determined whether expression of genes involved in both phospholipid and fatty acid metabolism were significantly enriched. Although the previous study examined scWAT after only 11 days of exercise, there was a marked increase in expression of gene pathways involved in phospholipid and fatty acid metabolism (Figure 3E). These data indicate that the changes in genes involved in phospholipid and fatty acid metabolism are consistent with different lengths of exercise duration (11 days versus 3 weeks), suggesting that these exercise-induced adaptations are of physiological importance.

It is possible that exercise alters the composition of the scWAT depot, and that the changes in the non-adipocyte content of scWAT may contribute to the alterations in phospholipid species after exercise. Previous studies, including studies from our laboratory, have demonstrated an increase in vascularization and increased number of "beige" adipocytes in response to exercise (Stanford et al., 2015b). Immunohistochemistry revealed an increase in macrophages (F4/80) per 100 adipocytes in scWAT after 3 weeks of chronic exercise, indicating that exercise training increases the macrophage content of scWAT (Figures S3A and S3B). To determine whether the changes in phospholipid species after exercise were a reflection of the change in the composition of the scWAT depot, we measured gene expression of *Hsl*, *Pla2g1b*, *Ipla2g*, and *Pld1* in scWAT from sedentary versus trained mice. There was no difference in *Hsl* expression, but *Ipla2g* was significantly increased in trained scWAT. While we cannot discount the fact that a change in adipose tissue depot content may contribute to the alteration in phospholipid species, these data suggest that a change in lipid metabolism is responsible for the selective alterations and re-sculpting of phospholipid species as opposed to potential changes in adipose tissue depot composition (Figure 3D).

Phospholipid Species Are Increased in BAT with Exercise

In contrast to the exercise-induced decreases observed in several phospholipid species in scWAT, there are marked increases in numerous individual phospholipid species in BAT

Figure 3. Exercise-Induced Changes in Phospholipid Species, Acyl-Chain Composition, and Gene Expression in scWAT

(A) Concentration of phospholipid species significantly changed in scWAT after 3 weeks of exercise.

(B and C) The concentration of acyl chains associated with (B) PE and (C) PS/LPS phospholipids. Data are means ± SEM (n = 6/group; *p < 0.05).

(D) Expression of genes involved in phospholipid metabolism by qPCR in mouse scWAT after 3 weeks of exercise training. Data are presented as means ± SEM (n = 6/group; *p < 0.05).

(E) Microarray analysis of genes involved in phospholipid metabolism and fatty acid elongation after 11 days of exercise training in mice, n = 7/group.

after exercise. PC, PE, and CL make up 89% of the phospholipids in BAT (Senault et al., 1990) and underwent the most dramatic changes after exercise. The increase in total PC was driven by the significant increase of the highly abundant PC 36:2 species, as well as increases in numerous species of PC and PC-O (species that are significantly changed are displayed in Figure 4A, species that are non-significantly altered are in Table S1). Interestingly, cold-exposed BAT also had significant increases in several individual PC species, but there is no overlap in the specific PC molecular species that are increased in response to cold or exercise in BAT (Marcher et al., 2015). With cold exposure, 18:0 and 18:2 PC subclasses were significantly increased (Marcher et al., 2015), while exercise resulted in an increase of several longer PC species. The mechanistic importance of these differences is unclear, but these data indicate that cold and exercise cause species-specific adaptations that contribute to PC remodeling.

There was no overall change in abundance of PE, but many molecular species of PE were significantly increased after exercise in BAT. The individual PE species that are increased after exercise include the highly abundant 40:5 and 40:6 species, and the relatively highly abundant lysophosphatidylethanolamines (LPE) 20:1 species. Several less abundant PE species were significantly decreased after exercise, including 24:1 and 34:0. Interestingly, all PE-O species that were significantly changed were increased with exercise training. The PS species 16:0/16:1 was significantly decreased with chronic exercise training (Figure 4A; Table S1). Similar to the changes in the specific PC species, these exercise-specific changes were distinct from the phospholipid remodeling in BAT after cold exposure, where the 18:0/18:2 and 18:2 were the only PS molecular species significantly increased (Marcher et al., 2015). It is also interesting to note that the PC and PE species that are increased in BAT with exercise are not the same species that are decreased in scWAT with exercise. In scWAT, we detected decreases in PC species 30:1, 30:2, and chain lengths of 32–36 carbons, while in BAT there are significant increases in PC species of shorter (e.g., 28:1 and 30:0) and longer (40:6 and 44:0) chain lengths after exercise training. The decrease in the PS species 16:0/16:1, however, is observed in both BAT and scWAT after exercise. These data indicate that exercise-induced changes in the lipid species of BAT and scWAT are molecular species specific, and that the molecular species altered may have a direct contribution to the physiological role of the tissue, although establishing that mechanistic effect will require further investigation.

When grouped into unsaturated, monounsaturated (MUFA), polyunsaturated (PUFA), and total saturated groups, there were no changes in PA (Figure S4A) or PS/LPS (Figure S3B) acyl chains, but there were significant changes in the acyl chains in both PC and PE species. Polyunsaturated PC species, and PC species with medium chain lengths, were significantly increased after exercise in BAT (Figure 4B), while monounsaturated PE acyl chains were significantly decreased (Figure 4C). Acyl chain length and saturation determine the fluidity of the membrane; phospholipids with longer and more saturated chain lengths tend to aggregate and form less fluid membranes. The decrease in acyl chain length and increase in polyunsaturated PC species likely contributes to the remodeling of the BAT membrane after exercise.

The response of BAT to exercise is markedly different than that of scWAT, with several phospholipid species significantly increased after 3 weeks of chronic exercise. Also, in contrast to exercise-trained scWAT, expression of genes involved in phospholipid metabolism was significantly decreased in BAT after chronic exercise. Expression of genes involved in phospholipid remodeling, including *Agpat3*, *Gpd1*, *Lgpat1*, *Ptdss2*, and *Pld1*, is decreased significantly (Figure 4D). A previous study indicated that, after acute cold exposure these genes are significantly increased in BAT, and different molecular species of phospholipids are increased (Marcher et al., 2015). It is possible that these genes have a more specific regulation, acting more efficiently to increase certain molecular species than previously thought. This could also be a result of cold exposure causing an increase in BAT activity and heat production, which could increase specific phospholipids in the mitochondrial membrane (Forner et al., 2009; Marcher et al., 2015); while there may be no need for BAT-induced thermogenesis to occur during exercise, exercise is thus suppressing phospholipid metabolism in response to exercise.

Exercise may alter the cell composition of the BAT depot, causing a change in the non-adipocyte content of BAT that may contribute to the alterations in phospholipid species after exercise. Immunohistochemistry indicated an increase in macrophages (F4/80) per mm^2 in BAT after 3 weeks of chronic exercise, indicating that exercise training increases the macrophage content of BAT (Figures S5A and S5B). To determine whether the changes in phospholipid species after exercise were a reflection of the change in the composition of the BAT depot, we measured gene expression of *Hsl*, *Pla2g1b*, *Ipla2g*, and *Pld1* in BAT from sedentary versus trained mice. *Hsl* expression was not altered, but *Ipla2g* was significantly increased in trained BAT. Similar to scWAT, while we cannot discount that changes in adipocyte composition may affect the phospholipid species in BAT after exercise, these data suggest that the change in lipid metabolism is responsible for the selective alterations and remodeling of phospholipid species (Figure 4D).

Exercise Training Alters TAG Species in scWAT

Total TAGs were significantly decreased in trained scWAT, consistent with the increased energy demand of chronic exercise and results of previous studies (Jeukendrup, 2002; Jeukendrup et al., 1998a, 1998b, 1998c), as well as the decrease in scWAT mass observed after chronic exercise (Figure S1B). The response of individual TAG species to exercise, however, was varied. TAG species with chain lengths of 44–58 carbons were significantly decreased in trained scWAT (species that are significantly changed are displayed in Figures 5A–5C, species that are non-significantly altered are in Table S1). Notably, none of the significantly decreased individual species were unsaturated indicating that the enzymes acting to decrease TAG species (specifically *Elovl3* and *Elovl4*, which are selective for saturated and monounsaturated fatty acids) are preferentially acting on saturated TAGs. Polyunsaturated TAGs and short and medium acyl chain TAGs were significantly decreased following chronic exercise (Figure 5D). There was also a significant

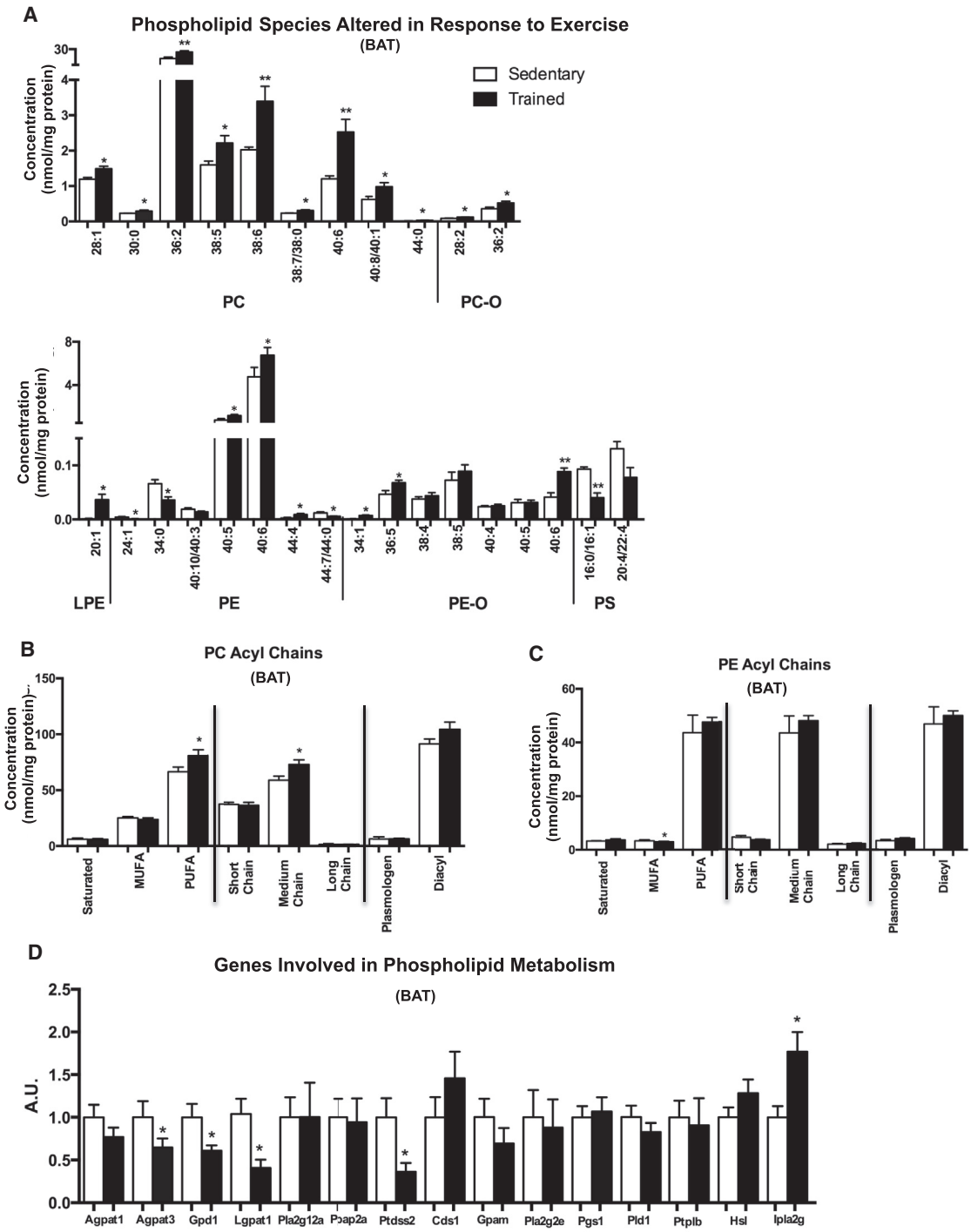

Figure 4. Exercise-Induced Changes in Phospholipid Species, Acyl-Chain Composition, and Gene Expression in BAT

(A) Concentration of phospholipid species significantly changed in BAT after 3 weeks of exercise.

(B and C) The concentration of acyl chains associated with (B) PC and (C) PE phospholipids. Data are presented as mean ± SEM (n = 6/group; *$p < 0.05$; **$p < 0.01$).

(D) Expression of genes involved in phospholipid metabolism by qPCR in mouse BAT after 3 weeks of exercise training. Data are presented as means ± SEM (n = 6/group; *$p < 0.05$).

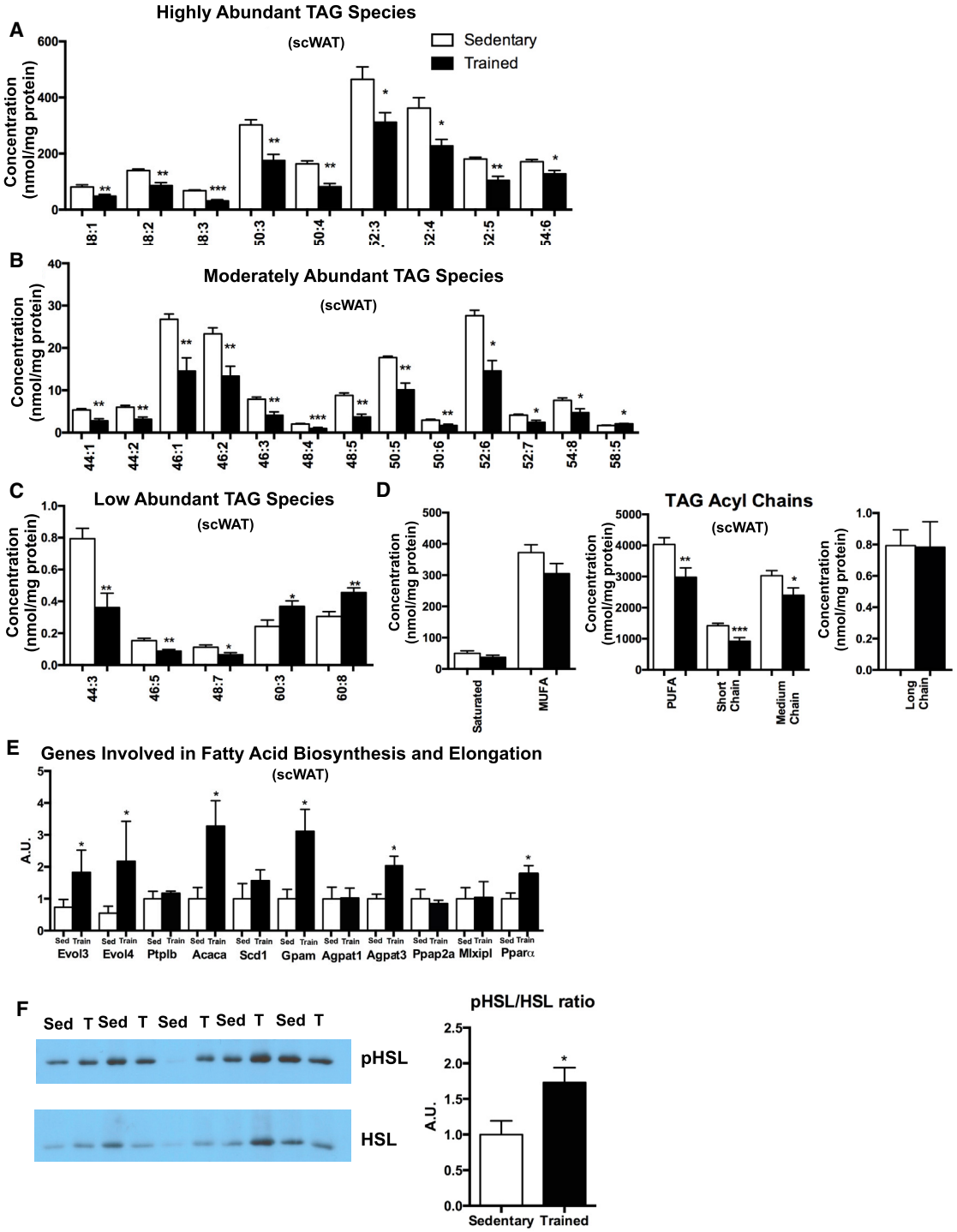

Figure 5. Exercise-Induced Changes in TAG Species, Acyl-Chain Composition, and Gene Expression in scWAT

(A–C) The concentration of (A) highly abundant, (B) moderately abundant, and (C) low-abundance TAG species significantly altered by exercise in scWAT.

(D) The concentration of acyl chains associated with TAG in scWAT from sedentary and exercise-trained mice. Data are presented as means ± SEM (n = 6/group; *p < 0.05; **p < 0.01; ***p < 0.001).

(E) Expression of genes involved in fatty acid biosynthesis and elongation measured by qPCR.

(F) Western blots of pHSL/HSL ratio in scWAT. Data are presented as means ± SEM (n = 6/group; *p < 0.05).

increase in the expression of Pparα and in the ratio of pHSL/HSL in trained versus sedentary scWAT, indicating an upregulation of lipolysis (Figures 5E and 5F). The overall decrease in TAGs in scWAT is consistent with the overall decrease in TAG concentration (Figure 1D) and is expected because a predominant role of scWAT during exercise is to increase lipid mobilization and provide fatty acids to be used as fuel for skeletal muscle (Jeukendrup, 2002; Jeukendrup et al., 1998a, 1998b, 1998c).

Interestingly, the response of individual TAG species to exercise was varied. The very long TAG species 60:3 and 60:8 are significantly increased in trained versus to sedentary mice (Figure 5C). Genes regulating fatty acid biosynthesis and elongation tended to be significantly increased in scWAT after exercise. The increase in the long TAG species 60:3 and 60:8 is likely related to the increased expression of *Elovl3* and *Elovl4*, which code for enzymes with key roles in elongation of long fatty acids (Figure 5E).

There was a significant increase in expression of *Acaca,* which codes for acetyl-coA carboxylase, the enzyme that catalyzes the rate-limiting step in fatty acid biosynthesis (Figure 5E). Analysis of microarray data from mice that had exercised for 11 days also revealed a significant increase in expression of genes involved in fatty acid synthesis and elongation (Figure 3G). Taken together, these data indicate that while TAGs are decreased after exercise, expression of genes involved in fatty acid synthesis and elongation are increased to make TAGs that can be utilized in the future. These exciting findings point to the unique ability of scWAT to concurrently enhance breakdown of fuel and increase the storage supply of TAGs for future use.

Exercise Training Alters TAG Species in BAT

Total TAGs are significantly decreased in BAT after chronic exercise, and this is likely due to decreases in highly abundant TAG species of 50, 52, and 54 total carbons (species that are significantly changed are displayed in Figure 6A, species that are non-significantly altered are in Table S1), as well as decreases in TAGs of medium abundance and similar chain length (48–56 total carbons) (species that are significantly changed are displayed in Figure 6B, species that are non-significantly altered are in Table S1). Interestingly, we observed an increase in long (58–60 carbons) chain TAG that mirrors the TAGs that were significantly increased in scWAT after exercise (species that are significantly changed are displayed in Figure 6C, species that are non-significantly altered are in Table S1). Total saturated, monounsaturated, polyunsaturated, and medium-length-chain TAGs are also significantly decreased in BAT after chronic exercise (Figure 6D). With cold exposure, the highly abundant TAGs were significantly increased in BAT, again indicating opposing roles of BAT in response to exercise and cold exposure (Marcher et al., 2015). After acute cold exposure, BAT increases TAG production in order to use energy from the TAGs as heat and contribute to non-shivering thermogenesis. In contrast, there is no need to increase heat production in BAT during or immediately after exercise, so there is no need for increased TAG production.

The decrease in abundance of TAGs in BAT after exercise is similar to the decrease in TAGs in scWAT after exercise, but expression of several genes involved in fatty acid biosynthesis and elongation were significantly decreased in exercise-trained BAT. *Acaca*, the enzyme that catalyzes the rate-limiting step in fatty acid biosynthesis, is significantly decreased in BAT, as are *Scd1*, *Agpat3*, *Dgkd*, and *Mlxipl* (Figure 6E).

These decreases likely contribute to the decrease in BAT TAG species in response to exercise training and also indicate that scWAT and BAT behave in opposite manners in response to exercise.

DISCUSSION

Exercise decreases adiposity and improves whole-body metabolic health. Recent studies have demonstrated the importance of exercise to improve metabolic health and function of WAT, but investigations regarding the effect of exercise on BAT have provided conflicting results. Here, we utilized MS/MS^{ALL} shotgun lipidomics for the analysis of mouse scWAT and BAT with the goal of providing a resource that describes the lipidomic profile of adipose tissue after exercise training. We found that 3 weeks of exercise significantly decreased TAGs in both scWAT and BAT and resulted in several species-specific changes in phospholipids in scWAT and BAT, albeit in opposite directions. While we did not see large shifts in total amounts of lipid subtypes, the specific changes in individual molecular species reflect selective remodeling in response to exercise training.

Lipidomics analysis revealed a significant decrease in overall abundance of PS in scWAT, as well as species-specific decreases in scWAT PA, PC, PE, and PS. The reason for the exercise-induced decrease in phospholipids in scWAT has not been investigated. The PCs account for nearly 50% of membrane phospholipids and are necessary for membrane structure and compartmentalization in the cell, as well as interaction with integral membrane proteins (Body, 1988). Regulation and metabolism of PC, PS, and PE can be protective against adipose tissue inflammation, hyperlipidemia, and obesity (Body, 1988). One class of enzymes that regulates the metabolism of phospholipids is phospholipases, enzymes that catalyze the cleavage of phospholipids and in some cases, the cleavage of TAGs (Body, 1988). Recent studies have shown a direct role of phospholipases, specifically the phospholipase A2 family members, in adiposity and insulin resistance. In obese mice, expression of phospholipase A2 group IIe (*Pla2g2e*) is significantly increased in WAT, and the absence of this gene (*Pla2g2e*$^{-/-}$ mice) results in reduced adiposity and improved glucose and insulin sensitivity. *Pla2g2e*$^{-/-}$ mice also have a decrease in overall phospholipid and TAG abundance. The absence of *Pla2g2e* also results in species-specific decreases in PE and PS (Sato et al., 2014). Interestingly, while we see a significant increase in several genes involved in phospholipid metabolism, *Pla2g2e* is significantly decreased in exercise-trained scWAT. It is possible that this decrease contributes to the regulation of certain phospholipid species and the resultant increase in WAT insulin sensitivity after exercise (Burstein et al., 1992; Koivisto and Yki-Jarvinen, 1987; Stanford et al., 2015b).

In another mouse model, the absence of phospholipase A_2y (iPLA$_2$y$^{-/-}$ mice) resulted in the reduction in high-fat-diet-induced weight gain, adipocyte hypertrophy, and insulin resistance. The WAT from these mice were analyzed by shotgun lipidomics and had a significant reduction in WAT triglyceride

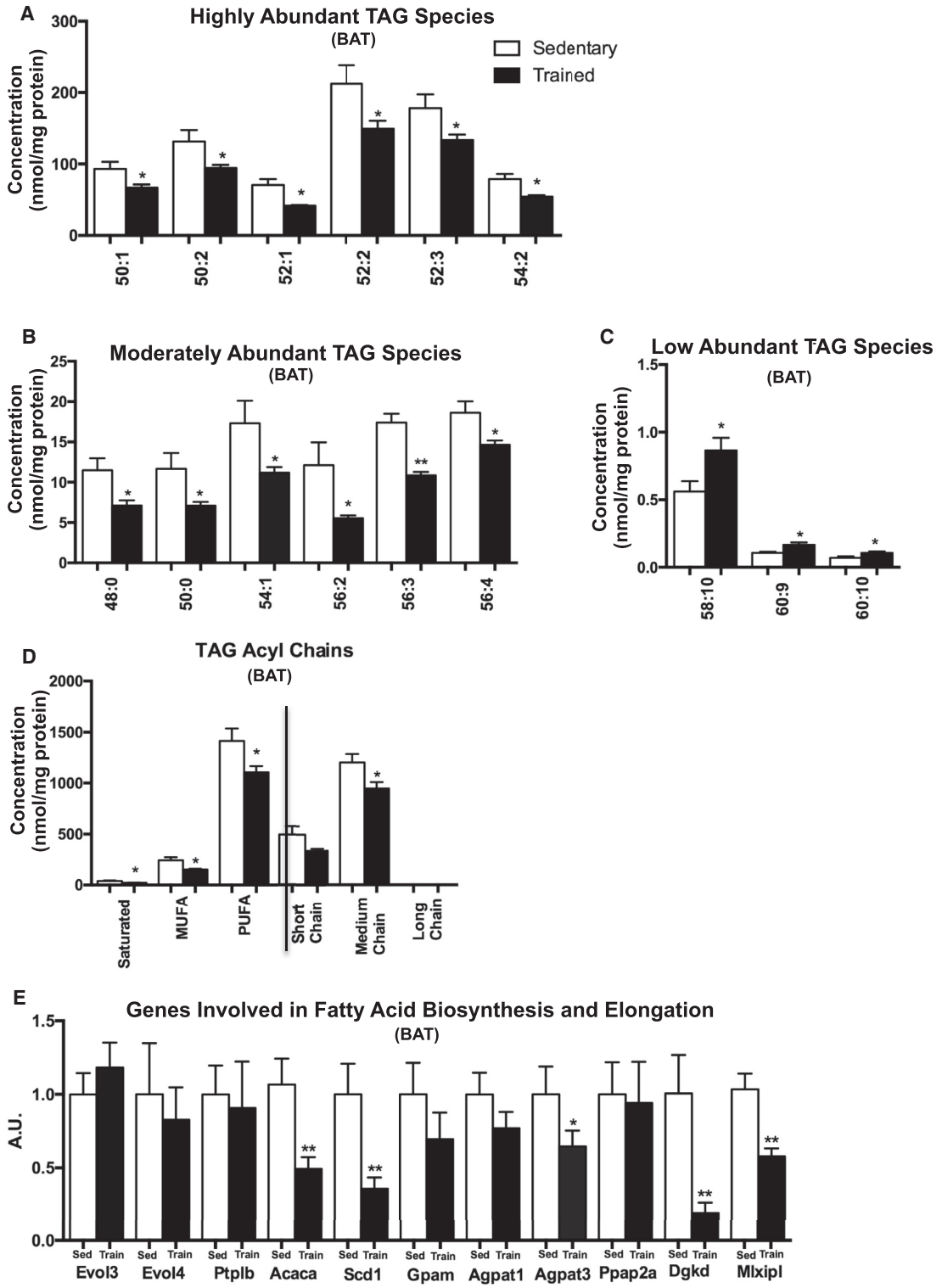

Figure 6. Exercise-Induced Changes in TAG Species, Acyl-Chain Composition, and Gene Expression in BAT

(A–C) The concentration of (A) highly abundant, (B) moderately abundant, and (C) low-abundance TAG species significantly altered by exercise in BAT.

(D) The concentration of acyl chains associated with TAG in BAT from sedentary and exercise-trained mice. Data are presented as means ± SEM (n = 6/group; *p < 0.05; **p < 0.01).

(E) Expression of genes involved in fatty acid biosynthesis and elongation measured by qPCR. Data are presented as means ± SEM (n = 6/group; *p < 0.05).

content accompanied by a shift to shorter-chain-length molecular species TAGs (Mancuso et al., 2010). Interestingly, these mice also have increased mitochondrial oxygen consumption in their WAT, accompanied by decreased mitochondrial oxygen consumption and increased TAG accumulation in skeletal muscle. These data suggest a role for iPLA$_2$γ in WAT and skeletal muscle communication and regulation of insulin sensitivity (Mancuso et al., 2010). While we have not directly investigated the role of iPLA2γ in scWAT after exercise, exercise-trained mice have increased scWAT mitochondrial oxygen consumption (Stanford et al., 2015b) and improved insulin sensitivity (Burstein et al., 1992; Koivisto and Yki-Jarvinen, 1987; Stanford et al., 2015b), providing a potential role for this phospholipase in the regulation of the exercise response. Further, the role of adipose triglyceride lipase (ATGL) has long been investigated for its regulation by insulin, expression alterations during obesity as well as exercise-induced enhancement for lipolysis in adipocytes. Thus, exercise likely induces a panoply of events that sculpt the lipidome of scWAT and BAT tissue (Burstein et al., 1992; Koivisto and Yki-Jarvinen, 1987; Stanford et al., 2015b; Jocken et al., 2007; Kershaw et al., 2006; Ogasawara et al., 2012). Together, these studies provide a rationale to study these phospholipases in the regulation of phospholipids and mitochondrial function in response to exercise (Jocken et al., 2007; Kershaw et al., 2006; Mancuso et al., 2010; Ogasawara et al., 2012); future studies will focus on exercise-induced regulation of these phospholipases and how they can potentially contribute to insulin sensitivity.

In contrast to exercise-induced changes in scWAT, BAT had a significant increase in overall abundance of PC, and species-specific increases in PC and PE. Interestingly, the only PS species that was significantly downregulated in BAT was also decreased in scWAT after exercise. It is not surprising that the lipidome of scWAT and BAT are changed in antagonistic directions in response to exercise. In rodents, exercise increases mitochondrial activity and expression of mitochondrial genes in scWAT but downregulates mitochondrial activity in BAT (Wu et al., 2014). This is likely a result of an increase in thermogenesis in scWAT to accommodate the working muscle and diet-induced thermogenesis, while BAT is decreasing thermogenic activity to core areas to compensate for the increase in heat generated with exercise (Wu et al., 2014). Consistent with this line of thought, the phospholipid pathway appears to be regulated differently in response to exercise and cold exposure in BAT. Cold exposure increases certain PC, PE, and PS species in BAT; however, the molecular species we have identified as increased after exercise are distinct from those upregulated after cold exposure. Expression of genes involved in phospholipid metabolism is significantly upregulated in BAT after cold exposure, while these same genes (*Agpat3*, *Gpd1*, *Lgpat1*, *Ptdss2*, and *Pld1*) are significantly decreased in BAT after exercise. It is important to note that the data investigating the effects of cold on the BAT lipidome was performed after short-term (4 hr) cold exposure, and the effects of chronic cold exposure on the BAT lipidome may be different. Our dataset provides evidence that the BAT lipidome responds differently to exercise than scWAT, and that exercise and cold exposure result in distinct species-specific adaptations to BAT. What the regulation of these different molecular species indicates in terms of function and insulin sensitivity will be a topic of future investigation.

Overall abundance of TAGs was significantly reduced in both scWAT and BAT after exercise. In scWAT, the decrease TAGs after exercise was largely a reflection of the reduction in the major TAG species. Interestingly, the long-chain TAGs were significantly increased in scWAT after exercise. Expression of genes involved in fatty acid biosynthesis and elongation were significantly elevated in scWAT. TAGs were also significantly reduced in BAT after exercise, again a reflection of the decrease in major and middle TAG species. Similar to scWAT, the long-chain TAGs were significantly increased in BAT after exercise. In contrast to scWAT, expression of genes involved in fatty acid biosynthesis and elongation are significantly decreased in BAT after exercise. It is likely that scWAT has a decrease in TAGs coupled with an increase in expression of genes involved in fatty acid biosynthesis because it is working to provide fuel for the working muscle. The decrease in the TAG content of BAT is a little less clear. It is possible that decreases in TAG molecular species are because the BAT is also releasing specific labile TAGs to be used as fuel for the working muscle during exercise. It is important to note that, in response to short-term cold exposure, the abundance of TAGs in BAT is not altered, but specific molecular species are significantly increased (Marcher et al., 2015); this increase in TAG species after cold-exposure functions to increase heat production. Long-chain TAGs are also increased in BAT with acute cold exposure, and it has been proposed that the accumulation of these long acyl chains are then channeled toward the α-oxidation pathway, which is normally used for oxidation of branched-chain fatty acids and then can enter the classical β-oxidation pathway (Marcher et al., 2015). Increased α- and β-oxidation of these TAGs results in increased heat production. Interestingly, long TAGs are also increased in BAT after exercise, but it is unlikely that the purpose is to increase heat production. Future studies will focus on the role of TAGs, and specifically long-chain TAGs, in BAT after exercise.

In summary, we determined exercise-induced adaptations in the structural lipidome of scWAT and BAT using MS/MS^{ALL} shotgun lipidomics analysis. Our data constitute a comprehensive resource that will stimulate new hypotheses regarding the physiological importance of exercise-induced changes in the lipidome of scWAT and BAT and provide further insight into functional differences in the lipidome of scWAT and BAT. This dataset will also allow for physiological insight into the differences in cold exposure and exercise on BAT activity. Future studies will investigate the role of decreased phospholipid species in scWAT, increased phospholipid species in BAT, the exercise-induced decrease in TAGs in both scWAT and BAT, and the physiological consequences of these changes.

EXPERIMENTAL PROCEDURES

Animals

Male, 10-week-old, C57BL/6 mice were obtained from Charles River Laboratory and fed a chow (21% kcal from fat; PharmaServ 9F5020). Mice were housed at room temperature (22°C). All procedures were followed as approved by the IACUC at The Ohio State University and Joslin Diabetes Center. BAT and scWAT were removed, frozen in liquid nitrogen immediately, and stored at −80C.

Exercise Training

Male mice at 10 weeks of age were divided into two groups. One group of mice was housed individually in wheel cages (24.5 cm in diameter and 8 cm in width; Nalgene), where voluntary access to physical activity was available at all times. The total number of wheel cage revolutions was monitored every day, and the accumulated running distance was calculated at the end of 3 weeks. Mice were given open access to wheel cage for 3 weeks and ran 145 ± 8 km/mouse). The age-matched control mice were maintained in individual cages and treated identically to the wheel-cage-housed mice, except that they did not have access to a running wheel. After 3 weeks, mice were removed from the wheel cages or static cages. Mice were anesthetized 4 hr after they were removed from the wheel cages or static cages, and scWAT and BAT were immediately removed. Any mouse that ran 10% less than the average of the trained group was excluded from analyses.

Lipidomic Profiling

All lipid standards were acquired from Cayman Chemical Company, Matreya, Cambridge Isotope Laboratories, NuChek Prep, Avanti Polar Lipid, or Sigma-Aldrich. All solvents are of high-performance liquid chromatography (HPLC) or liquid chromatography-mass spectrometry (LC/MS) grade and were acquired from Sigma-Aldrich, Fisher Scientific, or VWR International.

Liquid/Liquid Extraction of Structural Lipids

BAT and scWAT tissues were thawed in ten times diluted PBS and homogenized in Omni bead tubes with 2.8-mm ceramic beads in the Omni Bead Ruptor 24 with Cryo Cooling Unit (Omni International) at 4°C for 2 min. Protein concentration was determined by the bicinchoninic acid assay. 1 mg of protein from each sample was aliquoted, and a cocktail of deuterium-labeled and odd chain phospholipid standards from diverse lipid classes was added. Standards were chosen so that they represented each lipid class and were at designated concentrations chosen to provide the most accurate quantitation and dynamic range for each lipid species. 4 mL chloroform:methanol (1:1, by volume) was added to each sample, and lipidomic extractions were performed as previously described (Kiebish et al., 2010). Lipid extraction was automated using a customized sequence on a Hamilton Robotics STARlet system (Hamilton). Lipid extracts were dried under nitrogen and reconstituted in chloroform:methanol (1:1, by volume). Samples were flushed with nitrogen and stored at −20°C.

Direct Infusion MS/MS^{ALL} Structural Lipidomics Platform

Samples were diluted 50 times in isopropanol:methanol:acetonitrile:water (3:3:3:1, by volume) with 2 mM ammonium acetate in order to optimize ionization efficiency in positive and negative modes. Electrospray ionization-MS was performed on a TripleTOF 5600$^+$ (SCIEX), coupled to a customized direct injection loop on an Ekspert microLC200 system (SCIEX). 50 μL of sample was injected at a flow rate of 6 μL/min. Lipids were analyzed using a customized data independent analysis strategy on the TripleTOF 5600$^+$ allowing for MS/MS^{ALL} high-resolution and high-mass-accuracy analysis as previously described (Simons et al., 2012). Quantification was performed using an in-house library on MultiQuant software (SCIEX) and normalized to 1 mg protein.

qRT-PCR

BioRad Custom PrimePCR 384-well plates (Bio-Rad) were pretreated with 48 primers for genes of interest using primers shown in Table S2, as well as RT, PCR, gDNA, RQ1, and RQ2 controls. All qPCR gene expression was normalized to the housekeeping gene GAPDH.

Western Blotting

Tissue processing and immunoblotting were performed as previously described (Stanford et al., 2015b). The HSL and pHSL (Ser565) antibodies were obtained from a commercial source (Cell Signaling Technology).

Immunohistochemistry

Macrophages infiltrated in BAT and subcutaneous tissues were determined by immunohistochemistry as cells stained positive with anti-F4/80 antibodies (Serotec). Images were captured with a Zeiss Axioplan microscope, and the number of macrophages per 100 adipocytes (scWAT) or mm^2 (BAT) was determined using ImageJ. Data analyses were performed using GraphPad prism version 6.0 software.

Statistics

The data are presented as the mean ± SEM. Statistical significance was defined as $p \leq 0.05$ and determined by Student's t tests or two-way ANOVA and Bonferroni post hoc analysis. The number of samples used to determine statistical significance is indicated in the figure legends.

SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2017.01.038.

AUTHOR CONTRIBUTIONS

F.J.M., M.A.K., L.J.G., and K.I.S. conceived and designed the study. F.J.M., L.A.B., A.C.L., K.S., and K.I.S. performed mouse experiments. E.Y.C., F.G., N.R.N., and M.A.K. performed lipidomics experiments. L.G., A.R., and A.I.D. performed the immunohistochemistry experiments. F.J.M., L.A.B., and A.C.L. performed the gene expression experiments. F.J.M. and A.C.L. analyzed the lipidomics data with input from M.A.K. and K.I.S. F.J.M., M.A.K., and K.I.S. interpreted the results of experiments, prepared figures, and wrote the manuscript. All authors approved the final version of the manuscript.

ACKNOWLEDGMENTS

The authors thank Drs. Peter J. Mohler, Matthew D. Lynes, and Martha A. Belury for critical discussions. This work was supported by National Institutes of Health grants K01-DK-105109 (to K.I.S.), R01-DK101043 (to L.J.G.), and 5P30 DK36836 (to the Joslin Diabetes Center DERC), an American Heart Association Scientific Development grant 15SDG22990000 (to K.I.S.), USDA-2014-00654 (to A.I.D.), and funding provided by the Ross Heart Hospital and Davis Heart and Lung Research Institute TriFit Challenge (to K.I.S. and A.I.D.).

Received: August 30, 2016
Revised: November 23, 2016
Accepted: January 13, 2017
Published: February 7, 2017

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