Gut Derived Short Chain Fatty Acids Alter Plasma and Hepatic Lipid Metabolism in a Sex-Specific Manner in Long-Evans Rats

Shrushti Kaushikbhai Shah

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Department of Biochemistry

Memorial University of Newfoundland

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Abstract

Short chain fatty acids (SCFAs), generated from the microbial fermentation of dietary fibers, alter lipid metabolism. However, sex-specific effects of SCFAs on lipid metabolism and lipidomic profiles are not known. Male and female Long-Evans rats were either given a mixture of SCFAs (acetate, propionate, and butyrate; molar ratio 60:20:20), or vehicle for seven days intraperitoneally. SCFAs significantly decreased plasma and hepatic cholesterol, triacylglycerols and plasma glucose in males, while there was no effect in females compared to their respective controls. SCFAs showed a significant decrease in the mRNA expression of acetyl-CoA carboxylase in both males and females. SCFAs also altered the lipidomic profile of cholesteryl esters, triacylglycerols and phospholipids by decreasing the abundance of saturates and monounsaturates with a relative increase in polyunsaturated fatty acids. These findings demonstrate that SCFAs improve plasma and hepatic lipid profiles in a sex-specific manner, and may represent a cheaper therapeutic strategy against metabolic diseases.

Keywords: Short chain fatty acids, lipids, gene regulation, sex-specific effects, lipidomics.

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Abbreviations

AA	arachidonic acid
ACAT	acyl-CoA cholesterol acyltransferase
ACC	acetyl-CoA carboxylase
ALA	α-linolenic acid
CE	cholesteryl ester
CETP	cholesteryl ester transport protein
COX	cyclooxygenase
CYP7A1	cholesterol 7α-hydroxylase
CVD	cardiovascular disease
DGAT	diglyceride acyltransferase
DHA	docosahexaenoic acid
ELOVL	elongation of very long chain fatty acid
EPA	eicosapentaenoic acid
ESI	electrospray ionization mass spectrometry
FABP	fatty acid binding protein
FADS	fatty acid desaturase
FAO	fatty acid oxidation
FAS	fatty acid synthase
FATP	fatty acid transporter protein
FC	free cholesterol
FFAR	free fatty acid receptor
G3P	glucose-3-phosphate
GPCR	G protein-coupled receptor
HDL	high-density lipoprotein
HFD	high fat diet
HMGCR	3-hydroxy-3-methylglutaryl-CoA reductase
I.P.	intraperitoneal
LA	linoleic acid
LCAT	lecithin cholesterol acyltransferase
LCFA	longer chain fatty acid
LDL	low-density lipoprotein
LOX	lipoxygenase
LPAT	lysophospholipid acyltransferase
LPC	lysophosphatidylcholine
LPCAT-3	lysophosphatidylcholine acyltransferase 3
LPE	lysophosphatidylethanolamine
LPL	lipoprotein lipase
MALDI	matrix-assisted laser desorption/ionization
MGAT	monoacylglycerol transacylase
MS/MS	tandem mass spectrometry
MUFA	monounsaturated fatty acid
n-3 PUFA	omega-3 polyunsaturated fatty acid
n-6 PUFA	omega-6 polyunsaturated fatty acid
NEFA	non-esterified fatty acid

PA	phosphatidic acid
PC	phosphatidylcholine
PCA	principal component analysis
PE	phosphatidylethanolamine
PG	prostaglandin
PI	phosphatidylinositol
PL	phospholipid
Pls-	plasmalogen-linked
PS	phosphatidylserine
PPARγ	peroxisome proliferator activated receptor gamma
PUFA	polyunsaturated fatty acid
SCD-1	stearoyl CoA desaturase 1
SCFA	short chain fatty acid
SFA	saturated fatty acid
SREBP	sterol regulatory element binding protein
T2D	type 2 diabetes
TC	total cholesterol
TG	triacylglycerol
TLC	thin layer chromatography
UHPLC	ultra high-performance liquid chromatography
VLCFA	very long chain fatty acid
VLDL	very low-density lipoprotein

Chapter 1 - Introduction

1.1 Gut microbiota

1.1.1. Colonic composition of bacteria

The human gastrointestinal tract houses approximately 100 trillion microbes, known as the gut microbiome (Whitman, Coleman, and Wiebe 1998; Clemente et al. 2012; Boulangé et al. 2016). Bacteria are the most dominant and most diverse group, which comprises about 10^{14} colonies with more than 70 genera and 500-1000 different species (den Besten et al. 2013). A balanced and diversified microbiota is of utmost importance, as microbial composition leads to gut dysbiosis – a condition which causes obesity, inflammation, atherosclerosis, type 2 diabetes (T2D) or even cancer (Conlon and Bird 2015; Boulangé et al. 2016). These bacterial groups can be beneficial or harmful to the body, depending on the metabolites produced by them during fermentation (Conlon and Bird 2015). Following fermentation of complex carbohydrates (such as dietary fibers), the gut microbiota produce short chain fatty acids (SCFAs) as main metabolites (Topping and Clifton 2001; Mudgil and Barak 2013; David et al. 2014). SCFAs are of 2-6 carbons in length, and are known by the following names: acetic (C2), propionic (C3), butyric (C4), valeric (C5) and caproic (C6) acid. Of all, acetate, propionate and butyrate are considered as the three main SCFAs. Under symbiotic conditions, SCFAs are generated in the molar ratio of 60:20:20 in the colon (Cummings et al. 1987; Hijova and Chmelarova 2007; Ohira, Tsutsui, and Fujioka 2017).

Bacteroidetes, Firmicutes, and *Actinobacteria* are three of the most abundant phyla present in gut microbiota (den Besten et al. 2013). The proximal colon comprises of saccharolytic bacteria which belong to *Bacteroidetes and Firmicutes* phyla, and are responsible mainly for acetate, propionate and butyrate production in gut (Topping and Clifton 2001) whereas, the distal colon is comprised of proteolytic bacteria which are involved with production of branched-SCFAs, amines,

and some toxic compounds containing phenolic and sulfur chemical moieties (Millet et al. 2010).

SCFAs have direct and indirect influence on humans by affecting several tissue-specific functions, ranging from metabolism to immune regulation (Morrison and Preston 2016). For example, SCFAs have beneficial effects in energy homeostasis and appetite regulation (Frost et al. 2014; Byrne et al. 2015). Upon reaching various tissues, SCFAs affect lipid and glucose metabolism (den Besten et al. 2013). Moreover, SCFAs have a profound effect on the host cells involved in inflammatory and immune pathways (Aoyama, Kotani, and Usami 2010; Vinolo et al. 2011). The effects of SCFAs are affected by their concentration in gut, circulation, and other tissues where SCFAs are transported. The concentration of SCFAs in the proximal portion of the colon is about 70 to 140 mM, and it decreases to 20 to 70 mM in the distal colon (Wong et al. 2006). SCFA concentrations vary depending on the type of food consumed and dietary intake, and can have profound effects on the microbial community (Conlon and Bird 2015). The compositional change in the gut microbiota can vary SCFA concentrations and this microbial change can be a result of differences in colonization history, physiological conditions, diet, antibiotics, stress, alcohol consumption, environment, and genetic factors (Zoetendal A.D.L. Akkermans et al. 2001).

1.1.2 Factors influencing gut-microbiota composition

The gut-microbiota starts developing after birth in infants, with the help of maternal microbiota and environmental factors (Fouhy et al. 2012). The gut microbial composition remains quite unstable up to 3-4 years of age, but is altered later under the influence of diet and disease conditions (Rodrı et al. 2015). Thus, every individual has a unique microbiome depending primarily on host genotype and diet intake (Zoetendal A.D.L. Akkermans et al. 2001). High

consumption of dietary fibers increases the production of SCFAs in the gut. Few stable isotope studies have been able to prove that the consumption of dietary fibers in the concentration of 60 g/day generates 400-600 mmol/day SCFAs in the colon and provide approximately 10% calories to the body (Bergman 1990; Morrison and Preston 2016). However, the consumption of different types of dietary fibers (such as oligosaccharides, polysaccharides, and resistant starch) have significantly different effects on colonial microbiota composition, resulting in the generation of SCFAs in different concentrations (Edge 2016; Holscher 2017). Evidence suggests that dietary intake of indigestible fibers was greater than 100 g per day when humans consumed more of tubers, grasses and sedges. Consumption of dietary fibers is reduced to less than 15 g per day in the western diet (Mudgil and Barak 2013).

The chemical structure of SCFAs comprises of a carboxylic (–COOH) functional group imparting a mildly acidic character. The acidic property decreases the pH of the colon upon fermentation of microbiota and generation of SCFAs. A change in colonic pH has a direct correlation with the microbiota composition and these alternations consequently affect the concentration of SCFAs produced in gut (Morrison and Preston 2016). This indicates that along with the type of diet consumed by an individual, there is huge influence of colonic pH on gut microbiota and eventually on the concentration of SCFAs produced in colon. Studies have shown association of gut-dysbiosis with various metabolic and inflammatory diseases (Larsen and Vogensen 2010; Vrieze et al. 2010), confirming that the symbiotic relationship amongst microbial communities is a key factor to determine positive and negative effects of gut-microbiota and its metabolites on host health outcomes (Remely et al. 2014; Morrison and Preston 2016). Positive health benefits such as prevention of body-weight gain (Alard et al. 2015), increased lipid

oxidation (Mollica et al. 2017), anti-inflammatory role in autoimmune diseases (Opazo et al. 2018) etc. are associated with both the type of microbiota and the concentration of SCFAs in the body.

Studies have shown that T2D patients are relatively depleted in bacterial species producing SCFAs (Wang et al. 2012; Article et al. 2015). A study by Kong et al. (2014) showed that overweight and obese subjects with healthier dietary pattern (less consumption of confectionary and sugary drinks, more consumption of yogurts and soups) had lower inflammatory markers and also highest microbial gene richness when compared to subjects which consumed higher amounts of confectionary and sugary diets. Interestingly, the dietary intake, insulin sensitivity and plasma inflammatory marker (Interleukin 6; IL6) of obese subjects consuming heathier diet were closer to control group of lean subjects. Turnbaugh et al. (2005) suggested that the compositional changes in the *Bacteroidetes and Firmicutes* species are associated to obesity in animals. They demonstrated that obese animals have reduced abundance of *Bacteroidetes* with a relative increased abundance of *Firmicutes* (Turnbaugh et al. 2005) These results were supported by a human study which showed a relationship between *Firmicutes/Bacteroidetes* ratio and obesity (Koliada et al. 2017). Vrieze et al. (2010) suggested that there is direct interaction between diet, type of microbiota and metabolism of obese vs. lean subjects. These alterations in microbiota affects the levels of gut metabolites such as SCFAs (mainly acetate, propionate and butyrate) which are known to modulate inflammatory and anti-inflammatory conditions (Arpaia et al. 2014), and also ameriolates diseases such as obesity and T2D (Lin et al. 2012; Den Besten et al. 2015; Ohira, Tsutsui, and Fujioka 2017). Thus, the positive health effects of SCFAs on lipid and glucose metabolism and immunity might be due to symbiosis between bacterial colonies which generates SCFAs in the molar ratio of 60:20:20 (Vrieze et al. 2010; Conlon and Bird 2015).

1.2 Short Chain Fatty Acids

1.2.1 Concentration of SCFAs in gut

Microbiota act on non-digestible carbohydrates to produce oligosaccharides, which are converted to monosaccharides by the process of fermentation in the gut under anaerobic conditions. These monosaccharides are then converted to phosphoenolpyruvate (PEP) (Berg, Tymoczko, and Stryer 2002) either via Embden-Meyerhof-Parnas pathway or the pentose phosphate pathway. The PEP can be used to generate organic acids and alcohols. SCFAs (viz. acetate, propionate and butyrate) are major end products of these bacterial pathways (den Besten et al. 2013).

Of the three main SCFAs, acetate is found in the highest concentration because not only it is produced exogenously with the help of anaerobic bacteria, but also there is its endogenous release by the tissues and organs in the systemic circulation. With the help of bacteria, acetate is either produced by hydrolysis of acetyl-CoA or generated as end-product of the Wood-Ljungdahl pathway, where CO_2 is reduced to CO and is converted to acetyl-CoA with the help of methyl group and CoASH (Miller and Wolin 1996). On the other hand, propionate is formed either by the succinate pathway or by the acrylate pathway where lactate is reduced to propionate (Pryde et al. 2002). Butyrate is produced by condensation of two acetyl-CoA moieties, or from the conversion of butyryl-CoA to butyrate via butyryl-CoA:acetate CoA transferase (Duncan, Louis, and Flint 2004; Ragsdale and Pierce 2008). Pyruvate used to produce acetate via Wood-Ljungdahl pathway can be interconverted to lactate and used to produce propionate in acrylate pathway and vice-versa. The production of SCFAs depends on the availability of NADH and concentration of enzymes involved in these pathways such as acetyl-CoA transferase, propionyl-CoA synthase, butyryl-CoA

etc. (Pryde et al. 2002; den Besten et al. 2013).

Although, much of the biochemistry of SCFAs conversion is known, accurate production rates of acetate, propionate and butyrate in humans are not yet not known. This is because of paucity of data from human intestinal samples and variability in microbiomes due to diet. However, several studies on rodent and swine models (Mcneil, Cummings, and James 1978; Hijova 2007) have indicated the molar ratio of acetate:propionate:butyrate in the colon is approximately 60:20:20; human subjects fed various types of dietary fibers have supported these results upon analyzing their blood and fecal samples (Cummings et al. 1987; den Besten et al. 2013). Moreover, it has been observed that even though there is continuous variation in the concentrations of SCFAs throughout the body (highest concentration in colon region and least in systemic circulation), the ratio of acetate, propionate and butyrate remained consistent in colon and stool (Binder 2010; Cummings et al. 1987; Hijova 2007). In the current study, a mixture of acetate, propionate and butyrate in the molar ratio of 60:20:20 was administered in rats via intraperitoneal (I.P.) route. SCFAs once generated as metabolites of indigestible carbohydrates, are then transported via the portal circulation to liver and subsequently to other organs where they can be used as substrates or signaling molecules for lipid and glucose metabolism (Byrne et al. 2015; Schönfeld and Wojtczak 2016).

1.2.2. Transport of SCFAs

SCFAs are found in highest concentration when in the lumen of the intestine. The concentration decreases as the microbial metabolites are transported across the epithelial cells of colonocytes to the portal circulation, and are delivered to liver and other peripheral tissues such as

muscle, heart, kidney, adipose tissue and brain (Cummings et al. 1987; Ohira, Tsutsui, and Fujioka 2017). The apical uptake of SCFAs across the cells can either occur in its dissociated form ($SCFA^-$) via active transport or in undissociated form (SCFAs) via passive diffusion (Figure 1.1). However, due to the acidic nature, SCFAs mainly exist in a dissociated form, which is either exchanged with bicarbonate anions or transported with the help of monocarboxylate transporters (MCT1/2). Thus, it is likely that active transport plays a major role in apical uptake of SCFA-anions in comparison to passive diffusion, which occurs with undissociated SCFAs (Hadjiagapiou et al. 2000; Vidyasagar et al. 2005).

SCFAs act as fuel for colonocytes; especially butyrate is used up as energy source of these cells. Isolated colonocytes from rats, as well as humans, showed production rate of 0.6, 0.2, 0.4 μmol/min/g cell weight of acetate, propionate and butyrate respectively (Clausen and Mortensen 1995; Rey et al. 2010). However, the molar ratio found in circulation and fecal samples is 60:20:20 which explains most utilization of butyrate by colon cells. Remaining SCFAs are transported across the basolateral membrane to the blood compartment (portal or peripheral blood) with the help of MCT 4 and MCT 5 transporters (Halestrap A.P and Meredith D. 2004; den Besten et al. 2013).

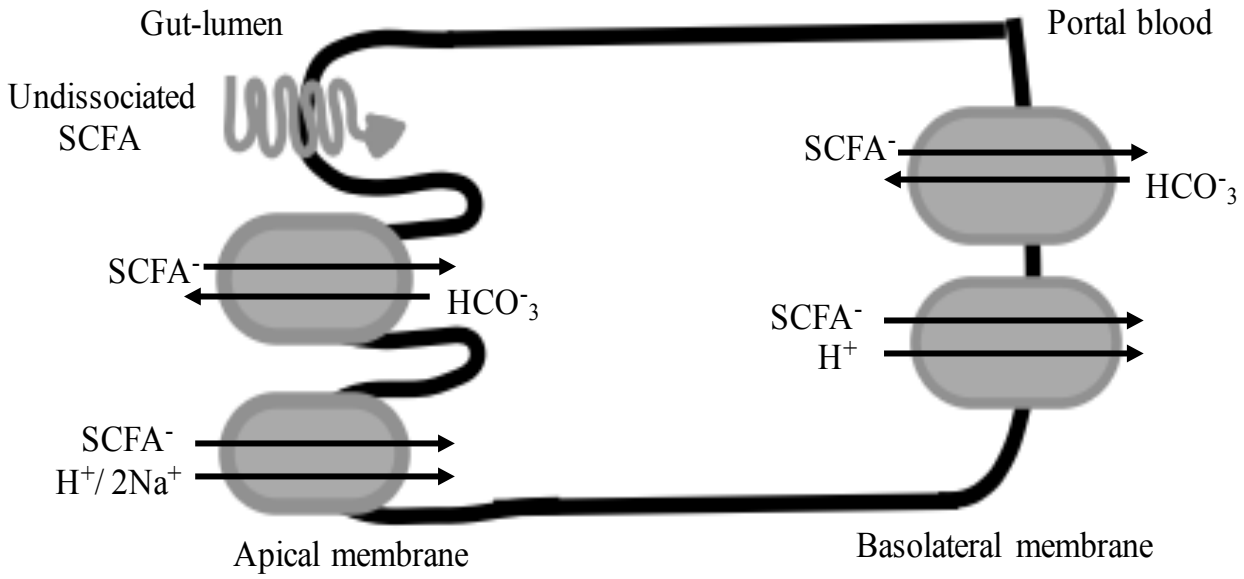


Figure 1.1 Schematics of transport of SCFAs across gut lumen. Majority of SCFAs are transported in dissociated form across the apical membrane of colonocytes by an HCO_3^- exchanger. Some SCFAs may be transported by passive diffusion. HCO_3^- - bicarbonate, $SCFA^-$ - anionic short chain fatty acid. Adapted from Gijs den Besten et al. J. Lipid Res. 2013; 54(9):2325-2340.

1.2.3. Role of SCFAs

The liver takes up most of the acetate (about 70%) from the circulation (Bloemen et al. 2009). The rest of the acetate is metabolized by peripheral organs, such as muscle, heart, kidney, and adipose tissue (Ballard 1974). In the liver, acetate provides energy to hepatocytes, and it also acts as a substrate for *de novo* synthesis of longer chain fatty acids (LCFAs) and cholesterol. Similar to acetate, propionate is also mostly taken up by liver from the portal circulation, and it can be used for hepatic gluconeogenesis (Cummings et al. 1987; Bloemen et al. 2009)**.** Butyrate provides energy to colon cells and it undergoes oxidation to generate ketone bodies and CO_2 (only if it reaches liver) (den Besten et al. 2013).

All three SCFAs, when administered individually, have been suggested to have a positive influence on lipid and glucose parameters, mainly by inhibiting cholesterol and triacylglycerol (TG) synthesis, increasing lipid oxidation and insulin sensitivity (Byrne et al. 2015; LeBlanc et al. 2017). This involves the role of G-protein coupled receptors GPCR 41 (aka free fatty acid receptor, FFAR 3) and GPCR 43 (aka FFAR 2) to which SCFAs bind and activate the pathways regulating lipid and glucose levels. A study by Kimura et al. (2013) found that mice overexpressing FFAR 2 specifically in adipose tissue are protected from diet-induced obesity. The study concluded that activation of adipose-specific FFAR 2 inhibits accumulation of fat in adipose tissue while promoting metabolism of lipid and glucose in other tissues (Kimura et al. 2013).

1.3. SCFAs mediated regulation of metabolic pathways

Dietary fibers and their metabolites have gained much interest recently due to their roles in the regulation of lipid and glucose regulation (den Besten et al. 2013; Byrne et al. 2015; Conlon

and Bird 2015; Ohira, Tsutsui, and Fujioka 2017)

1.3.1. SCFAs and lipid metabolism

1.3.1.1. Lipids

Lipids can be divided into eight categories – fatty acids, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids and polyketides (Barber, Price, and Travers 2005). Each lipid category has its own specific classes and sub-classes. Fatty acids are the building blocks of lipids. Fatty acids are classified based on the carbon chain length, and the number and position of double bonds.

1.3.1.1.1. Classification of fatty acids

The chain length in fatty acids varies from two carbons to 30 carbons. Fatty acids with two to six carbons are SCFAs, those with seven to 13 carbons are medium-chain fatty acids, those with 14 to 20 carbons are LCFAs, and those with 21 or more carbons are considered very long chain fatty acids (VLCFAs). On the basis of presence or absence of double bonds, fatty acids are sub-classified into unsaturated fatty acids and saturated fatty acids (SFAs), respectively. Unsaturated fatty acids containing one double bond in its structure are known as mono-unsaturated fatty acids (MUFAs); those fatty acids with two or more double bonds are known as poly-unsaturated fatty acids (PUFAs). The position of double bonds in PUFAs is typically at third, sixth or ninth carbon atom from the terminal methyl group, and accordingly they are classified into omega-3 (n-3), omega-6 (n-6) and omega-9 (n-9) PUFAs, respectively (Figure 1.2). All of these fatty acids are present as major components of dietary fats and have distinct role in digestion, absorption and transport of lipids.

1.3.1.1.2. Role of fatty acids

Fatty acids (FAs) are incorporated into blood lipids, storage lipids (e.g.: cholesteryl esters - ChE, TG), and also helps in the formation of membrane lipids (e.g.: phospholipids; PLs). The type of fatty acid and its quantity plays significant roles in contributing to health effects. Linoleic acid (18:2n-6, LA) and alpha linolenic acid (18:3n-3, ALA) are considered essential PUFAs. These fatty acids can only be obtained in the diet however, they can be used to synthesize longer chain PUFAs via the n-6 and n-3 pathways, involving elongation and desaturation (Figure 1.2). Arachidonic acid (20:4n-6, AA) is one of the most important fatty acid of the n-6 pathway, while eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) are important fatty acids of the n-3 pathway. The n-6 and n-3 PUFA (AA, EPA and DHA) generate metabolically active biomolecules such as eicosanoids, docosanoids, and resolvins, which play important physiological roles in cellular functions like inflammation, platelet aggregation, and chemotaxis (Figure 1.3). The n-6 PUFAs are generally considered pro-inflammatory, while n-3 PUFAs are anti-inflammatory (Ratnayake and Galli 2009). The metabolites generated from AA as a result of the action of cyclooxygenase (COX) and lipoxygenase (LOX) are the 4-series lipoxins (LXs), 4-series leukotrienes (LTs), 2-series prostaglandins (PGs), and 2-series thromboxanes (TXs), commonly known as eicosanoids. Those metabolites which are generated by the action of COX and LOX enzymes on EPA and DHA are known as resolvins and protectins. Each of these metabolites have a different physiological role (Table 1.1 and 1.2).

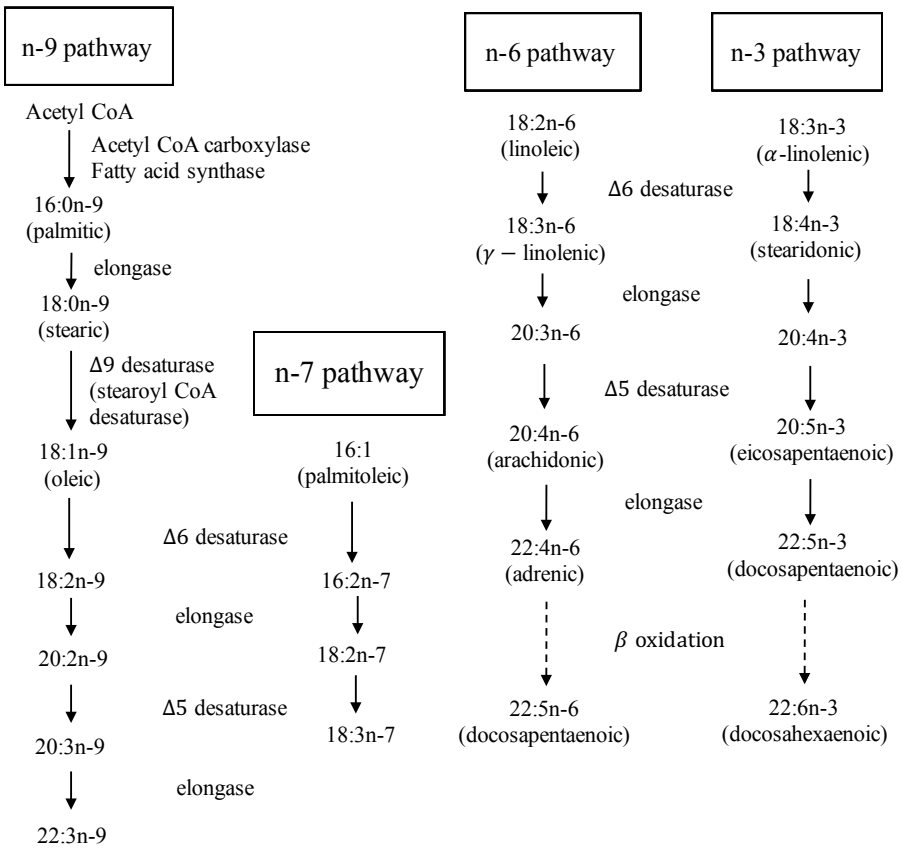
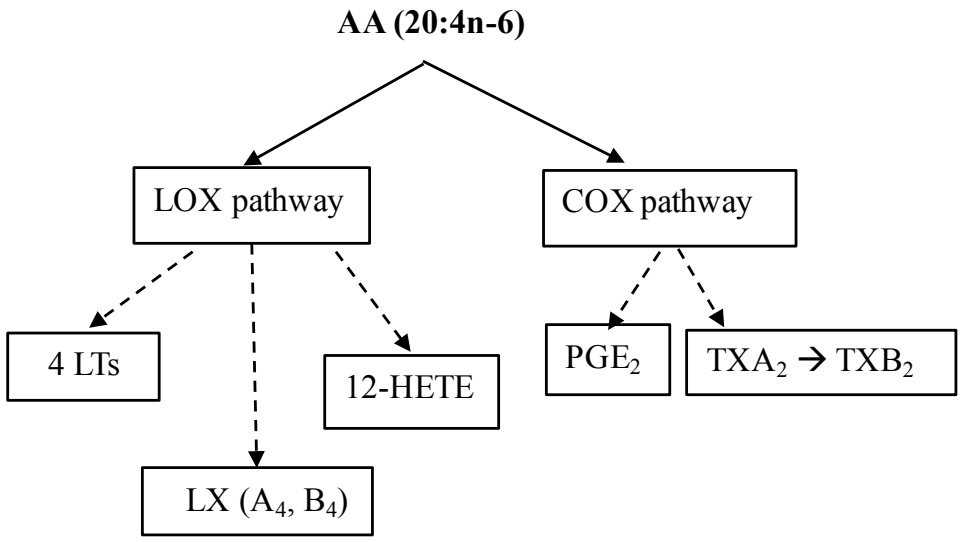


Figure 1.2 Schematic pathways of n-9, n-7, n-6 and n-3 fatty acid metabolism in mammals. Synthesis of monounsaturated fatty acid such as oleic acid (18:1n-9) occurs from acetyl Co-A with the help of acetyl-CoA carboxylase, fatty acid synthase, elongase and stearoyl CoA-desaturase, via omega (n)-9 pathway. The substrates oleic acid, palmitoleic acid (16:1n-7), linoleic acid (18:2n-6) and a-linolenic acid (18:3n-3) are primary fatty acids which undergoes elongation and desaturation to synthesis longer-chain fatty acids via n-9, n-7, n-6 and n-3 pathways respectively. 18:2n-6 and 18:3n-3 are essential fatty acids. Figure adapted from Ratnayake WM, et al. Ann.Nutr.Metab. 2009; 55:8-43

A.



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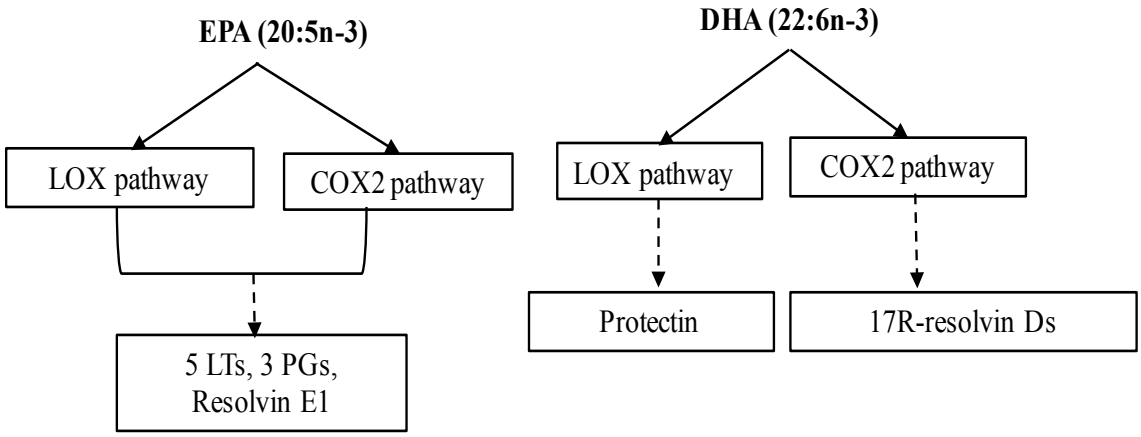


Figure 1.3 Synthesis of eicosanoids and docosanoids from AA (A), EPA and DHA (B). AA (20:4n-6), EPA (22:5n-3), and DHA (22:6n-3) undergoes either lipoxygenase or cyclooxygenase pathway to generate 2-, 3-, 4- and 5- series metabolites which have various physiological actions. AA – arachidonic acid, COX – cyclooxygenase, DHA – docosahexaenoic acid, EPA – eicosapentaenoic acid, HETE – hydroxyeicosatetraenoic acid, LOX – lipoxygenase, LTs – leukotrienes, LX – lipoxins, PGs – prostaglandins, TXs – thromboxanes.

Table 1.1: Physiological role of eicosanoids derived from arachidonic acid.

Type of eicosanoid	Biological activity
4-series LTs	Proinflammatory,
4-series LXs	Chemotaxis, superoxide anion generation
12-HETE	Stimulates glucose induced insulin secretion
PGE_2	Proinflammatory, pro-aggregatory, immune response suppresor.
2-series TXs	Potent vasoconstrictor, platelet aggregation

LTs – leukotrienes, LX – lipoxins, PGs – prostaglandins, TXs – thromboxanes

Table 1.2: Physiological role of eicosanoids and docosanoids derived from EPA and DHA

Eicosanoids from EPA and docosanoids from DHA	Biological activity
PGE_3	Vasodialatory
PGE_1	Mild anti-aggregatory
Resolvin E1	Potent anti-inflammatory
Protectin D	Potent anti-inflammatory
Protectin D1	Potent anti-inflammatory

DHA – docosahexaenoic acid, EPA – eicosapentaenoic acid, LTs – leukotrienes, LX – lipoxins, PGs – prostaglandins, TXs – thromboxanes.

1.3.1.1.3. Digestion, absorption and transport of lipids

Once dietary fat is hydrolyzed in the stomach, short- and medium-chain fatty acids are released into bloodstream, whereas LCFAs are emulsified and later stabilized by phospholipids in chylomicrons. Emulsified acylglycerols help in the secretion of bile and pancreatic juice; a *sn*-1/3-specific pancreatic lipase produces 2-monoacylglycerols and free fatty acids as final products and also 1,2- and 2,3- diacylglycerols in small proportion as intermediary products (Mu and Porsgaard 2005). The distribution of fatty acids at the *sn*-positions of TG influences their rates of hydrolysis and absorption (Karupaiah and Sundram 2007). The absorbed dietary lipids are utilized by either the monoacylglycerol pathway or the glycerol-3-phosphate (G3P) pathway for endogenous production of TGs (Lehner and Kuksis 1996). The monoacylglycerol pathway is the prominent one and occurs in smooth endoplasmic reticulum (ER) with the help of acyl-CoA synthetase, monoacylglycerol transacylase (MGAT) and diacylglycerol acyltransferase (DGAT). The G3P pathway is involved with generation of PLs along with re-synthesis of TG. However, G3P pathway is activated only when long-chain monoacylglycerols are present in dietary fats and occurs in rough ER. Figure 1.4 depicts the synthesis of TG and PLs via G3P pathway.

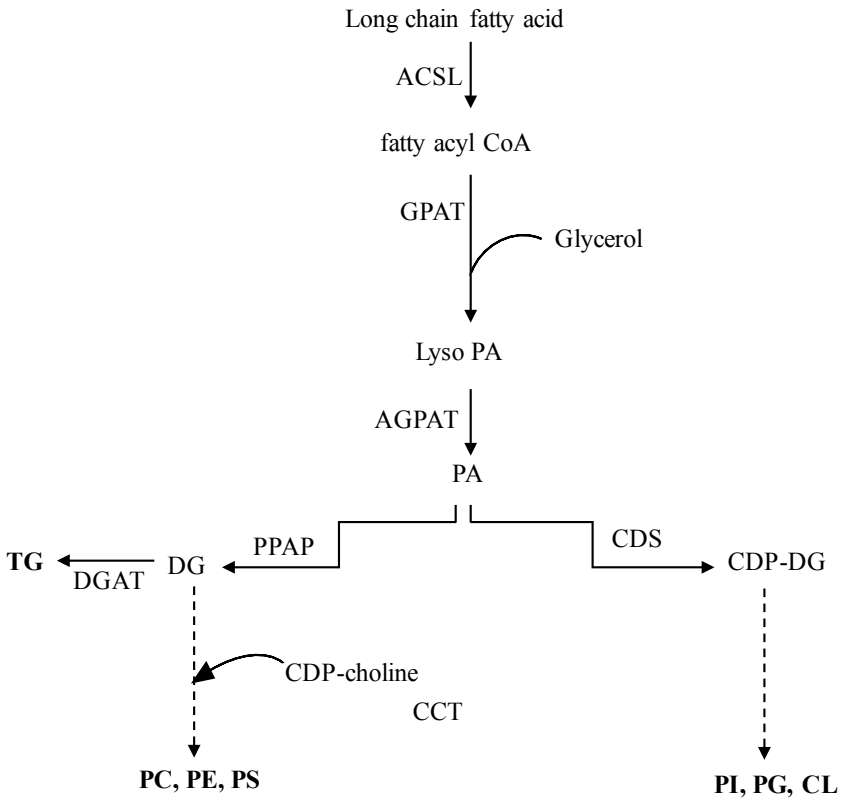


Figure 1.4: Synthesis of triacylglycerols (TG) and phospholipids via glucose-3-phosphate pathway. Endogenous production of phospholipids and re-synthesis of TG occurs via G3P pathway. ACSL: acyl CoA synthase (ligase); AGPAT: 1-acylglycerol-3phosphoacyltransferase; CDP-choline: cytidine-diphosphate; CDS: cytidine-diphosphate diacylglycerol synthase, CCT: cytidine-triphosphate: phosphocholine cytidyltransferase; DGAT: diacylglycerol acyltransferase; GPAT: glycerol-3-phosphoacyltransferase; PA: phosphatidic acid; PC: phosphocholine; PE: phosphoethanolamine; PG: phosphoglycerol; PI: phosphoinositol; PPAP: phosphatidic acid phosphatase; PS: phosphoserine. Adapted from Raphael W. and Sordillo L.M., 2013, Int. J. Mol. Sci. 14(10); 21167-21188.

Different enzymes are involved in the transport of LCFAs, lysophosphatidylcholine (LPC) and cholesterol. For example, lysolecithin acyl transferase is used for esterification of LPC to PC, whereas for the similar process of esterification of cholesterol in intestine and plasma, acyl-CoA acyltransferase (ACAT) and lecithin: cholesterol acyltransferase (LCAT) are used, respectively. LCAT functions to transfer *sn*-2 positioned fatty acids (mainly PUFAs) from PC to cholesterol. Similar to cholesterol, PUFAs at *sn*-2 position in TG help in the absorption of these fatty acids as 2-monoacylglycerols and thus, tend to retain PUFAs until subsequent processes of transport and biosynthesis of TG and PLs. Thus, the stereospecificity of FAs in the lipid structure is of significance.

1.3.1.1.4. Metabolism of fatty acids

The rate of fatty acid oxidation (FAO) is affected by the number of carbons and double bonds in the structure of the lipid molecule. For example, in case of SFAs, oxidation rates are in decreasing order starting laurate (C12:0) to stearate (C18:0). In general, lesser the number of carbon atoms, faster is the process of oxidation. Similarly, human as well as animal studies have reported that unsaturated fatty acids undergo faster oxidation than SFAs, indicating high chances of SFAs deposition in tissues than its oxidation (AASLD. 2014). Excess intake and deposition of SFAs in liver, adipose tissue, or in blood vessels can cause fatty liver disease and insulin resistance, thus increasing the risk of atherosclerosis, hyperlipidemia, T2D, obesity and few other diseases of metabolic syndrome (Cordain et al. 2005; Eaton 2006). However, it is not known whether SCFAs affect different lipid classes, and the fatty acyl composition of various lipids.

1.3.1.1.5. Fatty acyl composition of neutral lipids

Those lipids with dominant hydrophobic groups in its structure are classified as neutral lipids. Cholesterol, ChE and TG are three predominant neutral lipids in mammalian cells; with more abundance of free cholesterol (FC) in plasma membranes, while ChE and TG are mainly packaged in chylomicrons and transported to various tissues. Structurally, ChE consists of one fatty acid esterified to its sterol skeleton, whereas TG consists of three fatty acids at the *sn*-1, *sn*-2 and *sn*-3 positions of the glycerol backbone. These esterified fatty acids mainly comprise of 13-23 carbons and 0-6 double bonds (Karupaiah and Sundram 2007). It is not known whether SCFAs alter the fatty acyl composition of neutral lipids and the consequences on host lipid metabolism.

1.3.1.1.5.1. Effect of SCFAs on cholesterol metabolism

Cholesterol is either obtained from diet or synthesized *de novo* mainly in the liver and intestine (Field, Kam, and Mathur 1990; Feingold and Grunfeld 2015). The rate limiting enzyme in cholesterol synthesis is 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), which converts 3-hydroxy-3-methylglutaryl-CoA to mevalonate. An *in vitro* study showed that propionate lowers cholesterol synthesis rates due to a decrease in the activity of HMGCR in liver (Hara et al. 1999). These authors found that total cholesterol (TC) synthesis rates decreased when propionate was mixed in the administered diets. Isolated hepatocytes, when treated with propionate and butyrate separately, also showed that propionate inhibited fatty acid and cholesterol synthesis, whereas butyrate activated both pathways (Demigné et al. 1995). Fushimi et al. (2006) showed that when rats that were fed a diet with 1% (w/w) cholesterol, they showed a decrease in serum cholesterol levels upon supplementation with 0.3% (w/w) acetate. Acetate supplementation decreased the concentration of HMG-CoA enzyme, with increases in fecal bile acid excretion, but there was no

change in the mRNA expression of cholesterol 7α-hydroxylase (CYP7A1) (Fushimi et al. 2006). CYP7A1 is the rate-limiting enzyme in the pathway which involves breakdown of cholesterol to bile acids in liver. Plasma TC was decreased in male hamsters fed a high cholesterol diet (HCD) containing acetate, propionate or butyrate (Zhao et al. 2017). Although the effect of individual SCFAs have been studied on the regulation of cholesterol metabolism, no studies to date have investigated the effect of a mixture of SCFAs in a 60:20:20 molar ratio on cholesterol metabolism. The transport and storage of cholesterol mainly occurs in the form of ChE (Michael W King 2016), thus the type of fatty acid attached to cholesterol may be of prime importance in cholesterol metabolism (Rosqvist et al. 2017). No studies have investigated the effect of SCFAs on the fatty acyl composition of ChE.

1.3.1.1.5.2. Effect of SCFAs on TG metabolism

Dietary supplementation of SCFAs have been shown to prevent obesity induced by a high fat diet (HFD) (Lu et al. 2016). Obesity is a complex metabolic condition which involves elevated levels of glucose, TG, and high blood pressure. Individuals with obesity are at an increased risk of developing metabolic diseases like T2D and CVD and are clinically diagnosed for metabolic syndrome (Alberti, Zimmet, and Shaw 2005). Metabolic syndrome is characterized by abnormal fat accumulation, especially in adipose tissue and the liver. High levels of TG and fat accumulation in the liver and adipose tissue are considered very important biomarkers for metabolic dysfunction (Alberti, Zimmet, and Shaw 2005; I. Saito 2012).

Fatty acids in the TG species are either obtained directly from diet or synthesized *de novo* in the liver. Acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) are the rate limiting

enzymes in *de novo* fatty acid synthesis. In mammals, ACC has two isoforms – ACC1, which is involved in lipogenesis; and ACC2, which is involved in lipid oxidation. Both isoforms are highly expressed in liver (Barber, Price, and Travers 2005). For every odd-carbon in the newly synthesized fatty acids, the hydrogens are received from NADPH, whereas for every even-carbon in the fatty acids, the acetyl-CoA and water molecules contribute one hydrogen each, except for the methyl terminal position which receives all hydrogens from acetyl-CoA (Duarte et al. 2014). Along with *de novo* synthesis, the process of elongation and desaturation of lipids are also suggested to have implications in obesity (Wang et al. 2006). PUFAs, and its metabolites such as prostaglandins and eicosanoids, serve as ligands for peroxisome proliferator-activated receptor (PPAR), thereby regulating oxidation and/or storage of lipids (Moon, Hammer, and Horton 2009; Georgiadi and Kersten 2012). Lipids involved in inflammatory pathways such as those composed of n-6 PUFAs and generating eicosanoids may have negative effects on steatohepatitis, while those undergoing desaturation to MUFAs with the help of stearoyl-CoA desaturase (SCD-1) may protect against fatty liver (Scorletti and Byrne 2013). Thus, the effect of SCFAs on various lipid and fatty acyl species is important to understand the impact on the regulation of TG metabolism.

Mice fed a HFD supplemented with SCFAs showed a decrease in adipose tissue and hepatic PPARγ mRNA expression and activity, preventing excess storage of lipids in adipose (Den Besten et al. 2015). This study concluded that SCFAs protect against obesity by switching from hepatic lipogenesis to fat oxidation in adipose tissue. In another study, mice fed a cholesterol-rich diet supplemented with acetic acid showed decreased TG levels in plasma and the mRNA expression of hepatic FAS (Fushimi et al. 2006). However, no studies to date have investigated the effects of a mixture of SCFAs in the molar ratio of 60:20:20 on the regulation of TG metabolism.

1.3.1.1.6. Fatty acyl composition of phospholipids

Lipids with a polar head group in its structure along with hydrophobic aliphatic chains are classified as polar lipids. PLs, sphingolipids and glycolipids are classified under polar lipids. Of them, PLs comprise of 60% of lipid mass content and have at least one phosphate group at *sn*-3 position. Based on the head group, PLs are classified as: PC, phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylserine (PS), and phosphatidylinositol (PI). In all mammalian cell types, PC is the most abundant phospholipid, followed by PE. The other PLs (PG, PS, PI), represent minor components in the cell which collectively account for approximately 25% of total PLs (Pradas et al. 2018). A colossal diversity of PLs molecular species is found in mammalian cells and this is due to remodeling of acyl-chain constituents by the enzymatic action of phospholipases and lysophospholipid acyltransferases (LPAT). These alterations in the fatty acid composition of PLs imparts varied function/s in the regulation of lipids, lipoprotein and whole-body metabolism (van der Veen et al. 2017).

1.3.1.1.6.1. Functions of phospholipids

The major function of PLs is to maintain the structure and integrity of cell membranes. A recent study proposed that increased hepatic PC synthesis stimulates VLDL production (Martínez-Uña et al. 2015), resulting to increased hepatic accumulation of re-esterified TG (Van Der Veen, Lingrell, and Vance 2012). Interestingly, along with the amount of hepatic PC, the fatty acyl composition of PC in hepatic membranes also regulates VLDL secretion. Studies have found that the hepatic enzyme lyso phosphatidylcholine acyltransferase 3 (LPCAT3) remodels newly synthesized PC in such a way that there is enrichment of AA in its *sn*-2 position (Hishikawa et al. 2008; Rong et al. 2015). It has also been reported that there is increased membrane fluidity in cells

with PLs containing PUFAs in the *sn*-2 position (Vásquez et al. 2014; Rosqvist et al. 2017). Thus, identification of the fatty acyl composition of PLs is of significance to understand the regulation of lipid metabolism. There are no studies to date on the effect of SCFAs on phospholipids fatty acyl composition.

1.3.1.1.6.2. Sub-classification of phospholipids

PC and PE are further classified to three sub-classes (phosphatidyl, plasmenyl and plasmanyl PLs) on the basis of ester, vinyl ether and alkyl ether linkages of the aliphatic chain on *sn*-1 position of the glycerol moiety (Horrocks and Sharma 1982). The PC and PE moieties with ester linkages, are known as diacylated PLs and are the predominant PLs in the cell membranes of most cell types, except for sarcolemma and neuronal cells, in which alkyl ether linkages predominate. The plasmenyl PLs are more commonly known as plasmalogens (PlsEtn/PlsCho) and these lipid species contain a fatty alcohol with a vinyl-ether bond at sn-1 position and are enriched with PUFAs at *sn*-2 position (Braverman and Moser 2012).

Plasmalogens constitute about 15-20% of total PLs mass in humans. The brain has the highest abundance of plasmalogen species (Han., Holtzman., and Mckeel Jr., 2001) constituting about 90% of total PE species followed by heart and immune cells (about 50% of total PE); the liver has the least abundance. The lower abundance in liver relative to other tissues could be explained by the synthesis of plasmalogens in liver and transport to other tissues with the help of lipoproteins (J. E. Vance 1990). Due to varied tissue distribution, the identification of physiological roles of plasmalogen species have been quite challenging, as it is likely that the functions are specific to a particular tissue. Few of the known physiological roles of plasmalogens are the maintenance of membrane fluidity (Han and Gross 1990), and prevent oxidation of PUFAs

(Broniec et al. 2011). Plasmalogens also act as reservoirs for AA and DHA, and as biologically active mediators in cell-signaling pathways (Stables and Gilroy 2011).

1.3.1.2. Lipidomics

Lipidomics is rapidly growing analytical tool for the comprehensive identification and characterization of lipid constituents mediating life processes. In other words, lipidomics is a part of omics science that facilitates the study of lipid molecular species, also known as lipidome, in biological samples (plasma, blood, urine, tissue) (Han and Gross 2005). Lipidomics can be divided into two approaches: 1) targeted lipidomics, which focuses on the analysis of definite lipid groups of interest in a sample; and 2) untargeted lipidomics, which detects all possible lipid groups in a sample. Both of these approaches can characterize the identified molecular species in an absolute quantitative (expressed in molar units) or semi-quantitative manner (expressed in arbitrary units) (Cajka and Fiehn 2016). The targeted analyses rely on specific isolabeled internal standards that are added to a sample during lipid extractions, which allow for quantitative analyses. In contrast, untargeted lipidomics is not a quantitative approach and it can be performed with or without internal standards. The analyses obtained by untargeted lipidomics cannot be directly compared to other studies but are accurate for treatment/control comparisons. For each approach, until the mid-2000s, there has been lack of highly sensitive analytical techniques for accurate measurement of lipidome (Han and Gross 2005). However, advances in mass spectrometry technologies have facilitated great knowledge and understanding in terms of information on elemental composition and molecular structure of lipid species in a biological sample (Watson and Sparkman 2007). Techniques using electrospray ionization mass spectrometry (ESI/MS) and Orbitrap technology were used within the current study to investigate the effects of SCFAs on lipidomics profile.

1.3.2. SCFAs and glucose metabolism

The balance between glucose intake and its utilization is of utmost importance to maintain the normal plasma glucose levels. Activation of FFAR 2 and 3 receptors by SCFAs increases gut hormones, which have a direct effect on plasma glucose levels (den Besten et al. 2013; Morrison and Preston 2016). SCFAs have also been shown to reduce plasma free fatty acids (FFAs) levels by activating FAO, inhibiting lipolysis in adipose tissue and *de novo* synthesis in liver (Morrison and Preston 2016; Ohira, Tsutsui, and Fujioka 2017). Acetate, propionate and butyrate prevented HFD induced obesity and insulin resistance in mice (Lin et al. 2012). Butyrate and propionate, but not acetate, reduced food intake by inducing gut hormones. Acetate and propionate when administered orally in diabetic hyperglycemic mice reduced glycaemia (Sakakibara et al. 2006). Moreover, propionate and butyrate generated by feeding soluble fibers in rats promoted reduction in body-weight by activating intestinal gluconeogenesis (De Vadder et al. 2014). Others have also shown that acetate, propionate and butyrate increase insulin sensitivity (Yamashita et al. 2007; Z. Gao et al. 2009). However, it is yet not known if the mixture of SCFAs in a molar ratio of 60:20:20 will improve plasma glucose and non-esterified fatty acid (NEFA) concentrations.

1.4. Sex-specific differences in metabolism

Majority of the studies conducted to date on the effects of SCFAs on glucose and lipid metabolism have used males. There are undisputable evidences suggesting differences in metabolism of males and females. It is well known that the sex-specific differences in metabolism are mainly due to genetic make-up and varied concentration of sex hormones like estrogens and androgens (Oleg Varlamov, Bethea, and Roberts 2014). Due to a difference in sex chromosomes X and Y; a clear difference in parameters like food intake, adiposity, fat storage and systemic

glucose homeostasis in mouse model have been demonstrated between males and females (Link et al. 2013). Estrogens and progesterone in females, and testosterone in males, are well documented to regulate energy intake and energy utilization in rodents as well as humans (Sugiyama and Agellon 2012). Females tend to store more lipids, whereas males oxidize more of body fat and therefore, women have more percentage of body fat but lesser visceral white adipose tissue (V-WAT). Studies have shown that lesser V-WAT and higher subcutaneous WAT in body fat distribution is a favorable condition for homeostasis in lipid and glucose metabolism, and thus prevents metabolic diseases associated to abdominal obesity (Vague 1956; Snijder et al. 2005; Hernandez et al. 2011). FABP-2 deficient male mice are more vulnerable to develop fatty liver compared to female mice when fed a HFD (Sugiyama and Agellon 2012). FABP-2 deficiency causes an increase in fatty acids released into the portal circulation, which could lead to an overload of lipids in male livers, whereas the protection in female mice could be a result of compensatory mechanisms in female intestines.

It is also known that females have a higher sensitivity towards insulin compared to males (O Varlamov and Bethea 2015). When male and female rats were treated with 0.1-10 nmol/L insulin, there was stimulation of glucose uptake in males whereas inhibition of glucose uptake was observed with female rats. Estrogen is known to reduce hepatic lipogenesis and increase lipolysis in adipocyte and thus decrease *de novo* synthesis. (Bryzgalova et al. 2008; D'Eon et al. 2005; H. Gao et al. 2006). Although men have lower body fat mass, there is a higher risk of T2D and obesity due to greater whole-body insulin sensitivity in women (Frias et al. 2001; Soeters et al. 2007). Human studies have also shown that there is more utilization of glucose in women compared to men (Hale, Wright, and Nattrass 1985; Basu et al. 2006). Animal studies have shown that female

rats have greater resistance towards HFD and higher insulin sensitivity (Corsetti et al. 2000; Macotela et al. 2009; Trevaskis et al. 2008). One of the main reason for more storage as well as utilization of glucose and lipids in females is increased energy utilization during phases of menstruation and lactation.

Another proposed mechanism for changes in lipid and glucose metabolism by SCFAs is the effect of gut hormones during the digestion and absorption of dietary fibers (Freeland and Wolever 2010). When SCFAs were infused in the colon of male animals or given as dietary fibers, there was an increase in gut hormone concentrations in blood (Cuche, Cuber, and Malbert 2000; Zhou et al. 2008), suggesting a role of SCFAs and gut hormones in communication between tissues. A study by Freeland and Wolever (2010) showed that when female subjects were administered sodium acetate via intravenous and rectal routes, there was an increase in plasma Peptide YY (PYY) and glucagon-like peptide (GLP) levels to regulate weight management (Freeland and Wolever 2010). It is therefore important to understand whether SCFAs have sex-specific effects on the regulation of glucose and lipid metabolism.

1.5 Rationale

To date, several studies have shown beneficial effects of individual SCFAs on the regulation of lipid and glucose metabolism. However, the effect of SCFAs mixture (acetate, propionate and butyrate; 500mg/kg), in the molar ratio of 60:20:20 (produced under symbiotic conditions) on the regulation of lipid and glucose metabolism is not known. Moreover, the sex-specific effects of SCFAs on the regulation of lipid and glucose metabolism are not known. In the current study, male and female Long-Evans rats were injected intraperitoneally with sodium salts

of acetate, propionate and butyrate in the molar ratio of 60:20:20 for seven days. I.P. administration mimics the transport pathway of endogenously generated SCFAs, whereby SCFAs once produced in gut are transported to the liver via portal circulation. Moreover, administration by I.P. route eliminates the effects of SCFAs derived in gut with the help of microbiota.

The animal model in the study was previously used by our collaborator, Dr. Raymond Thomas (Department of Environmental Science, Memorial University of Newfoundland), to investigate the effects of SCFAs on brain health. Dr. Thomas's group used tissues from the current study to investigate the regulation of lipids and lipidomic profile of the brain. An untargeted lipidomics approach was used to evaluate the effects of SCFAs on plasma and hepatic lipid metabolism in Long-Evans rats.

There are several drug therapies to prevent and/or treat metabolic diseases caused by the dysregulation of lipid and glucose metabolism. One of the main drawbacks of these modern medicines is cost, as many of these medications are not affordable by people of all socio-economic classes. Establishing the beneficial effects of SCFAs may suggest these as a cheaper therapeutic strategy for chronic diseases which require long-term treatment. To understand the role of SCFAs on lipid and glucose metabolism, and to investigate their sex-specific effects, the following hypotheses and objectives were designed.

1.6 Hypotheses and Objectives

Hypothesis 1: SCFAs when given intraperitoneally in the molar ratio of 60:20:20 will decrease plasma and hepatic cholesterol and TG levels, and plasma glucose levels in a sex-specific manner, by regulating genes involved in hepatic lipogenesis.

Objective 1a: To measure plasma and hepatic lipid levels, plasma glucose levels, and to investigate sex-specific effects of SCFAs on lipid and glucose metabolism;

Objective 1b: To measure the mRNA expression of genes regulating cholesterogenesis and lipogenesis.

Hypothesis 2: SCFAs, due to its role in biosynthesis of LCFAs, will alter plasma and hepatic lipidomic profile of various lipid classes.

Objective 2: To measure fatty acyl composition of various lipid classes.

Chapter 2 - Materials and Methods

2.1 Animals

Long-Evans rats (12 males and 12 females; 45-50 days old) were obtained from Charles River Laboratories, Canada. All the animal procedures were in accordance with the Guidelines of the Canadian Council on Animal Care and were approved by the Animal Care Committee of Memorial University of Newfoundland (protocol #16-01-RT). The animals were housed in individual cages in a well-ventilated room and maintained under standard laboratory conditions (21℃ and 12 hours light/dark cycle). After one week of acclimatization, male and female rats were divided into control and treatment groups (n=6 in each group). Control group received 0.1 M phosphate-buffered saline (PBS; pH 7.4) solution at 2 mg/kg body mass, whereas treatment groups received a mixture of sodium acetate (Cat # S2889, Sigma-Aldrich, USA), sodium propionate (Cat # P1880, Sigma Life Science, USA), and sodium butyrate (Cat # B6887, Sigma-Aldrich, USA), dissolved in 0.1 M PBS in the molar ratio of 60:20:20, respectively. Treatment was given at a dose of 500 mg/kg body mass via I.P. route, each day for seven days at 7:00 AM. Animals had free access to chow diet (Cat #7012; Teklad Laboratory Animal Diets, USA) and water throughout the experimental period. Animals were weighed each morning before administration of I.P. injection, and the amount of vehicle control and treatment was calculated according to the body weight. Food intake was measured daily after three hours of injection.

2.2 Blood and tissue sampling

After one week of treatment, rats were euthanized 20 mins post last injection in a non-fasted condition. Rats were subsequently killed by cardiac puncture under isoflurane. Blood samples were collected into EDTA-coated (0.1 M, 300 μl) tubes and centrifuged for 10 mins at 2100 $\times g$ (4℃) to separate plasma. All biochemical assays were performed within one week, using fresh

plasma stored on ice at 4℃. Remaining plasma samples were stored at -80℃ for further analysis. Liver was collected, freeze-clamped and weighed immediately. Brain and abdominal fat was isolated, weighed, snap-frozen in liquid nitrogen. Tissues were stored at -80℃ until further analysis. A small portion of liver, right brain and plasma for each animal was shipped on dry ice to Dr. Thomas's laboratory, Department of Environmental Sciences, Memorial University of Newfoundland, for lipidomics analysis by UHPLC-MS/MS.

2.3 Lipid Extraction

2.3.1 Folch Extraction for biochemical analysis

Total lipids were extracted from liver and adipose tissue (100 mg tissue) using 300 μl of 50 mM sodium chloride solution and 1.5 ml chloroform: methanol (2:1 v/v) mixture according to the Folch method (Folch, Lees, and Stanley 1957). The bottom organic layer was transferred to pre-weighed glass vials and dried under nitrogen. Extracted lipids were re-suspended in isopropanol and used for biochemical analysis.

2.3.2. Lipid Extractions Using Bligh-Dyer method for lipidomics

Lipids for UHPLC-MS/MS analysis were extracted using a modified Bligh and Dyer method (Bligh, E.G. and Dyer 1959; Vidal et al. 2018). In this method, 1 ml methanol containing 0.01% butylated hydroxytoluene was added prior to homogenization to prevent oxidation of lipids. Homogenized samples were treated with 1 ml chloroform and 0.8 ml water, and centrifuged for 10 mins at 10,000 x *g*. The organic layer was recovered and dried under nitrogen gas. The extracted lipids were resuspended in 1 ml chloroform: methanol (1:1 v/v) for further analysis.

2.4. Analysis of biochemical parameters

2.4.1 Plasma and hepatic total cholesterol

TC was assayed according to manufacturers' instructions using commercially available kit # 234-60 (Genzyme Diagnostics, Canada). The assay uses cholesterol esterase enzyme, which hydrolyzes ChE to FC; the generated FC is oxidized to cholest-4-ene-3-one generating hydrogen peroxide (H_2O_2) molecule using cholesterol oxidase enzyme. The H_2O_2 generated during this reaction yields chromogen as a result of condensation reaction with 4-aminoantipyrine and p-hydroxybenzoate. The absorbance of a generated chromogen was measured at 500 nm. All assays were performed in duplicates and the average absorbance values were used for calculations. The concentration of the samples was calculated with the help of slope obtained from the standard curve that was performed for each independent assay run.

2.4.2 Plasma and hepatic free cholesterol and cholesteryl esters

FC and ChE were assayed in plasma and liver samples using thin layer chromatography coupled to a flame ionization detector (TLC-FID) (Zhau S., 2003). A solvent system made of benzene:chloroform:formic acid (70:30:0.5 v/v/v) was poured into a TLC tank and allowed to incubate at room temperature for atleast one hour. A standard mixture containing 1 mg/ml of cholesterol, TG, ChE, FFA, monoacylglycerols, and diacylglycerols was used. Before the spotting of standard and samples, blank scanning was performed on a rack containing ten Chromarods on an Iatroscan TLC-FID analyzer using a scanning speed of 4mm/sec, airflow of 2000ml/min and hydrogen flow of 160 ml/min. Standard mixture ($2\mu l$) was applied on first rod of each rack with ten Chromarods followed by $2\mu l$ of sample solution on the following nine rods, using a pressure locked syringe. The rack containing the spotted Chromarods was then placed into a constant

humidity chamber containing saturated NaCl solution and was allowed to incubate for 10 mins. The Chromarod rack was immediately transferred to the TLC chamber developed using the solvent system of benzene:chloroform:formic acid (70:30:0.5 v/v/v) and incubated for 45 mins at room temperature. Chromarod rack was dried for 2-3 mins at 100℃ in oven and later, scanned on an Iatroscan TLC-FID analyzer using same settings as described for blank scans. Analysis of peak areas was done using Iantroscan MK-6s along with Peak 432-64bit software.

2.4.3 Plasma, hepatic and adipose tissue triacylglycerols

TG assay was performed according to manufacturers' instructions using commercially available kit #236-17 (Genzyme Diagnostics, Canada). The assay uses lipase enzyme which hydrolyzes TG to glycerol and FFAs. The generated glycerol is further phosphorylated to glycerol-1-phosphate with the help of ATP and enzyme glycerolkinase. The glycerol-1-phosphate is further oxidized with the help of glycerol phosphate oxidase and produces H_2O_2, that yields a chromogen after coupling with 4-aminoantipyrine and p-chlorophenol. The absorbance of the chromogen was measured at 520 nm. All assays were performed in duplicates and the average absorbance values were used for calculations. The concentration of the samples was calculated with the help of slope obtained from the standard curve that was performed for each independent assay.

2.4.4 Plasma non-esterified fatty acids

NEFA was assayed according to manufacturers' guidelines using commercially available kit # 278-76491 (WAKO chemicals, USA). The assay uses acyl-CoA synthetase enzyme to form thiol esters of CoA known as acyl-CoA. The produced acyl-CoA further undergoes oxidation reaction

generating H_2O_2. The H_2O_2 generated during this reaction yields purple colored chromogen as a result of coupling with 4-aminoantipyrine and 3-methyl-N-ethyl-N-(B-hydroxyethyl)-aniline. The absorbance of the chromogen was measured at 550 nm. All assays were performed in duplicates and the average absorbance values were used for calculations. The concentration of the samples was calculated with the help of slope obtained from the standard curve that was performed for each independent assay.

2.4.5. Plasma glucose

Glucose was assayed according to manufacturers' guidelines using commercially available kit # 10009582 (Cayman chemicals, USA). The assay uses glucose oxidase enzyme to produce delta-gluconolactone with simultaneous production of reduced flavin adenine dinucleotide molecule and H_2O_2. The H_2O_2 generates pink colored chromogen as a result of condensation with 3,5-dichloro-2-hydroxybenzenesulfonic acid and 4-aminoantipyrine. The absorbance of the chromogen was measured at 514 nm. All assays were performed on plasma samples (5 μl) in duplicates and the average absorbance values were used for calculations. The concentration of the samples was calculated with the help of slope obtained from the standard curve that was performed for each independent assay.

2.5 Gene expression analysis

Total RNA was extracted from liver and adipose tissue samples using Trizol reagent (Cat #15596026; Thermo Fisher Scientific, USA). The integrity of extracted RNA was assessed using gel electrophoresis (1.2% agarose gel). The concentration of extracted RNA was determined with the help of NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). Genomic DNA

contamination was removed after treatment with DNase (Cat #M610A 12358503; Promega, USA). Reverse transcription was performed to generate cDNA (Cat #A3500; Promega, USA), using 1μg of RNA which was later amplified for quantitative real-time PCR (qPCR) using iQ-SYBR Green Supermix (Cat #170-8880; Bio-Rad Laboratories. Inc., USA). All qPCR reactions were run at a reaction volume of 10 μl using 50ng cDNA per reaction. Samples were run using the CFX96TM Real-Time System, and data output was managed using the CFX ManagerTM Software Version 3.0. Primers for lipogenic and adipogenic genes (Table 2.1) were designed using NCBI primer blast (www.ncbi.nlm.gov/tools/primerblast/) and obtained from IDT technologies (IA, USA). Relative mRNA expressions for hepatic genes were standardized to β-actin as the housekeeping gene, whereas glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene for adipose tissue. The $\Delta\Delta C_T$ method (Livak and Schmittgen 2001) was used to calculate relative gene expression.

Table 2.1: Primers for the gene of interest and housekeeping genes

Name of the gene	Base pairs	Gene sequence	Primer Efficiency (%)	Gene accession number
HMG CoA reductase (Hmgcr)	98	Forward ATT GCA CCG ACA AGA AAC CTG CTG Reverse TTC TCT CAC CAC CTT GGC TGG AAT	119.72	NM_013134.2
Acetyl CoA carboxylase (Acaca)	101	Forward TGT AGA AAC CCGAAC CGT GG Reverse CTG GAA ACC AAA CTT GC CG	115.03	NM_022193.1
Fatty acid synthase (Fasn)	98	Forward GCT GCT ACA AAC AGG ACC AT Reverse TCC ACT GAC TCT TCA CAG ACC A	110.45	NM_017332.1
Cholesterol 7$\boldsymbol{\alpha}$ hydroxylase (Cyp7a1)	89	Forward ACG CAC CTC GCT ATT CTC Reverse AGG CTG CTT TCA TTG CTT CA	107.83	NM_012942.2
Peroxisome proliferator activated receptor (Pparg)	89	Forward TGA TAT CGA CCA GCT GAA CC Reverse TCA GCG GGA AGG ACT TTA TG	112.17	NM_013124.3
β-actin (Actb)	85	Forward AGC GTG GCT ACA GCT TCA CC Reverse AAG TCT AGG GCA ACA TAG CAC AGC	114.13	NM_031144.3
Glyyceraldehyde-3-phosphate dehydrogenase (Gapdh)	99	Forward TGC CAC TCA GAA GAC TGT GG Reverse TTC AGC TCT GGG ATG ACC TT	97.87	NM_017008.4

2.6 Lipidomics Analysis

HPLC grade acetic acid, formic acid, ammonium acetate, and ammonium formate were obtained from Sigma-Aldrich, Canada. HPLC grade acetonitrile, chloroform and methanol were purchased from Fisher Scientific, Canada, and deionized water was purchased from PURELAB Purification System (ELGA Lab-water, Canada). For internal standards, the synthetic odd chain PC 17:0/14:1(9Z), PE 17:0/14:1(19Z), PS 17:0/14:1(9Z) and PI 17:/14:1(9Z) were used. In order to verify the method, both natural as well as synthetic external standards were used. A complex lipid standard mixture (10μl) was prepared using individual lipid classes. The standard mixture included lipids from natural sources as plant sulfoquinovosyl diacylglycerol (SQDG) 16:0/16:0, plant monogalactosyldiacylglycerol (MGDG) 16:3 (7Z,10Z,13Z)/18:3(9Z,12Z,15Z), chicken egg PG 18:0/20:4(5z,8z,11z,14z), porcine brain PC 18:0/20:4(5Z,8Z,11Z,14Z), porcine brain plasmalogen LPE P-18:0, porcine brain SM d18:1/18:0, bovine liver PI 18:0/20:4(5Z,8Z,11Z,14Z), bovine heart dilysocardiolipin (DLCL) 18:2(9Z,12Z)/18:2(9Z,12Z), and bovine heart CL 18:2/18:2/18:2/18:2. Synthetic lipid standards included - PA 18:1(9Z)/18:1(9Z), LPA 20:4(5Z,8Z,11Z,14Z), LPE 18:0, LPC 18:1(9Z), plasmalogen PC P-18:0/20:4(5Z,8Z,11Z,14Z), plasmalogen PE P-18:0/20:4(5Z,8Z,11Z,14Z), monomethylphosphatidylethanolamine (MMPE) 16:0/16:0, dimethylphosphatidylethanolamine (DMPE) 16:0/16:0, diether PC O-18:0/O-18:0, diether DG O-20:0/O-20:0, DG 18:0/20:4(5Z,8Z,11Z,14Z), medium chain TGs 8:0/8:0/8:0 and long chain TG 16:0/16:0/16:0. Each lipid class was purchased from Avanti Polar Lipids (Alabaster, USA).

The complex lipid standard mixture and lipids extracted from the samples (as per the method discussed in section 2.3.2) were subjected to C30 reverse phase (C30 RP) chromatography,

coupled with ultra-high performance liquid chromatography and heated electrospray ionization high resolution tandem mass spectrometry (UHPLC-C30RP-HESI-HR-MS/MS) using a Q-Exactive Orbitrap mass spectrometer (Thermo Scientific, USA) (Vidal et al. 2018). XCalibur software 4.0 (Thermo Scientific, USA) was used for instrument operation. Linear standard curves for each standard within the range of 1-10 μg/mL was used to provide quantification of the observed lipids. These outputs displayed excellent linear relationships between lipid concentration and the extracted ion chromatogram (XIC) peak areas.

The extracted lipids were analyzed using a Orbitrap mass spectrometer coupled to an automated Dionex Ultimate 3000 UHPLC system, controlled by Chromeleon software; the entire instrument was controlled by XCalibur software 4.0. Full scans of mass spectra were obtained in negative and/or positive modes. The parameters listed in table 2.2 were used for the Orbitrap mass spectrometer.

Table 2.2: Parameters used for Orbitrap mass spectrometer

Parameter	
Sheath gas	40
Auxiliary gas	2
Ion spray voltage	3.2 kV
Capillary temperature	300 ^{o}C
S-lens RF	30 V
Mass range	200-2000 m/z
Resolution (full scan mode)	70,000 m/z
Resolution (top-20 data dependent MS/MS)	35,000 m/z
Collision energy	35 (arbitrary unit)
Injection time	35 minutes
Isolation window	1 m/z
Automatic gain control	1 e5 (w/ dynamic exclusion setting of 5.0s)
Target database	Q-Exactive
Precursor tolerance	5 ppm
Product tolerance	5ppm
Product ion threshold	5 %
m-score threshold	2 %
Quan m/z tolerance	±5 ppm
Quan RT range	1 minute
Adduct ions for positive ion mode	[M+H]+ and [M+NH4]+
Adduct ions for negative ion mode	[M-H]-, [M+HCOO]-, [M+CH3COO]- and [M-2H]2-

XCalibur 4 and LipidSearch 4.1 (Mitsui Knowledge Industry, Japan) software were used to acquire and process all MS data. The identification and semi-quantification of all the lipid classes and its sub-classes, along with lipid molecular species involved with each class were conducted using LipidSearch software. PC, LPC, TG, ChE lipid classes were identified in positive ion mode ($[M+H]^+$ and $[M+NH_4]^+$), whereas PE, LPE, PS, PI and PA classes were identified in negative ion mode ($[M-H]^-$, $[M+HCOO]^-$, $[M+CH_3COO]^-$ and $[M-2H]^{2-}$). Following identifications, the observed lipid classes and molecular species were aligned and merged using the alignment parameters. The alignment parameters were first optimized using lipid standards followed by application to untargeted lipidomic analysis. Positions of the fatty acids present in the molecular species found in each lipid class of the samples evaluated were identified based on the fragmentation patterns of the MS/MS spectra, and manually confirmed using XCalibur 4.

2.7 Data Analysis

Statistical analyses were performed using two-way analysis of variance (ANOVA) (treatment and sex as the two parameters) followed by Tukey's post-hoc analysis using GraphPad Prism (Version 6.0; GraphPad Software Inc., USA). The significance level was set at $P < 0.05$. The Grubbs' outlier test was performed for all lipidomics data using XLSTAT software. Principal component analysis (PCA) were used to group the lipid outputs base on similarities or differences; more than 60 % of variance was taken into account for each lipid class analyzed for lipidomics. Two-way ANOVA was used to assess the significance of the clusters or groups observed on selected lipid molecular species, following PCA analysis. Lipid species clustered around control and treatment quadrants in the PCA biplot were selected for post-hoc analysis. PCA has been widely adapted by mathematicians and statisticians to down-sample high-dimensional data sets

and minimize re-projection error. However, due to assumptions made in this technique for linearity, larger covariance and orthogonality (Parsons, Cooper, and Albertson 2009), and also, the inability of PCA to detect slightest of invariance (Karamizadeh et al. 2013), few important components are treated as noise and removed during analysis. In the current study, analysis was also performed on TG lipid species not found in control and treatment quadrants of the PCA output as some of these species are well known for their involvement in the regulation of metabolic pathways.

Chapter 3 - Results

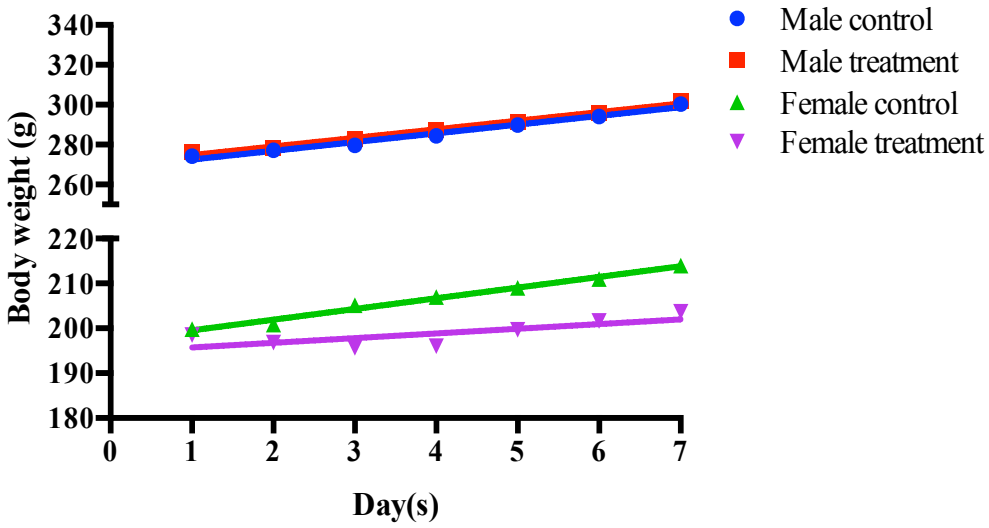
3.1. Effects of SCFAs on the regulation of metabolic pathways

3.1.1. Effect of SCFAs on body weight, organ weight, food intake, plasma glucose and NEFA concentrations

Body weight and food intake of Long-Evans, for each day, over the seven days period is shown in Figure 3.1; while the body weight and food intake at day seven is presented in Table 3.1. There was no significant difference in body weight (Figure 3.1A) or food intake (Figure 3.1B) between control and treatment groups, for both males and females, on each day of the treatment including day seven (Table 3.1). However, males (both control and treatment groups) had significantly higher body weight ($p<0.0001$), as well as food intake ($p<0.001$), compared to female control and treatment groups over the seven days period and at the end of the study period (Table 3.1).

Organ (liver, abdominal fat, brain) weights of male and female rats, on day seven, are given in Table 3.1. There was no significant effect of treatment on liver weight of both male and females, compared to their respective control groups. However, males (both control and treatment groups) had significantly higher liver weight ($p<0.0001$), compared to female control and treatment groups, respectively. No significant difference was observed in abdominal fat in males or females, compared to their respective control groups; however, males treated with SCFAs had higher abdominal mass ($p<0.01$), compared to SCFAs treated females. Males treated with SCFAs showed significantly lower ($p<0.05$) levels of plasma glucose, compared to its control group; however, there was no effect of SCFAs on plasma glucose levels of females, compared to its control group. There was no effect of SCFAs treatment on NEFA levels in both males and females, compared to their respective control groups (Table 3.1).

A.



B.

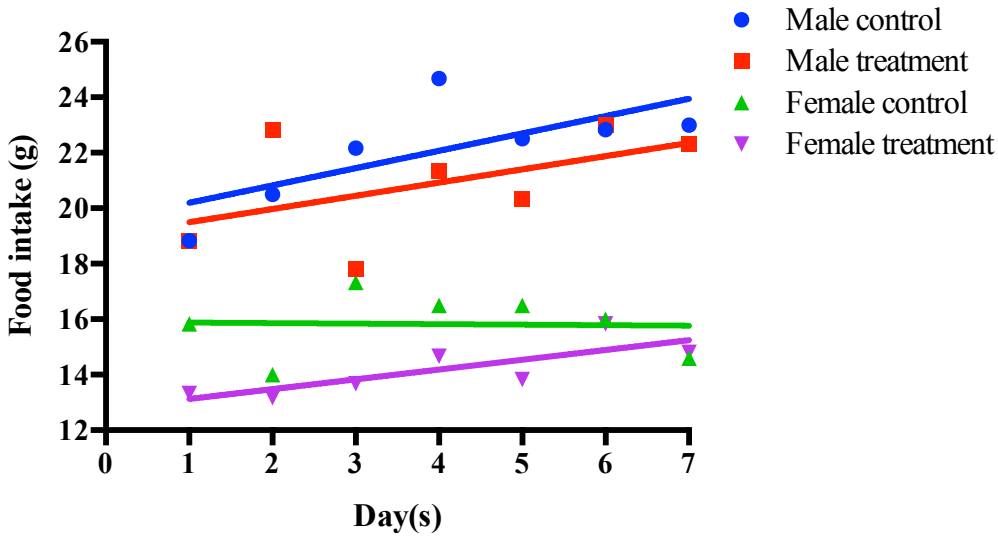


Figure 3.1: Effect of short chain fatty acids on A) body weight and B) food intake of animals from day 0 to day 7 of the study period. Data were analyzed using Dunn's test for linear regression. All data are expressed as mean ± SD. $P < 0.05$ was considered significant (n=6).

Table 3.1: The effect of SCFAs on body weight, food intake, organ weight, plasma glucose and NEFA concentrations of Long Evans rats on day 7.

Parameter	MC	MT	FC	FT
		(grams)		
Body weight	300.33 ± 18.79[a]	301.83 ± 8.57[a]	214 ± 7.72[b]	203.667 ± 5.58[b]
Food intake	23.00 ± 1.75[a]	22.33 ± 1.87[a]	14.60 ± 1.07[b]	14.80 ± 0.88[b]
Liver weight	12.588 ± 1.24[a]	12.723 ± 1.24[a]	8.494 ± 0.45[b]	8.975 ± 0.44[b]
Abdominal fat weight	2.032 ± 0.56[ab]	2.343 ± 0.43[a]	1.743 ± 0.28[ab]	1.431 ± 0.26[b]
Brain weight	1.971 ± 0.16	1.979 ± 0.19	1.859 ± 0.13	1.824 ± 0.198
		(mmol/L)		
Plasma glucose	6.38 ± 0.78[a]	4.76 ± 0.68[b]	5.52 ± 0.33[b]	6.01 ± 0.82[ab]
Plasma NEFA	0.73 ± 0.07	0.68 ± 0.16	0.65 ± 0.12	0.60 ± 0.09

Values are expressed as mean ± SD. Data were analyzed using two-way ANOVA followed by Tukey's *post-hoc* test. Different superscripts indicate significant differences amongst groups within columns. $P < 0.05$ was considered significant (n=6). SCFAs – short chain fatty acids, MC – male control, MT – male treatment, FC – female control, FT – female treatment, NEFA – non-esterified fatty acids.

3.1.2. Effect of SCFAs on cholesterol metabolism

TC, FC and ChE concentration in plasma and liver, are given in Figure 3.2. There was a significant decrease in plasma TC and FC ($p<0.01$ concentrations in males treated with SCFAs, compared to their respective controls (Figure 3.2A and B, respectively). However, SCFAs had no effect on plasma TC and FC concentrations in females, compared to their respective controls (Figure 3.2A and B, respectively). Male control and treatment groups had significantly higher plasma TC concentrations ($p<0.0001$), compared to female control and treatment groups (Figure 3.2A). There was no effect of SCFAs on plasma ChE levels in both males and females, compared to their respective controls (Figure 3.2C).

SCFAs significantly decreased ($p<0.01$) hepatic TC levels in males, compared to its control group; however, there was no effect of treatment in females compared to its respective control (Figure 3.2D). There was no effect of SCFAs treatment on hepatic FC and ChE in both males and females, compared to their respective controls (Figure 3.2E and F, respectively).

The relative mRNA expression of genes involved in regulating cholesterol metabolism is given in Figure 3.3. There was no effect of SCFAs treatment on the mRNA expression of HMG-CoA reductase in both males and females, compared to their respective control groups (Figure 3.3A). Furthermore, there was no effect of SCFAs on the mRNA expression of CYP7A1; however, male control and treatment groups had significantly lower ($p<0.01$) CYP7A1 mRNA expression, compared to female control and treatment groups (Figure 3.3B).

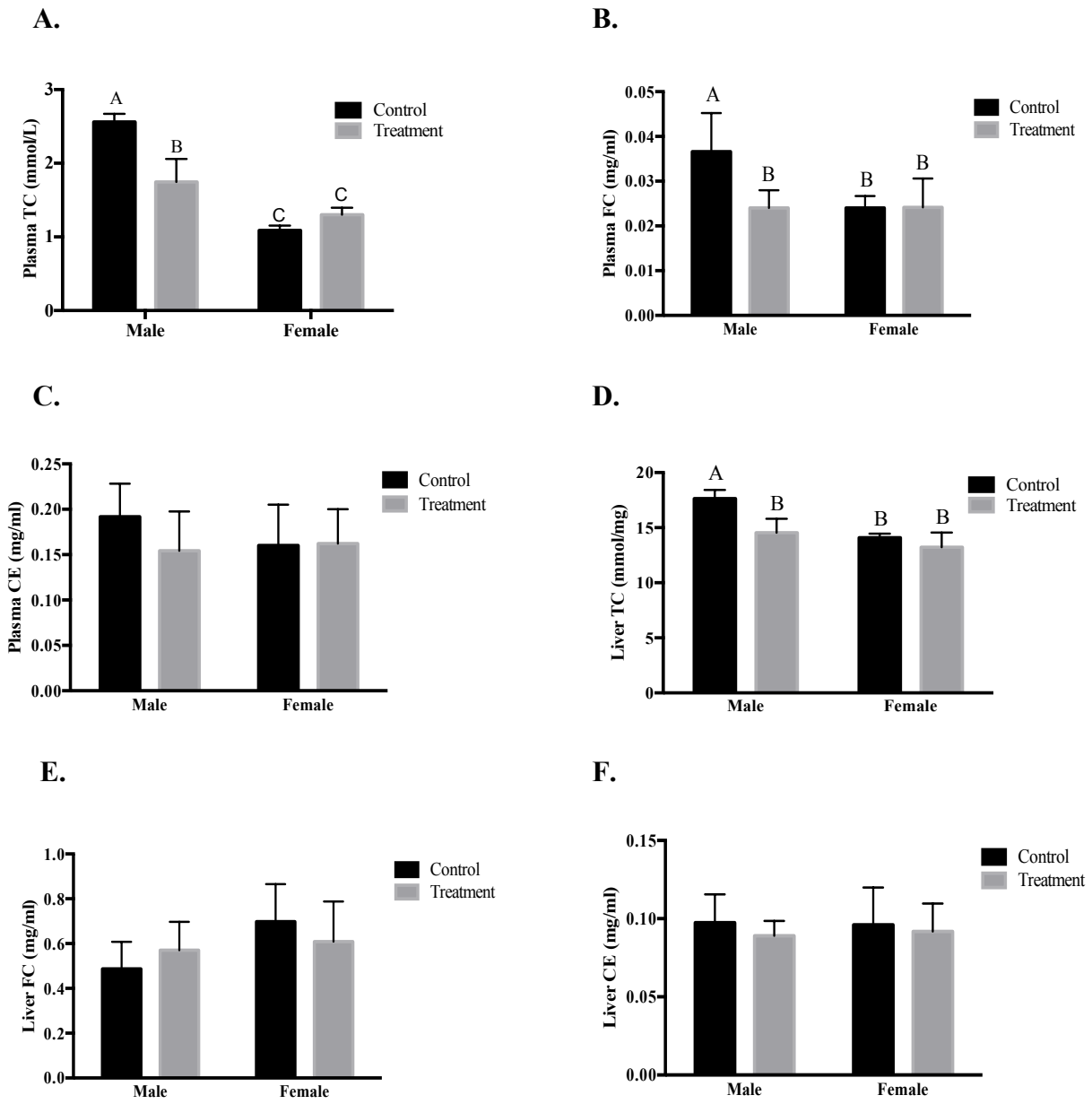


Figure 3.2: Effects of short chain fatty acids on A) plasma TC, B) plasma FC, C) plasma ChE, D) hepatic TC, E) hepatic FC and F) hepatic ChE. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed as mean ± SD. Different superscripts indicate significant differences amongst groups. $P < 0.05$ was considered significant (n=6). TC – total cholesterol, FC – free cholesterol, ChE – cholesteryl esters.

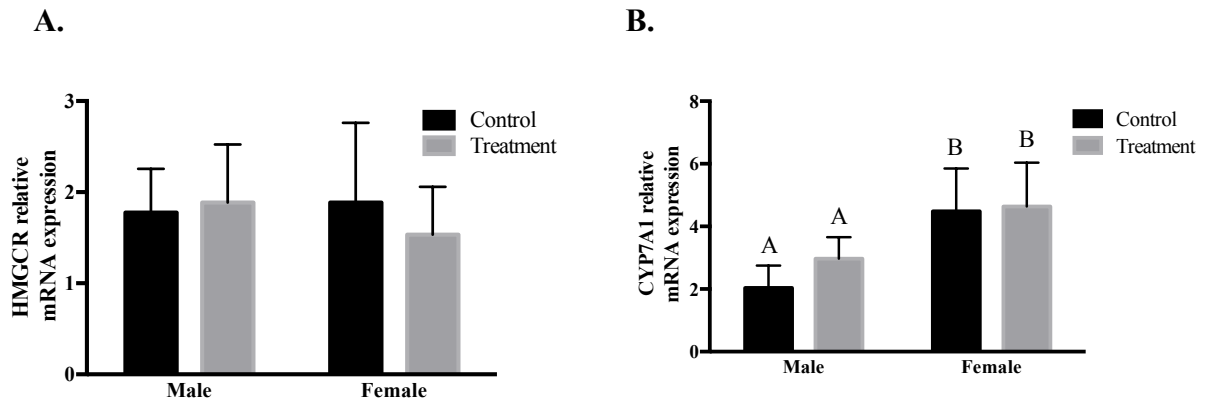


Figure 3.3: Effects of short chain fatty acids on the relative mRNA expression of A) HMGCR and B) CYP7A1. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are presented relative to β-actin and expressed as mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). HMGCR – 3-hydroxy-3-methylglutaryl-CoA reductase, CYP7A1 – cholesterol 7α hydroxylase

3.1.3. Effect of SCFAs on TG metabolism

Plasma and liver TG concentrations and the relative mRNA expression of genes involved in regulating TG synthesis are given in Figure 3.4. There was a significant decrease in plasma and hepatic TG (p<0.05) concentrations in males treated with SCFAs, compared to their control group (Figure 3.4A and B, respectively). However, SCFAs had no effect on plasma and hepatic TG concentrations in females, compared to their respective control group (Figure 3.4A and B, respectively). SCFAs treatment significantly decreased the mRNA expression of hepatic ACC1 in both males and females (p<0.001), compared to their respective controls (Figure 3.4C). However, there was no effect of SCFAs treatment or sex on the hepatic FAS mRNA expression (Figure 3.4D).

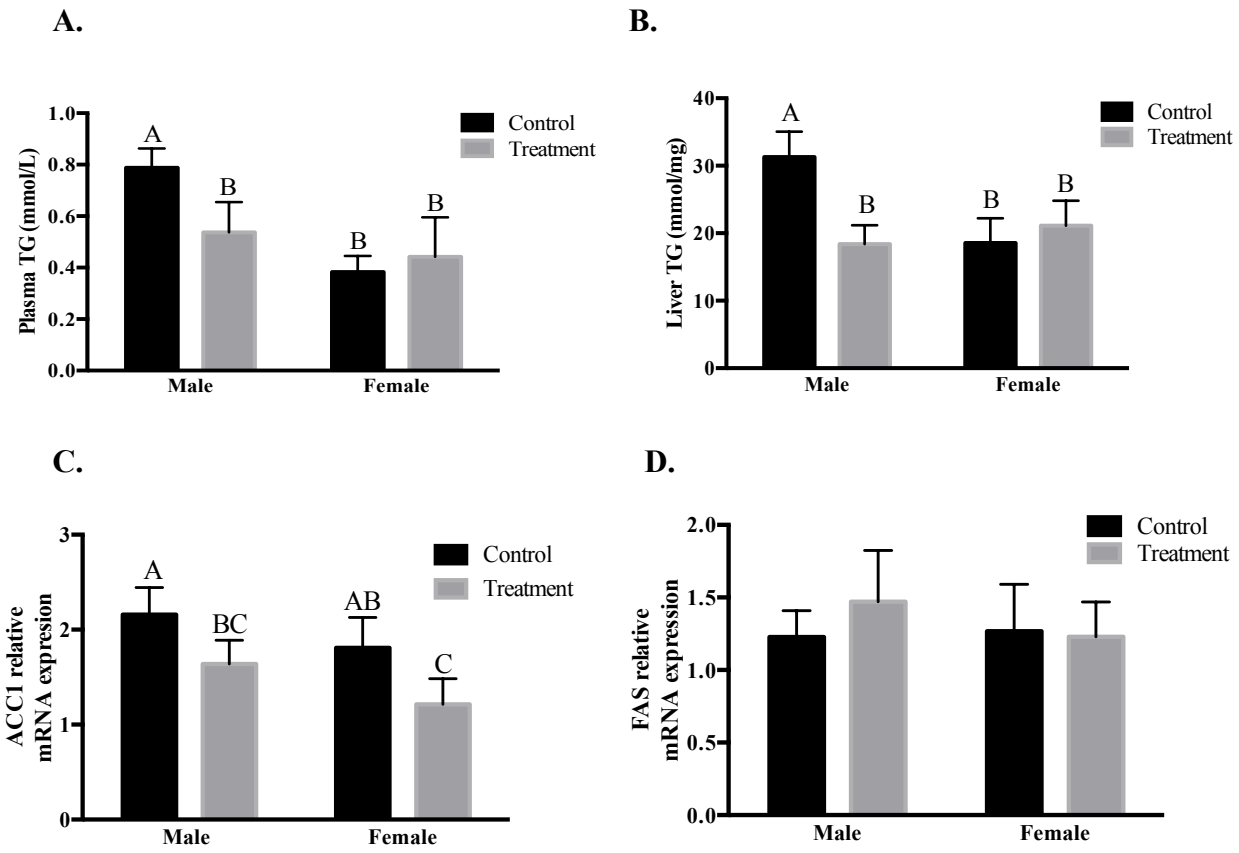


Figure 3.4: Effect of short chain fatty acids on A) plasma TG, B) hepatic TG, C) the relative mRNA expression of ACC1 and D) FAS. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. The mRNA expression data is presented as relative to β-actin. All data are expressed as mean ± SD. Different superscripts indicate significant difference among groups. $P < 0.05$ was considered significant (n=6). TG – triacylglycerols, ACC1 – acetyl-CoA carboxylase subform1, FAS – fatty acid synthase.

Adipose tissue TG concentration and the relative mRNA expression of PPARγ are given in Figure 3.5. There was no effect of SCFAs treatment on adipose tissue TG concentrations in both male or females, compared to their respective controls; however, male control group had significantly higher (p<0.05) TG, compared to female control group (Figure 3.5A). Furthermore, there was no effect of SCFAs treatment on adipose tissue mRNA expression of PPARγ in both males and females, compared to their respective controls (Figure 3.5B).

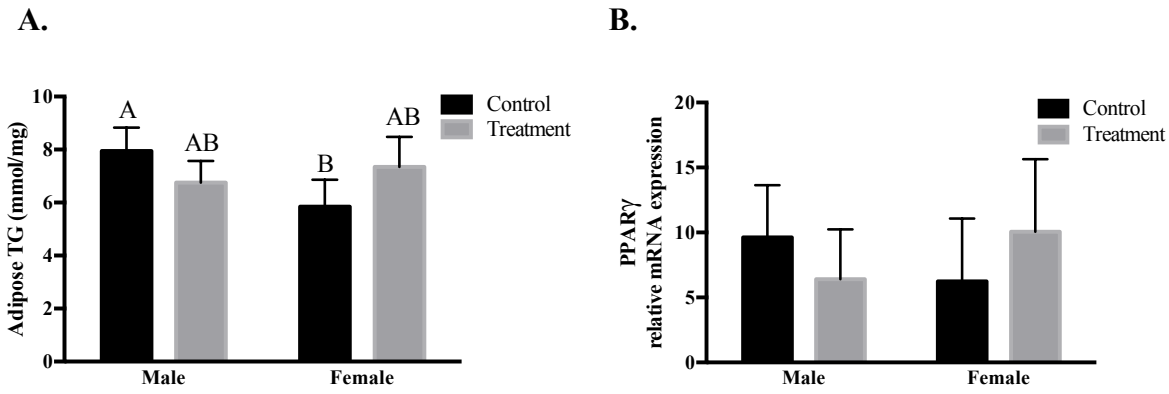


Figure 3.5: Effect of short chain fatty acids on A) adipose tissue TG, and B) the relative mRNA expression of PPARγ. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. The mRNA expression data is presented as relative to GAPDH. All data are expressed as mean ± SD. Different superscripts indicate significant difference among the groups. $P < 0.05$ was considered significant (n=6). TG – triacylglycerols, PPARγ – peroxisome proliferator-activated receptor gamma.

3.2 Effect of SCFAs on ChE, TG and phospholipids fatty acid composition

3.2.1. Effect of SCFAs on fatty acyl composition of ChE

There was no detection and/or identification of ChE species composed of SFAs (16:0 or 18:0) either in plasma or liver samples; therefore, the effect of SCFAs is only reported for the relative abundance of ChE – MUFAs, n-6 PUFAs and n-3 PUFAs.

3.2.1.1 Effect of SCFAs on ChE fatty acid composition in plasma

The observation chart and biplot obtained by PCA for plasma ChE is given in the Appendix (Figure I A and B, respectively). PCA output for plasma ChE showed a significant effect of SCFAs treatment in both males and females, compared to their respective controls. Each of the four groups (male control – MC, male treatment –MT, female control – FC, female treatment – FT) were found in different quadrants (Appendix figure I A). 20:4 ChE was the only lipid species found in the control quadrant (MC and FC). All the other plasma ChE species (ChE - 18:1, 20:4 and 22:6) were clustered in the treatment quadrants (MT and FT) (Appendix figure I B).

The effect of SCFAs on the relative abundance of plasma ChE species composed of MUFAs is given in Figure 3.6A. Males treated with SCFAs showed significantly higher (p<0.001) relative abundance of plasma 18:1 ChE, compared to its control group; however, females showed no effect of SCFAs on ChE 18:1, compared to its control group (Figure 3.6A). Males treated with SCFAs showed no effect on 20:4 ChE or 22:6 ChE in plasma, compared to their respective controls (Figure 3.6B and C, respectively). However, females treated with SCFAs showed significantly lower relative abundance of 20:4 ChE (p<0.001) (Figure 3.6B) and significantly higher abundance of 22:6 ChE (p<05) (Figure 3.6C), compared to their respective controls. Furthermore, there was

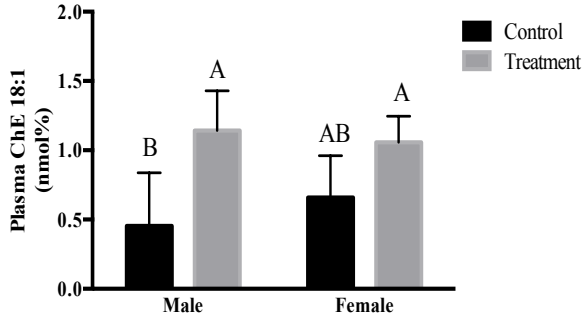
no effect of treatment or sex on 18:2 and 18:3 ChE, compared to the controls (Figure 3.6B and C, respectively).

3.2.1.2. Effect of SCFAs on hepatic ChE fatty acid composition

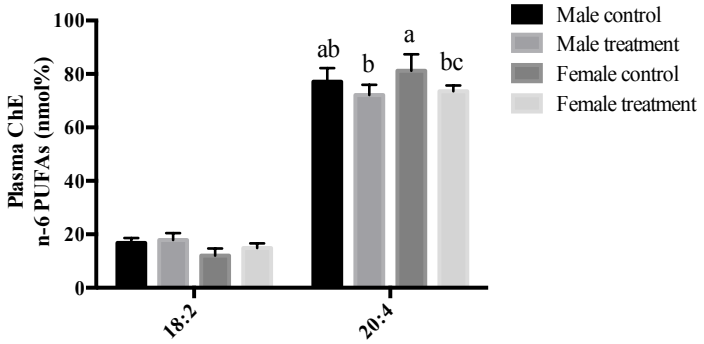
The observation chart and biplot obtained by PCA for hepatic ChE is given in the Appendix figure II A and B, respectively. PCA output for liver ChE showed a significant effect of SCFAs treatment in both males and females, compared to their respective controls. Each of the four groups were found in different quadrants (Appendix figure II A). Hepatic 18:1, -18:2, -18:3 and -20:5 ChE were clustered in the control quadrants (MC and FC), while ChE species composed of other PUFAs (20:3, 20:4, 22:4, 22:5 and 22:6) were clustered in the treatment quadrants (MT and FT) (Appendix figure II B).

The relative abundance of ChE species composed with 18:1, n-6 PUFAs and n-3 PUFAs in liver is given in Figure 3.7. There was no effect of SCFAs treatment on ChE species containing 18:1 (Figure 3.7A), n-6 PUFAs – 18:2 and 20:4 (Figure 3.7B) and n-3 PUFAs – 18:3, 20:5, 22:5 and 22:6 (Figure 3.7C) in both males and females, compared to their respective controls.

A.



B.



C.

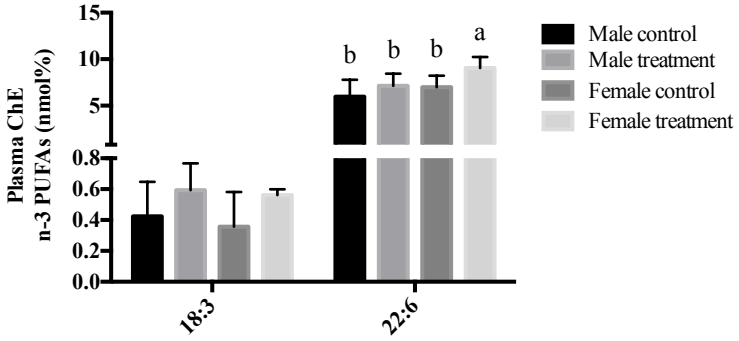
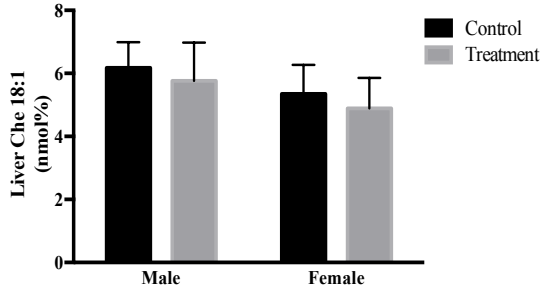
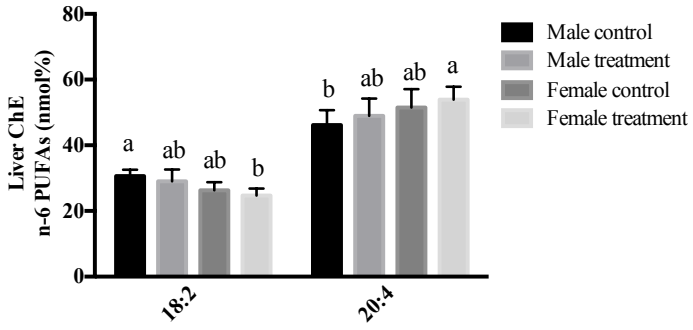


Figure 3.6: Effect of short chain fatty acids on the relative abundance of plasma ChE composed of A) MUFAs, B) n-6 PUFAs and C) n-3 PUFAs. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed in mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). ChE – cholesteryl esters, MUFAs – monounsaturated fatty acids, PUFAs – polyunsaturated fatty acids.

A.



B.



C.

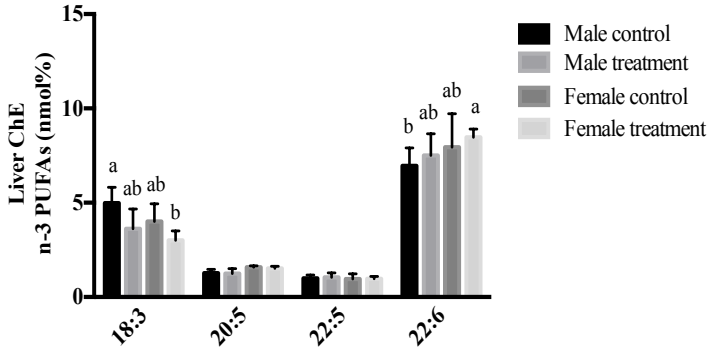


Figure 3.7: Effect of short chain fatty acids on the relative abundance of hepatic ChE composed of A) MUFA, B) n-6 PUFAs and C) n-3 PUFAs. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed in mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). ChE – cholesteryl esters, MUFAs – monounsaturated fatty acids, PUFAs – polyunsaturated fatty acids.

3.2.2. Effect of SCFAs on TG fatty acid composition

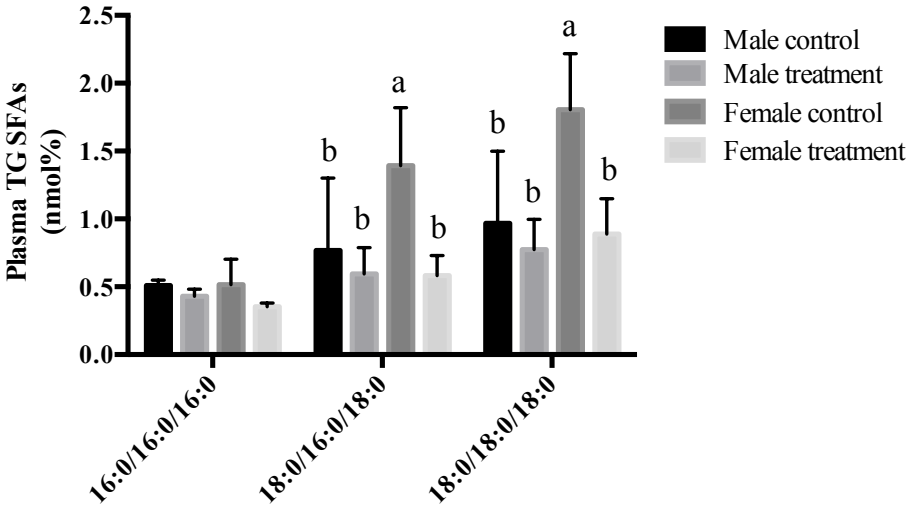
3.2.2.1 Effect of SCFAs on plasma TG fatty acid composition

The observation chart and biplot obtained by PCA for plasma TG is given in the Appendix figure III A and B, respectively. PCA output for plasma TG showed a significant effect of SCFAs treatment only in females, compared to its controls. MC, MT and FT groups were found in same PCA quadrant and the FC group was found in another PCA quadrant (Appendix figure III A). Plasma TG species 18:0/16:0/16:0, 18:0/16:0/18:0 and 18:0/18:0/18:0 were clustered in the control quadrant (FC), and all the other TG species with MUFAs and 18:2 were clustered in the other three PCA quadrants (Appendix figure III B).

The relative abundance of TG species composed with SFAs is given in Figure 3.8A. There was no effect of SCFAs treatment on plasma TG composed with SFAs and MUFAs in males, compared to its controls. However, SCFAs treated females showed significantly lower (p<0.0001) relative abundance of species composed of SFAs at all 3 *sn*-positions, TG - 16:0/16:0/16:0, -18:0/16:0/18:0 and -18:0/18:0/18:0 (Figure 3.8A), and significantly higher (p<0.05) relative abundance of TG composed of MUFA at *sn*-2 position – 16:1/18:1/18:2 (Figure 3.8B), compared to its controls.

Even though no TG species composed of n-6 PUFAs (such as 20:4) or n-3 PUFAs (such as 20:5 or 22:6) were found in either of the PCA quadrants (Appendix, Figure III B) due to physiological significance of these fatty acids, the effect of SCFAs is presented on the relative abundance of TG composed of 20:4, 20:5 and 22:6 at *sn*-2 or *sn*-3 position. There was no effect of SCFAs on plasma TG with PUFAs at *sn*-2 or *sn*-3 position (18:2, 20:4 and 22:6) in both males and females, compared to their control groups (Appendix figure IV A - D).

A.



B.

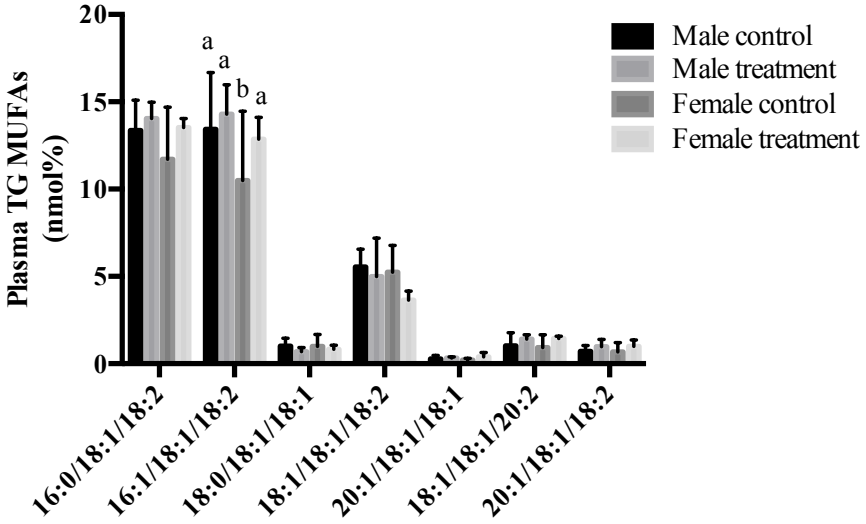


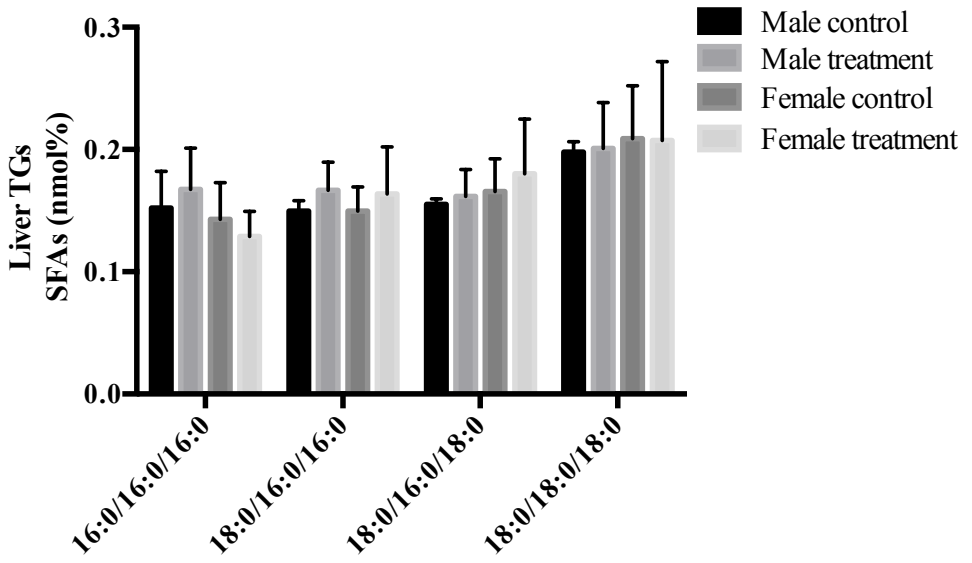
Figure 3.8: Effect of short chain fatty acids on the relative abundance of plasma TG composed of A) SFAs and B) MUFAs at *sn*-2 position. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed as mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). TG – triacylglycerol, SFAs – saturated fatty acids, MUFAs – monounsaturated fatty acids.

3.2.2.1. Effect of SCFAs on hepatic TG fatty acid composition

The observation chart and biplot obtained by PCA for hepatic TG is given in the Appendix (Figure V A and B, respectively). PCA output for hepatic TG showed a significant effect of SCFAs treatment in males, compared to its control group; MC and MT groups were found in different PCA quadrants (Appendix figure V A). However, PCA output showed no significant differences between female control and treatment groups as FC and FT groups were found in the same PCA quadrant (Appendix figure V A). Plasma TG species composed with 18:2 were clustered in the control quadrant, while TG species composed of SFAs and MUFAs were clustered in the treatment quadrant (MT) (Appendix figure V B). No species were found in the FC-FT quadrant.

The relative abundance of TG species composed with SFAs and MUFAs is given in Figure 3.9A and B, respectively. There was no effect of SCFAs treatment on plasma TG composed with SFAs in both males and females, compared to their respective controls (Figure 3.9A). Moreover, there was no effect of SCFAs on hepatic TG with MUFAs (Figure 3.9B), n-6 and n-3 PUFAs at *sn*-2 or *sn*-3 position (Appendix figure VI).

A.



B.

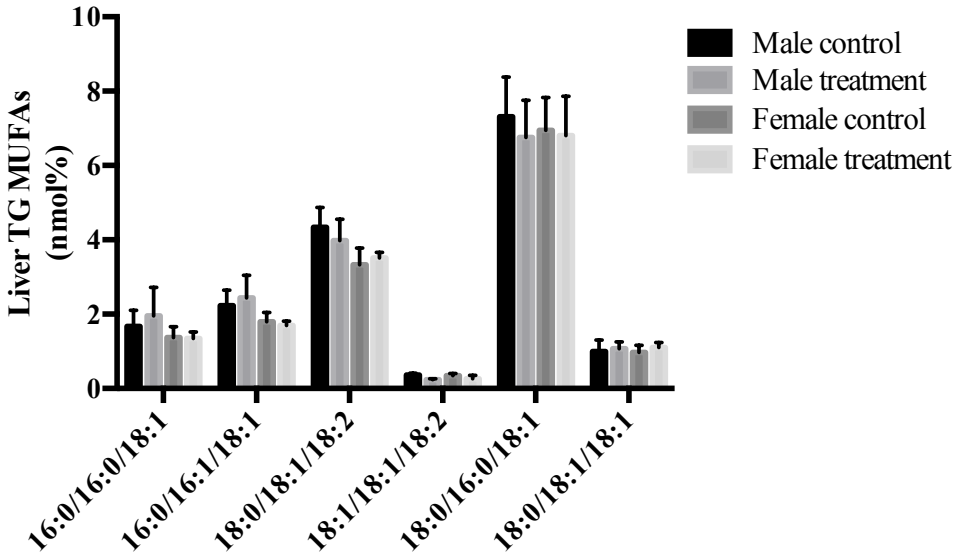


Figure 3.9: Effect of short chain fatty acids on the relative abundance of hepatic TG composed of A) SFAs and B) MUFAs. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed as mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). TG – triacylglycerols, SFAs – saturated fatty acids, MUFAs – monounsaturated fatty acids.

3.2.3. Effect of SCFAs on PC fatty acid composition

3.2.3.1. Effect of SCFAs on plasma PC fatty acid composition

The relative abundance of total diacylated, plasmalogen and ether-linked PC species is given in Figure 3.10. SCFAs treatment significantly increased (p<0.0001) relative abundance of PC diacylated species in both males and females, compared to their respective controls. However, there was no effect of SCFAs treatment on relative abundance of plasma PlsPC and PC-ether species, in both males and females, compared to their respective controls.

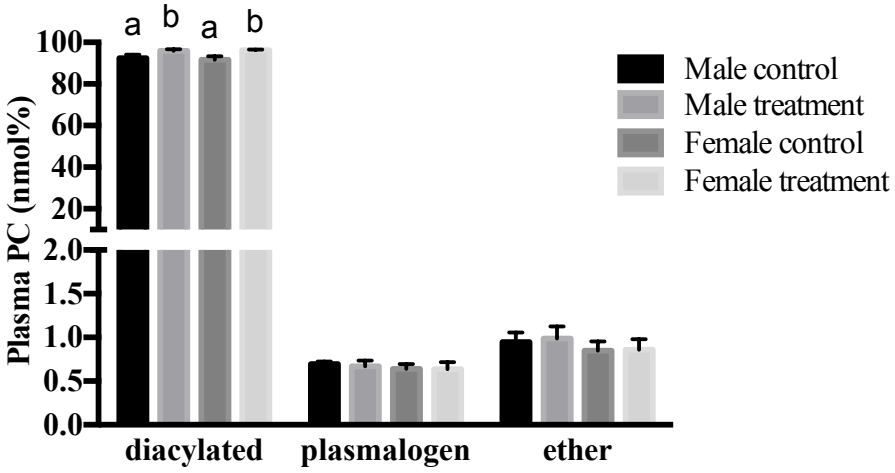


Figure 3.10: Effect of short chain fatty acids on relative abundance of plasma total PC species. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed as mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). PC – phosphatidylcholine.

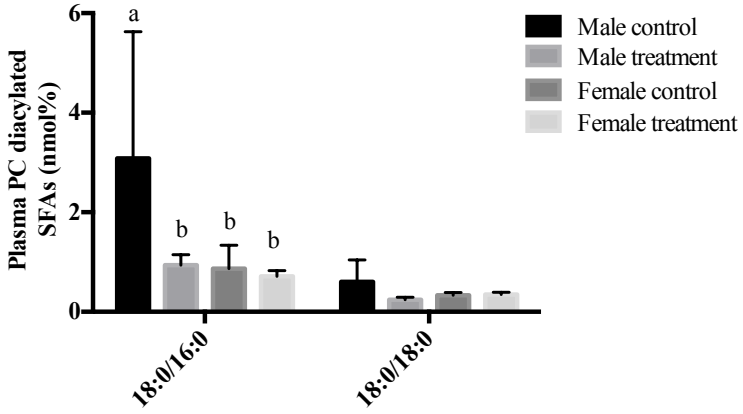
There were very few PlsPC (36:4p and 38:4p) and ether-linked PC (32:0e, 34:1e, 36:4e and 38:4e) species detected in plasma, contributing no more than 2% of total PC (Figure 3.10). Furthermore, there was no effect of SCFAs on plasmalogen- and ether-linked PC species (data not shown). PCA was carried out on plasma PC-diacylated species, which contributed ~98% of total PC. The observation chart and biplot obtained by PCA for plasma PC-diacylated species is given in Appendix (Figure VII A and B, respectively).

PCA output for plasma PC showed a significant effect of SCFAs treatment on both males and females, compared to their respective controls. Each of the four groups (MC, MT, FC and FT) were found in different quadrants (Appendix figure VII A). PC species with 16:1 and 18:1 were clustered in the control quadrant (MC), while PC species composed of n-6 PUFAs (18:2 and 20:4) and n-3 PUFAs (22:5 and 22:6) were clustered in the treatment quadrants (MT and FT), respectively (Appendix figure VII B).

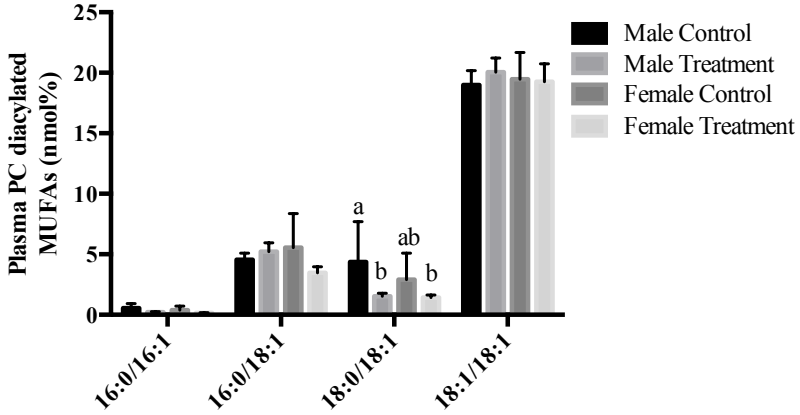
The effect of SCFAs on the relative abundance of SFAs and MUFAs species in plasma PC is given in Figure 3.11A and B, respectively. Males treated with SCFAs showed a significant decrease ($p<0.05$) in the relative abundance of 18:0/16:0 PC (Figure 3.11A) and 18:0/18:1 PC (Figure 3.11B), compared to their control; however, females showed no effect of SCFAs on PC-18:0/16:0 (Figure 16A) or 18:0/18:1 PC (Figure 3.11B), compared to their control. There was no effect of treatment on the relative abundance of 18:0/18:0 PC (Figure 3.11A) as well as 16:0/16:1, 16:0/18:1 and 18:1/18:1 PC (Figure 3.11B) in both males and females, compared to their respective controls.

The effect of SCFAs on the relative abundance of n-6 and n-3 PUFAs species in plasma PC is given in Figure 3.11C and D, respectively. Males treated with SCFAs showed a significant increase (p<0.01) in the relative abundance of 16:0/18:2 PC but there was no effect on 16:0/20:4 or -18:0/20:4 PC (Figure 3.11C) abundance, compared to its controls. On the other hand, SCFAs treated females showed no effect of SCFAs on either of the n-6 PUFA species. Moreover, males treated with SCFAs also showed a significant increase (p<0.01) in the relative abundance of 16:0/22:6, 18:0/22:5 PC and 18:0/22:6 PC (Figure 3.11D), compared to its controls. However, females treated with SCFAs only showed a significant increase (p<0.01) in the relative abundance of 18:0/22:6 PC but no effect of SCFAs on 16:0/22:6 and 18:0/22:5 PC, compared to its control group (Figure 3.11D). Furthermore, there was significantly lower (p<0.0001) relative abundance of 18:0/20:4 PC in male control and treatment groups, compared to female control and treatment groups, respectively (3.11C).

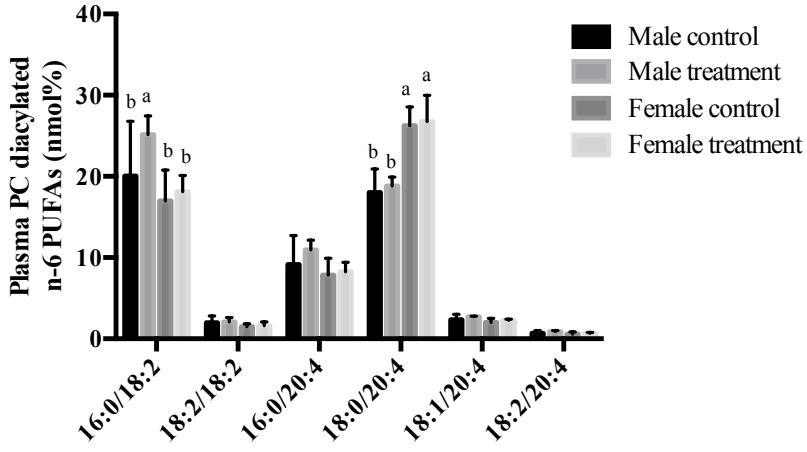
A.


Plasma PC diacylated SFAs (nmol%)
6
4
2
0
a
b
b
b
18:0/16:0
18:0/18:0
Male control
Male treatment
Female control
Female treatment

B.


Plasma PC diacylated MUFAs (nmol%)
25
20
15
10
5
0
a
b
ab
b
16:0/16:1
16:0/18:1
18:0/18:1
18:1/18:1
Male Control
Male Treatment
Female Control
Female Treatment

C.


Plasma PC diacylated n-6 PUFAs (nmol%)
40
30
20
10
0
b a
b b
b b
a a
16:0/18:2
18:2/18:2
16:0/20:4
18:0/20:4
18:1/20:4
18:2/20:4
Male control
Male treatment
Female control
Female treatment

D.

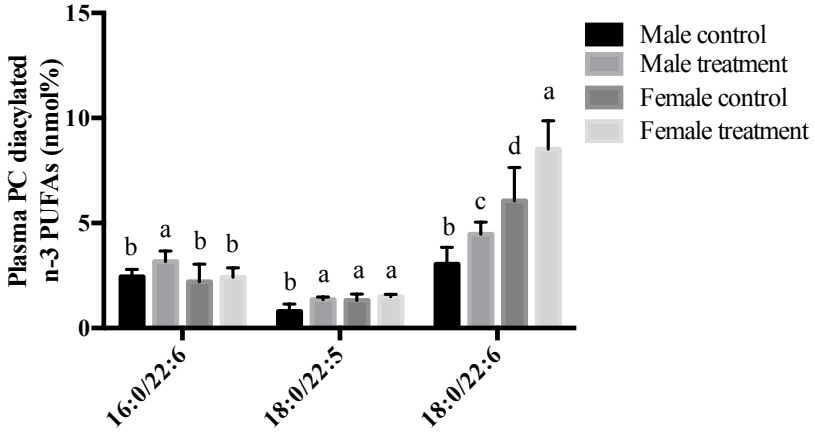


Figure 3.11: Effect of short chain fatty acids on relative abundance of plasma PC-diacylated composed of A) SFAs, B) MUFAs, C) n-6 PUFAs and D) n-3 PUFAs. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed as mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). PC – phosphatidylcholine, SFAs – saturated fatty acids, MUFAs – monounsaturated fatty acids, PUFAs – polyunsaturated fatty acids.

3.2.3.2. Effect of SCFAs on hepatic PC fatty acid composition

The relative abundance of total diacylated and ether-linked lipid species in hepatic PC is given in Figure 3.12. There was no effect of SCFAs treatment on the relative abundance of hepatic PC-diacylated and ether-linked lipid species, in both males and females, compared to their respective controls (Figure 3.12). Unlike plasma, no PlsPC species were detected in liver samples.

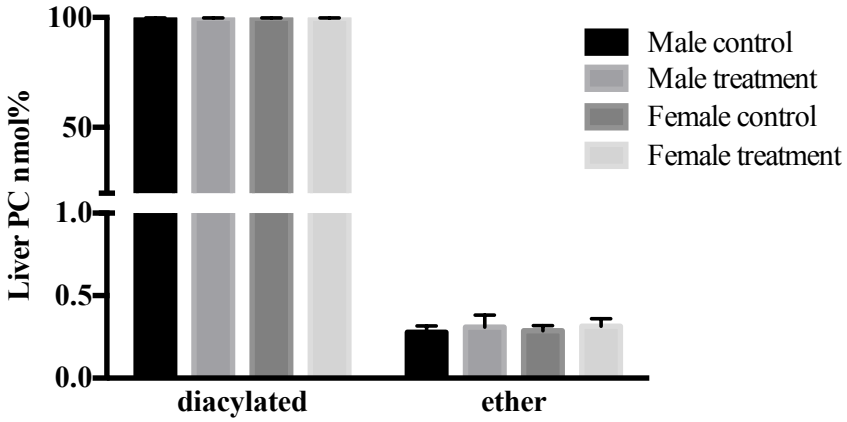
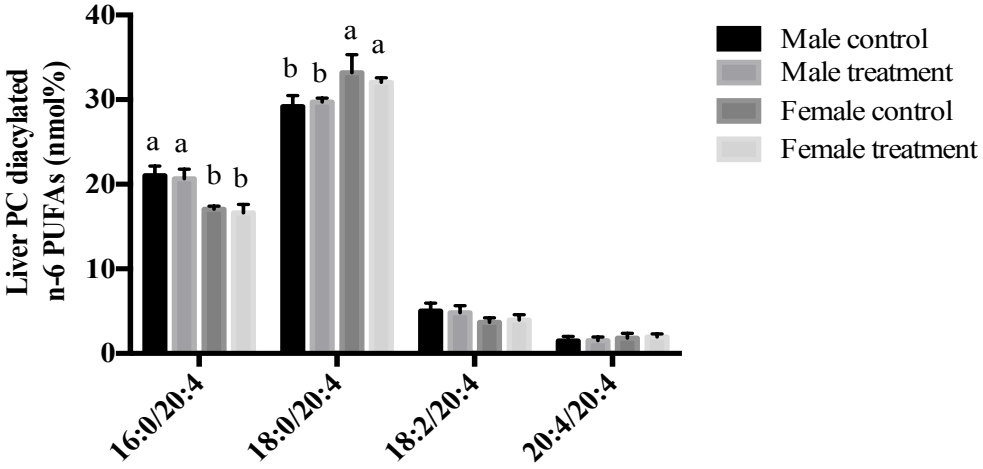


Figure 3.12: Effect of short chain fatty acids on the relative abundance of hepatic total PC species. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed as mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). PC – phosphatidylcholine.

Very few ether-linked PC species (16:0e/16:0 and 16:0e/18:0) were detected in the liver samples, which contributed <0.5% of total PC abundance (Figure 3.12); there was no effect of SCFAs on these PC species (data not shown). Therefore; the PCA output was carried out only on hepatic PC-diacylated species. The observation chart and biplot obtained by PCA for hepatic PC is given in the Appendix figure VIIIA and B, respectively. PCA output for hepatic PC showed no significant effect of SCFAs treatment on males, compared to its control group; MC and MT groups were found in the same PCA quadrant (Appendix figure VIII A). However, the PCA output revealed differences between female control and treatment groups; FC and FT were found in different quadrants (Appendix figure VIII A) Furthermore, PC species composed of n-6 PUFAs were clustered in the MC-MT quadrant, while PC species composed of n-3 PUFAs were clustered in the FC quadrant (Appendix figure VIII B).

PC species fragmented to SFAs and MUFAs were not identified in the liver samples. The effect of SCFAs on the relative abundance of n-6 PUFAs and n-3 PUFAs species in hepatic PC is given in Figure 3.13A and B, respectively. Male control and treatment groups showed significantly higher (p<0.0001) relative abundance of hepatic 16:0/20:4 PC, compared to female control and treatment groups (Figure 3.13A). On the other hand, male control and treatment groups had lower (p<0.0001) abundance of 18:0/20:4 PC (Figure 3.13A), compared to female groups. There was no effect of treatment or sex on the relative abundance of liver 18:2/20:4 and 20:4/20:4 PC (Figure 3.13A). Furthermore, male groups (both control and treatment) had lower (p<0.0001) abundance of 18:0/22:6 PC compared to both female groups but there was no effect of SCFAs on 16:0/22:6 and -18:0/22:5 PC (Figure 3.13B)

A.



B.

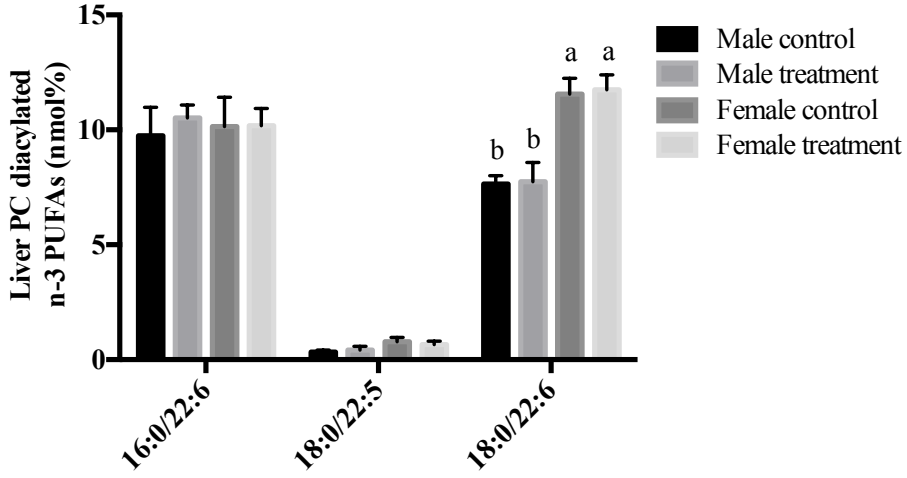


Figure 3.13: Effect of short chain fatty acids on the relative abundance of hepatic PC-diacylated composed of A) n-6 PUFAs and B) n-3 PUFAs. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed as mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). PC – phosphatidylcholine, PUFAs – polyunsaturated fatty acids.

3.2.3.3. Effect of SCFAs on plasma LPC fatty acid composition

The observation chart and biplot obtained by PCA for plasma LPC species is given in the Appendix (Figure IX A and B, respectively). PCA output for plasma LPC showed a significant effect of SCFAs treatment in both males and females, compared to their respective controls. MC and FC groups were found in the same PCA quadrant, while MT and FT were found in two different PCA quadrants (Appendix, Figure IX A). All plasma LPC species were clustered in the treatment quadrants (MT and FT), except for 22:5 and 20:2 LPC that were clustered in the control quadrant (MC-FC) (Appendix, Figure IX B).

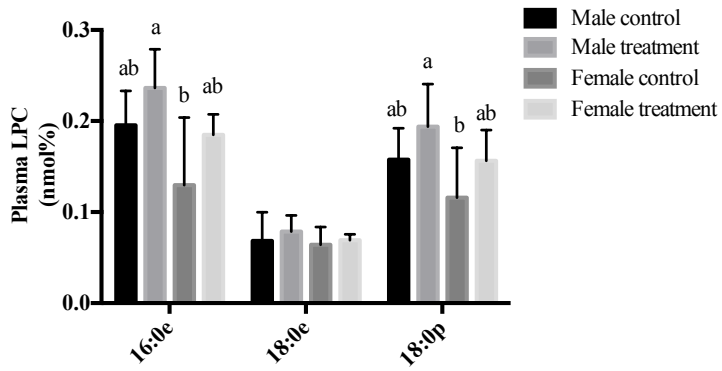
The relative abundance of plasma LPC composed of ether- and plasmalogen-linked SFAs is given in Figure 3.14A. There was no effect of SCFAs treatment on plasma 16:0e, 18:0e or 18:0p LPC (Figure 3.14A) on either males or females, compared to their respective control groups. However, there was significantly higher (p<0.001) relative abundance of plasma 16:0e and 18:0p LPC in males treated with SCFAs, compared to their respective female control groups (Figure 3.14A).

The effect of SCFAs on the relative abundance of plasma LPC composed of SFAs and MUFAs is given in Figure 3.14B and C, respectively. SCFAs treatment had no effect on plasma LPC species composed with SFAs (16:0, 18:0 and 20:0 LPC) in either males or females, compared to their respective control groups (Figure 3.14B). However, there was significantly lower (p<0.0001) relative abundance of plasma 18:0 LPC, in male control and treatment groups, compared to female control and treatment groups (Figure 3.14B). Furthermore, there was no effect of SCFAs on plasma LPC species composed with MUFAs (16:1, -18:1 or -20:1 LPC) in both males

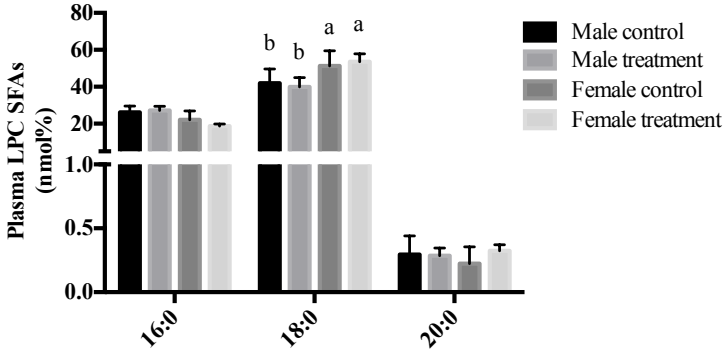
and females, compared to their respective controls (Figure 3.14C). However, male control and treatment groups showed significantly higher (p<0.0001) relative abundance of plasma 18:1 LPC, compared to female control and treatment groups (Figure 3.14C).

The effect of SCFAs on the relative abundance of plasma LPC composed of n-6 PUFAs and n-3 PUFAs is given in Figure 3.14D and E, respectively. There was no effect of SCFAs treatment on the relative abundance of plasma LPC n-6 PUFAs (18:2, -20:3 and -20:4 LPC) or n-3 PUFAs (22:5 and -22:6 LPC) in either males or females; compared to their respective controls (Figure 3.14D and E, respectively).

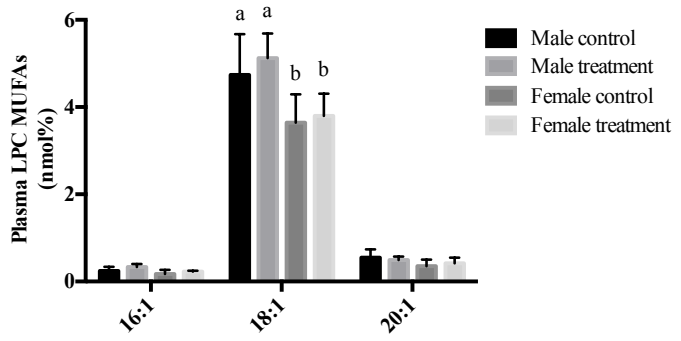
A.



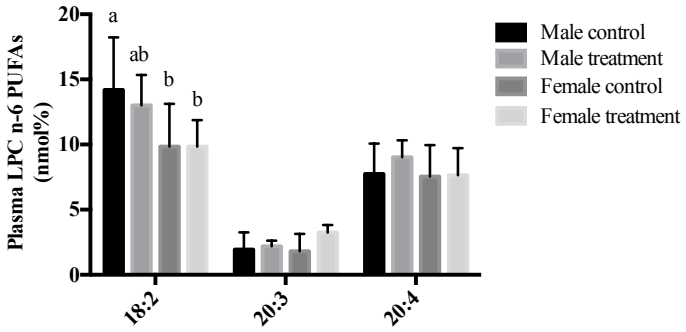
B.



C.



D.



E.

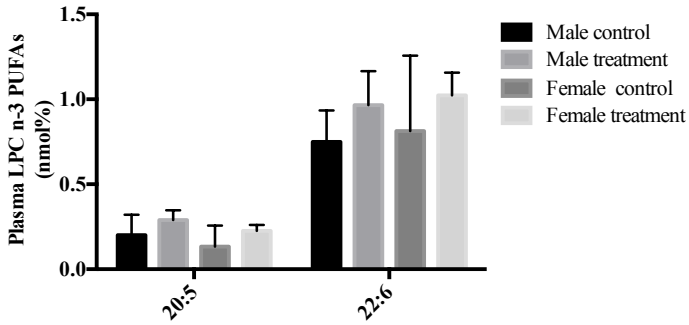


Figure 3.14: Effect of short chain fatty acids on the relative abundance of plasma LPC composed of A) plasmalogen- and ether-linked SFAs, B) SFAs without linkage, C) MUFAs, D) n-6 PUFAs, and E) n-3 PUFAs. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed as mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). LPC – lyso phosphatidylcholine, SFAs – saturated fatty acids, MUFAs – monounsaturated fatty acids, PUFAs – polyunsaturated fatty acids.

3.2.3.4. Effect of SCFAs on hepatic LPC fatty acid composition

The observation chart and biplot obtained by PCA for hepatic LPC is given in the Appendix (Figure X A and B, respectively). PCA output showed a significant effect of SCFAs treatment in both males and females, compared to their respective control groups. Each of the four groups were found in different quadrants (Appendix figure X A). LPC species with SFAs (16:0, 20:0 and 22:0), n-6 PUFAs (18:2, 20:3 and 20:4) and n-3 PUFAs (22:5 and 22:6) were clustered in the control quadrants (MC and FC), while LPC species with MUFAs (18:1 and 20:1), plasmalogen- (18:0p) and ether-linked LPC (16:0e and 18:0e) were clustered in the treatment quadrants (MT and FT) (Appendix figure X B).

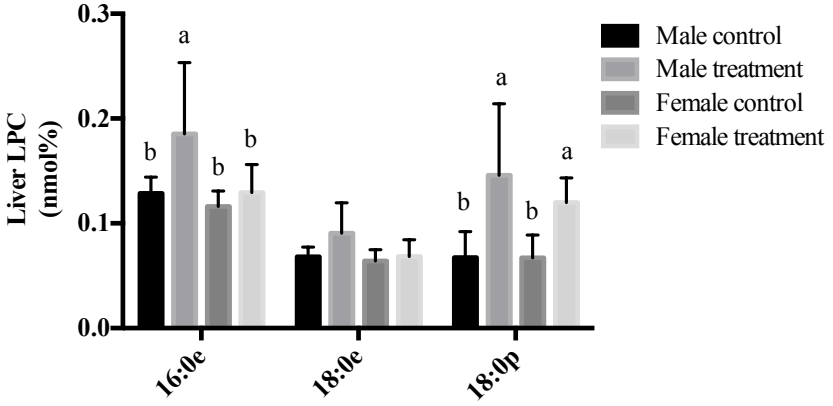
The relative abundance of ether- and plasmalogen- linked SFAs in hepatic LPC is given in Figure 3.15A. Males treated with SCFAs showed significantly higher (p<0.0001) relative abundance of hepatic 16:0e and -18:0p LPC, compared to its respective controls (Figure 3.15A). However, females treated with SCFAs showed significantly higher (p<0.0001) relative abundance of 18:0p LPC, and there was no effect on 16:0e LPC, compared to its control (Figure 3.15A).

The relative abundance of hepatic LPC composed with SFAs and MUFAs is given in Figure 3.15B and C, respectively. There was no effect of SCFAs treatment on hepatic LPC composed of SFAs (16:0, 18:0, 20:0 and 22:0 LPC) in males, compared to its respective control group (Figure 3.15B). However, there was significantly lower (p<0.05) relative abundance of hepatic – 16:0, in female treatment group, compared to its control group (Figure 3.15B). Moreover, there was significantly lower (p<0.05) relative abundance of 18:0 LPC in male control and treatment groups compared to female control and treatment groups (Figure 3.15B). Males treated

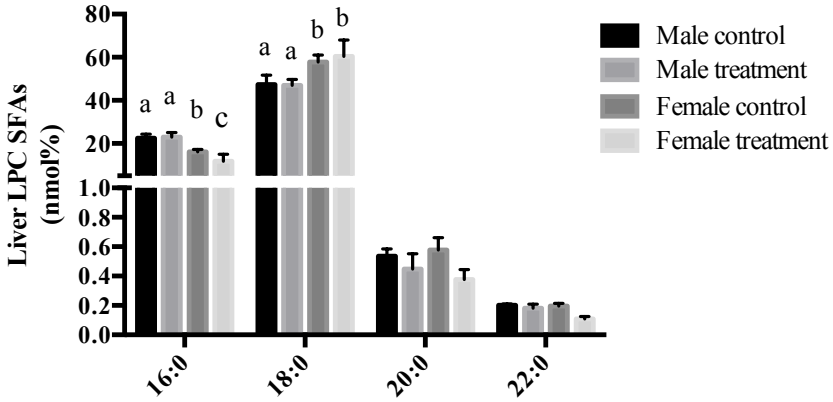
with SCFAs showed significantly higher (p<0.0001) relative abundance of hepatic 18:1 LPC, while there was no effect on 20:1 LPC, compared to its control group. On the other hand, females treated with SCFAs showed no effect on 18:1 or 20:1 LPC, compared to its control (Figure 3.15C).

The relative abundance of hepatic LPC composed of n-6 PUFAs and n-3 PUFAs is given in Figure 3.15D and E, respectively. There was no effect of SCFAs treatment on hepatic LPC with n-6 PUFAs (18:2, 20:3 and 20:4 LPC) in males, compared to its control. However, females treated with SCFAs showed significantly higher (p<0.01) abundance of 20:4 LPC, and there was no effect of treatment on 18:2 and 20:3 LPC, compared to its control (Figure 3.15D). There was no effect of treatment on n-3 PUFAs (22:5 and 22:6 LPC) in both males and females, compared to their respective controls (Figure 3.15E). However, there was significantly lower (p<0.01) relative abundance of 22:6 LPC in male control and treatment groups, compared to female control and treatment groups (Figure 3.15E).

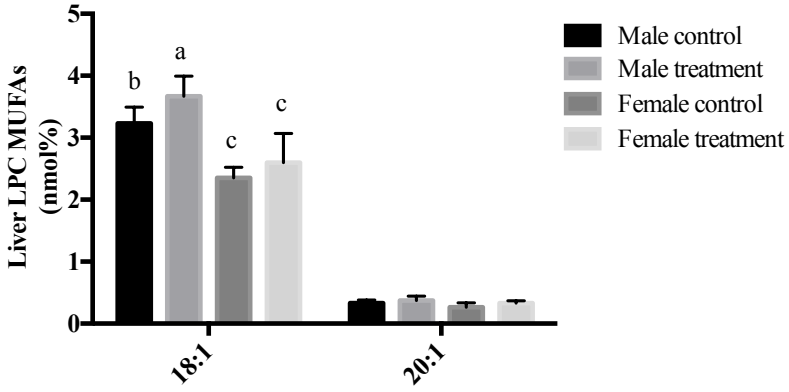
A.


Liver LPC (nmol%)
0.3
0.2
0.1
0.0
a
b
b
b
a
b
b
a
16:0e
18:0e
18:0p
Male control
Male treatment
Female control
Female treatment

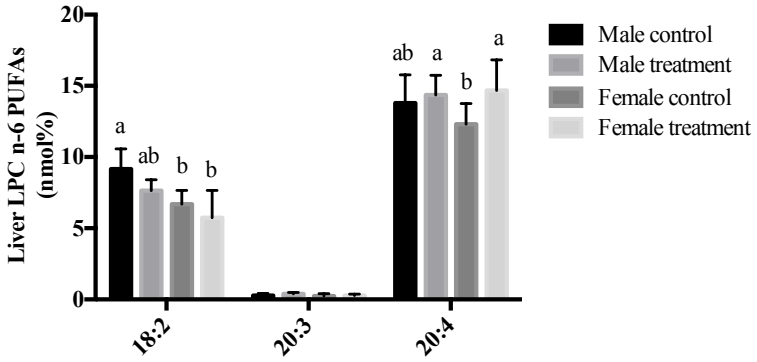
B.


Liver LPC SFAs (nmol%)
80
60
40
20
1.0
0.8
0.6
0.4
0.2
0.0
a
a
b
c
a
a
b
b
16:0
18:0
20:0
22:0
Male control
Male treatment
Female control
Female treatment

C.


Liver LPC MUFAs (nmol%)
5
4
3
2
1
0
b
a
c
c
18:1
20:1
Male control
Male treatment
Female control
Female treatment

D.



E.

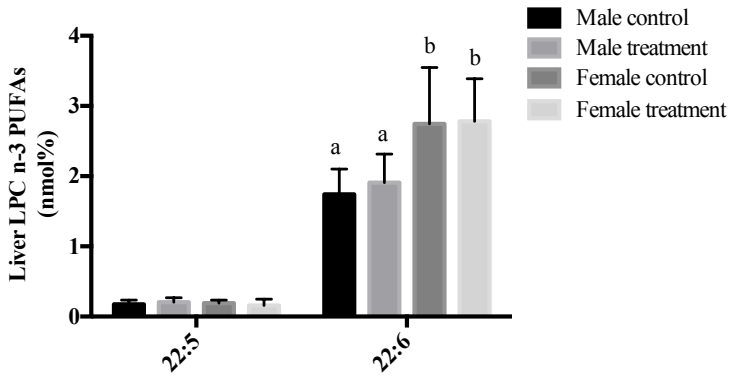


Figure 3.15: Effect of short chain fatty acids on hepatic LPC composed of A) ether- and plasmalogen-linked LPC, B) SFAs, C) MUFAs, D) n-6 PUFAs and E) n-3 PUFAs. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed as mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). LPC – lyso phosphatidylcholine, SFAs – saturated fatty acids, MUFAs – monounsaturated fatty acids, PUFAs – polyunsaturated fatty acids.

3.2.4. Effect of SCFAs on PE fatty acid composition

3.2.4.1. Effect of SCFAs on plasma PE fatty acid composition

The relative abundance of plasma total PE-diacylated, PlsPE and PE-ether species is given in Figure 3.16. Males and females treated with SCFAs showed significantly higher (p<0.0001) relative abundance of PE-diacylated species, compared to their respective controls (Figure 3.16). There was a significant decrease (p<0.0001) in relative abundance of total PlsPE in both males and females, compared to their respective control groups. However, there was no effect of SCFAs or sex on ether-linked PE species (Figure 3.16).

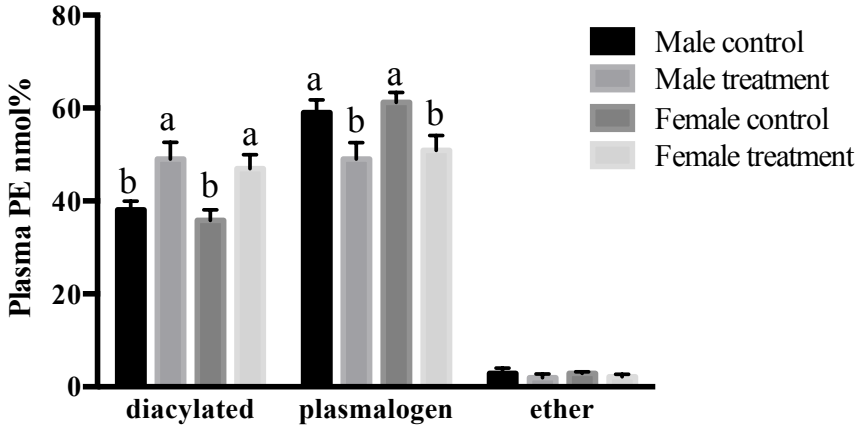


Figure 3.16: Effect of short chain fatty acids on the relative abundance of plasma total PE species. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed as mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). PE – phosphatidylethanolamine.

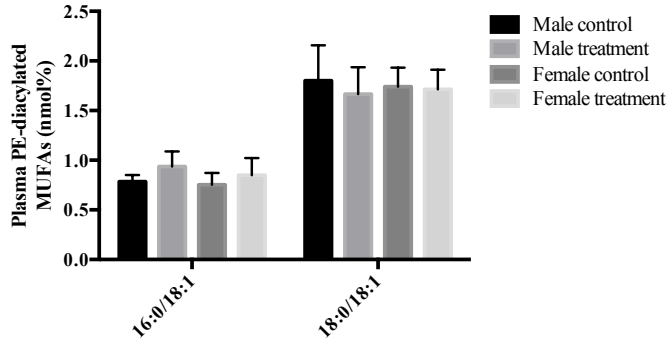
3.2.4.1.1. Effect of SCFAs on plasma PE-diacylated fatty acid composition

The observation chart and biplot obtained by PCA for plasma PE-diacylated species is given in Appendix (Figure XI A and B, respectively). PCA output for plasma PE-diacylated showed a significant effect of SCFAs treatment in both males and females, compared to their respective controls. MC and FC groups were found in same PCA quadrant, while MT and FT groups were found in a different PCA quadrant (Appendix figure XI A). No species were clustered in control quadrant, while PE-diacylated species composed with PUFAs (18:2, 20:4, 22:4 and 22:6) were clustered in the treatment quadrant (Appendix figure XI B).

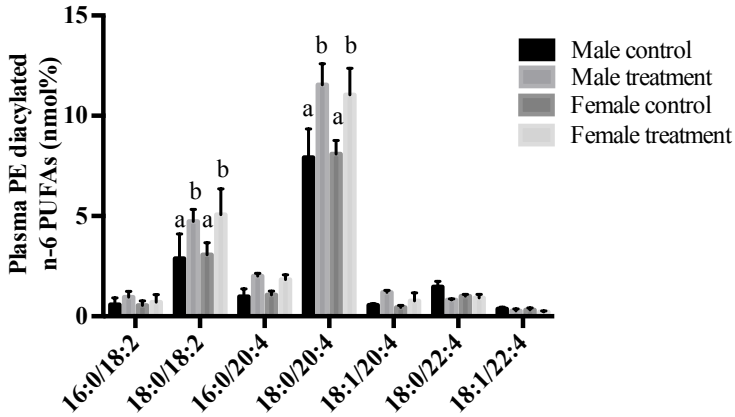
PE-diacylated species composed of SFAs were not detected in the plasma samples. The effect of SCFAs on the relative abundance of PE-diacylated species composed of MUFAs is given in Figure 3.17A. There was no effect of SCFAs treatment or sex on the relative abundance of plasma PE-diacylated species composed of MUFAs (16:0/18:1 and 18:0/18:1 PE) in both males and females, compared to their respective controls (Figure 3.17A).

The effect of SCFAs on the relative abundance of plasma PE-diacylated n-6 PUFAs and n-3 PUFAs is given in Figure 3.17B and C, respectively. SCFAs treatment showed a significant increase ($p<0.01$) in the relative abundance of n-6 PUFAs (18:0/18:2 and 18:0/20:4 PE), in both males and females; compared to their respective controls (Figure 3.17B). Males treated with SCFAs showed significantly higher ($p<0.01$) relative abundance of diacylated 16:0/22:6 PE, while there was no effect on 18:0/22:6 PE, compared to its control group. On the other hand, females treated with SCFAs showed no effect on n-3 PUFAs (16:0/22:6 or 18:0/22:6 PE), compared to their controls (Figure 3.17C).

A.



B.



C.

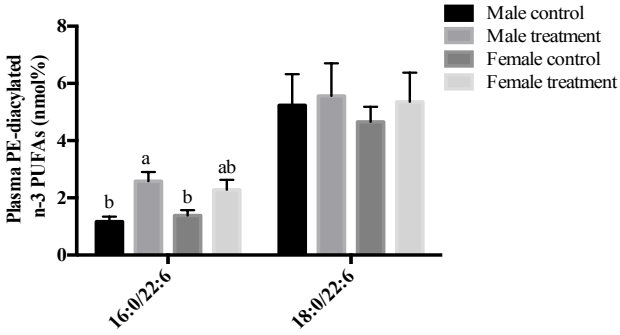


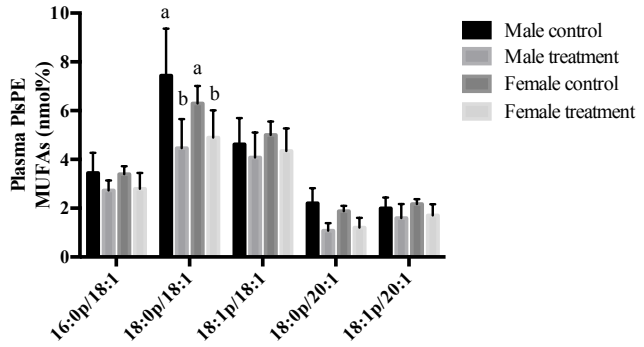
Figure 3.17: Effect of short chain fatty acids on the relative abundance of plasma PE-diacylated composed of A) MUFAs, B) n-6 PUFAs and C) n-3 PUFAs. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed as mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). PE – phosphatidylethanolamine, MUFAs – monounsaturated fatty acids, PUFAs – polyunsaturated fatty acids.

3.2.4.1.2. Effect of SCFAs on plasma PE-plasmalogen fatty acid composition

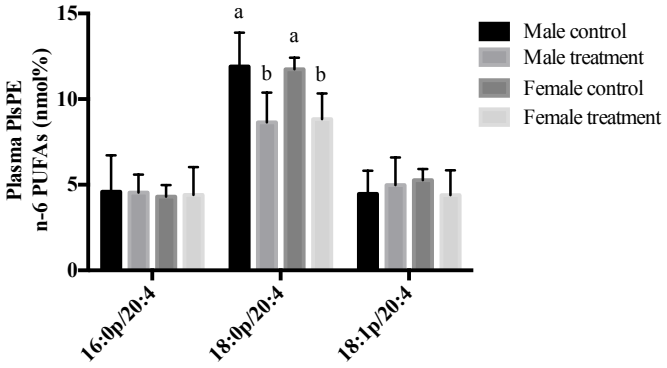
The observation chart and biplot obtained by PCA for plasma PlsPE is given in the Appendix (Figure XII A and B, respectively). PCA output for plasma PlsPE showed a significant effect of SCFAs treatment on both males and females, compared to their respective controls. MC and FC groups were found in the same PCA quadrant, while MT and FT groups were found in different PCA quadrants (Appendix figure XII A). PlsPE species with MUFAs were clustered in the control quadrant, while PlsPE species composed with 22:5 were in the treatment quadrant (Appendix figure XII B).

PlsPE with 20:4 and 22:6 were clustered in other PCA quadrants (Appendix figure XII B). PlsPE species with SFAs were not detected in plasma samples. The effect of SCFAs on the relative abundance of plasma PlsPE composed of MUFAs is given in Figure 3.18A. Males and females treated with SCFAs showed significantly lower ($p< 0.0001$) relative abundance of 18:0p/18:1 PE, compared to their respective controls (Figure 3.18A). However, there was no effect of SCFAs treatment or sex on the relative abundance of other plasma PlsPE species composed of MUFAs (16:0p/18:1, 18:1/p/18:1, 18:0p/20:1 and 18:1p/20:1 PE) in both males and females, compared to their respective controls (Figure 3.18A).

A.



B.



C.

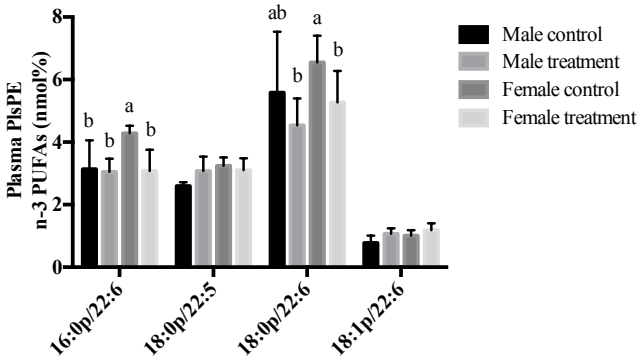


Figure 3.18: Effect of short chain fatty acids on the relative abundance of plasma PlsPE species composed of A) MUFAs, B) n-6 PUFAs and C) n-3 PUFAs. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed as mean ± SD. Different superscripts indicate a significant difference amongst groups. $P < 0.05$ was considered significant (n=6). PlsPE – phosphatidylethanolamine plasmalogen, PlsPE – plasmalogen PE, MUFAs – monounsaturated fatty acids, PUFAs – polyunsaturated fatty acids.

The effect of SCFAs on the relative abundance of plasma PlsPE composed of n-6 PUFAs and n-3 PUFAs is given in 3.18B and C, respectively. Males and females treated with SCFAs showed significantly lower (p<0.05) relative abundance of 18:0p/20:4 PE, while there was no effect on 16:0p/20:4 and 18:1p/20:4 PE, compared to their respective controls (Figure 3.18B). Furthermore, males treated with SCFAs showed no effect on PlsPE n-3 PUFAs (16:0p/22:6, 18:0p/22:5, 18:0p/22:6 and 18:1p/22:6), compared to their respective controls (Figure 3.18C). On the other hand, females treated with SCFAs showed significantly lower (p<0.001) relative abundance of 16:0p/22:6 and -18:0p/22:6 PE, while there was no effect on 18:0p/22:5 and 18:1p/22:6, compared to its control (Figure 3.18C).

3.2.4.2. Effect of SCFAs on hepatic PE fatty acid composition

There was no detection of ether-linked PlsPE in liver samples. The relative abundance of total hepatic PE-diacylated and PE-plasmalogen (PlsPE) species is given in Figure 3.19. Males treated with SCFAs showed no effect on total PE-diacylated or PlsPE, compared to its respective controls. However, females treated with SCFAs showed a significantly higher (p<0.05) relative abundance of PE-diacylated species, and lower (p<0.05) relative abundance of PlsPE, compared to their respective controls (Figure 3.19).

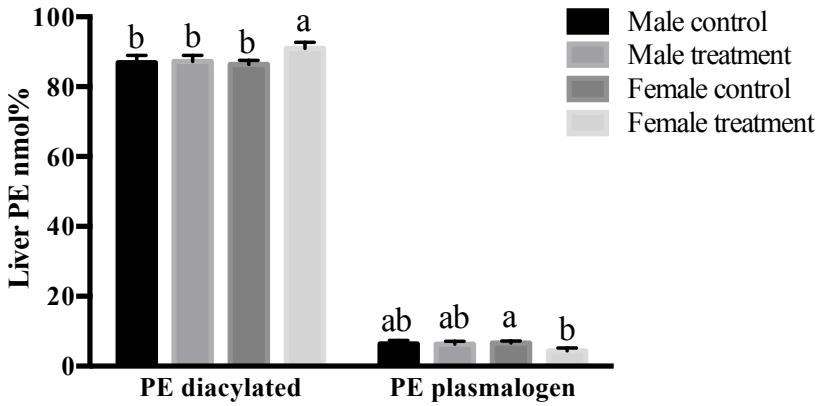


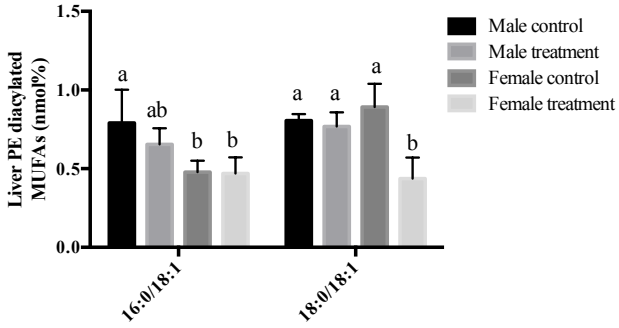
Figure 3.19: Effect of short chain fatty acids on the relative abundance of hepatic total PE species. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed as mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). PE – phosphatidylethanolamine.

3.2.4.2.1. Effect of SCFAs on hepatic PE-diacylated fatty acid composition

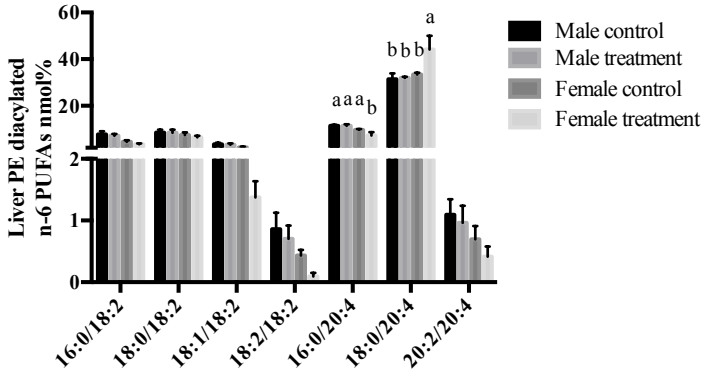
The observation chart and biplot obtained by PCA for hepatic PE is given in the Appendix (Figure XIII A and B, respectively). PCA output for hepatic PE-diacylated showed no significant effect of SCFAs treatment on males, compared to their respective controls as MC and MT groups were found in the same PCA quadrant (Appendix figure XIII A). However, the PCA output revealed differences between female control and treatment groups as FC and FT groups were found in different quadrants (Appendix figure XIII A). Furthermore, hepatic PE-diacylated species with 18:2 were clustered in MC-MT quadrant, while PE-diacylated species with other PUFAs (20:4, 22:5 and 22:6) were clustered in other PCA quadrants (Appendix figure XIII B).

The PE-diacylated composed of SFAs was not detected in liver samples. The effect of SCFAs on the relative abundance of hepatic PE-diacylated MUFAs is given in Figure 3.20A. Males treated with SCFAs showed no effect of treatment on the relative abundance of PE-diacylated MUFA; however, females treated with SCFAs showed a significant decrease in the relative abundance of hepatic diacylated 18:0/18:1 PE ($p<0.0001$), compared to its control group (Figure 3.20A). The effect of SCFAs on the relative abundance of n-6 PUFAs and n-3 PUFAs is given in Figure 3.20B and C, respectively. Males treated with SCFAs showed no effect of treatment on the relative abundance of PE-diacylated PUFAs (both n-6 and n-3); however, females treated with SCFAs showed a significant decrease in the relative abundance of hepatic diacylated 16:0/20:4 PE ($p<0.05$), while there was a significant increase in the relative abundance of diacylated 18:0/20:4 PE (Figure 3.20B), compared to the respective control. Females treated with SCFAs also showed a significant ($p<0.001$) decrease in 18:0/22:5, and a significant increase in -18:0/22:6 PE (Figure 3.20C), compared to their respective controls.

A.



B.



C.

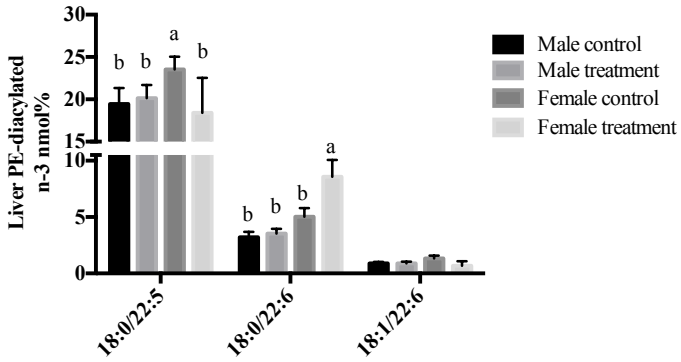


Figure 3.20: Effect of short chain fatty acids on the relative abundance of hepatic PE-diacylated species composed of A) MUFAs, B) PE-diacylated n-6 PUFAs and C) n-3 PUFAs. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed as mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). PE – phosphatidylethanolamine, MUFAs – monounsaturated fatty acids, PUFAs – polyunsaturated fatty acids.

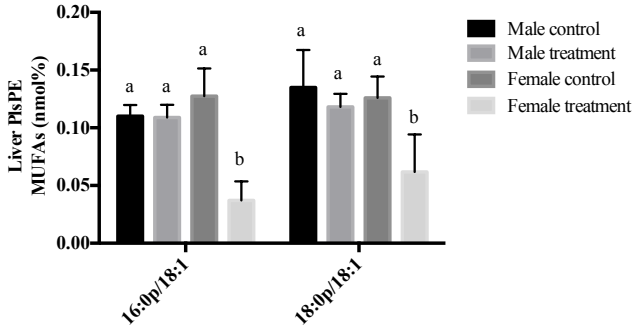
3.2.4.2.2. Effect of SCFAs on hepatic PE-plasmalogen fatty acid composition

The observation chart and biplot obtained by PCA for hepatic PlsPE is given in the Appendix (Figure XIV A and B, respectively). PCA output for PlsPE showed a significant effect of SCFAs treatment for both males and females, compared to their respective controls. MT and FC groups were found in the same PCA quadrant, while MC and FT groups were found in two different PCA quadrants (Appendix figure XIV A). PlsPE species with MUFAs were clustered in the control quadrant (MC), while PlsPE species with PUFAs (20:4, 22:5 and 22:6) were clustered in the MT-FC quadrant (Appendix figure XIV B). No species were clustered in FT quadrant (Appendix figure XIV B).

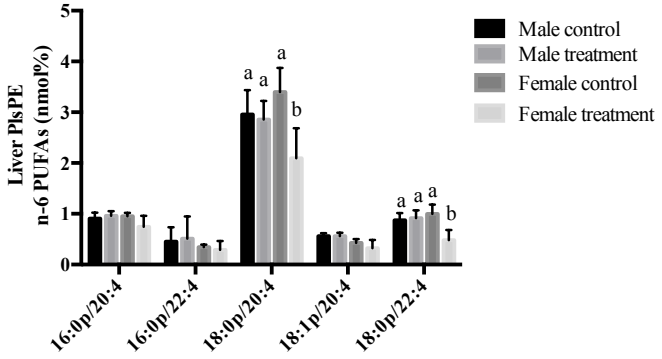
PlsPE species composed of SFAs were not detected in liver samples. The effect of SCFAs on the relative abundance of hepatic PlsPE composed of MUFAs is given in Figure 3.21A. Males treated with SCFAs showed no effect on PlsPE MUFAs; however, females treated with SCFAs showed a significant decrease ($p<0.0001$) in the relative abundance of 16:0p/18:1 and 18:0p/18:1 PE, compared to its respective control (Figure 3.21A).

The effect of SCFAs on the relative abundance of hepatic PlsPE composed of n-6 PUFAs and n-3 PUFAs is given in Figure 3.21B and C, respectively. Males treated with SCFAs showed no effect on PlsPE composed of n-6 and n-3 PUFAs, compared to their respective controls (Figure 3.21B and C, respectively). Females treated with SCFAs showed significantly lower ($p<0.0001$) relative abundance of n-6 PUFAs, such as 18:0p/20:4 and 18:0p/22:4 PE (Figure 3.21B), and n-3 PUFAs, such as 16:0p/22:6, 18:0p/22:5 and 18:0p/22:6 PE ($p<0.0001$) (Figure 3.21C), compared to their respective controls.

A.



B.



C.

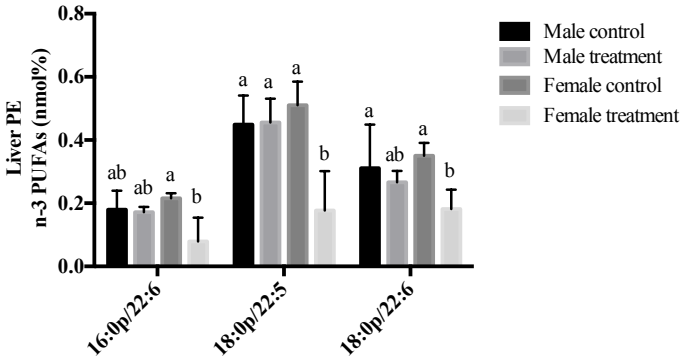


Figure 3.21: Effect of short chain fatty acids on the relative abundance of hepatic PlsPE composed of A) MUFAs, B) n-6 PUFAs and C) n-3 PUFAs. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed as mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). PE – phosphatidylethanolamine, PlsPE – phosphatidylethanolamine plasmalogen, MUFAs – monounsaturated fatty acids, PUFAs – polyunsaturated fatty acids.

3.2.4.3. Effect of SCFAs on plasma and hepatic LPE fatty composition

The effect of SCFAs on the relative abundance of plasma and hepatic LPE is given in Figure 3.22A and B, respectively. LPE species composed of MUFAs, n-6 PUFAs and n-3 PUFAs were not detected in the plasma and liver samples. As very few LPE species were identified, there was no need of PCA output for either plasma or hepatic LPE.

Males treated with SCFAs showed significantly higher (p<0.01) relative abundance of 18:0 LPE; compared to its control group; however, females treated with SCFAs showed no effect on the relative abundance of plasma LPE (Figure 3.22A), compared to control. Males showed no effect of SCFAs treatment on the relative abundance of hepatic LPE (16:0 and 18:0), compared to their control; whereas, females treated with SCFAs showed a significantly lower (p<0.001) relative abundance of hepatic 16:0 and -18:0 LPE; compared to their respective controls (Figure 3.22B).

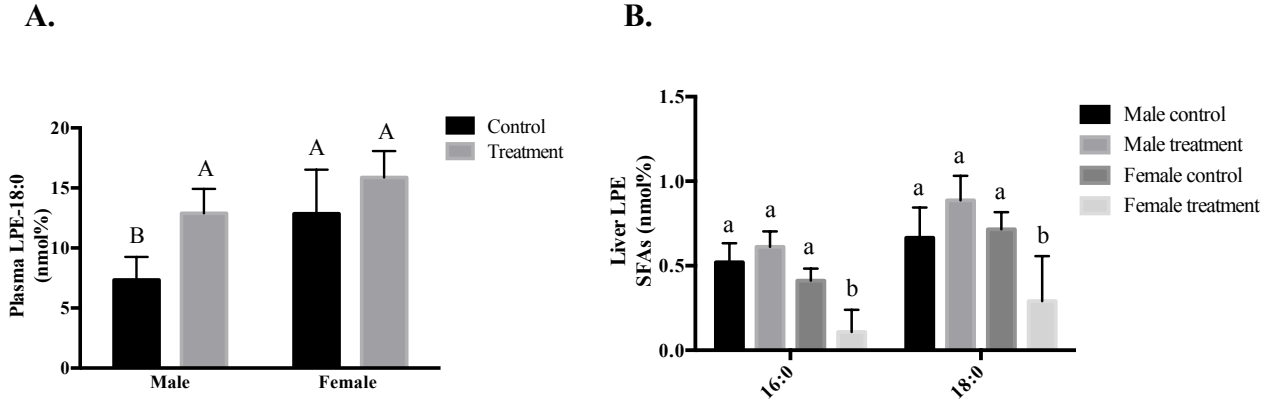


Figure 3.22: Effect of short chain fatty acids on the relative abundance of A) plasma LPE and B) liver LPE. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed as mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). LPE – lyso phosphatidylethanolamine, SFAs – saturated fatty acids.

3.2.5. Effect of SCFAs on PI fatty acid composition

There was no detection and/or identification of PI species linked with plasmalogen or ether moieties in either plasma or liver; therefore, the effect of SCFAs was only observed on the relative abundance of PI-diacylated species. Furthermore, lyso species were also not detected for plasma or hepatic PI. Moreover, PI species composed of SFAs and MUFAs were not detected in either plasma or liver samples; therefore, the effect of SCFAs was only observed on the relative abundance of PI species composed of n-6 and n-3 PUFAs.

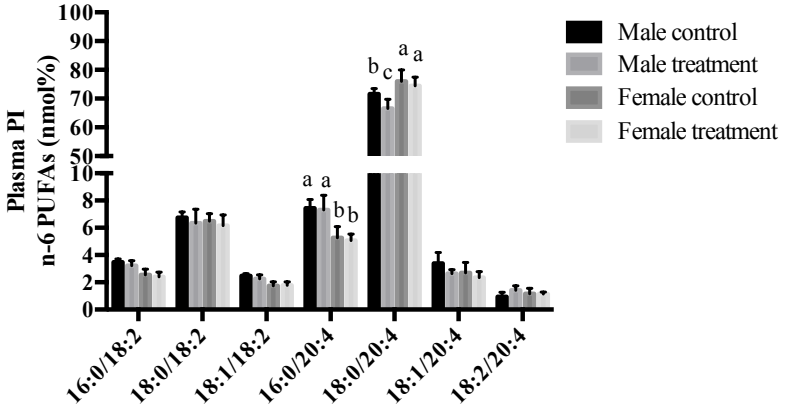
3.2.5.1. The effect of SCFAs on plasma PI fatty acid composition

The observation chart and biplot obtained by PCA for plasma PI is given in the Appendix (Figure XV A and B, respectively). PCA output for plasma PI showed a significant effect of SCFAs treatment on males, compared to their controls; MC and MT groups were found in different PCA quadrants (Appendix figure XV A). However, the PCA output showed no differences between female control and treatment groups as FC and FT groups were found in the same PCA quadrant (Appendix figure XV A). PI species composed with PUFAs such as 18:2, 20:4, and 22:6 were clustered in the control quadrant (MC) (Appendix figure XV B); however, no species were clustered in the FC-FT quadrant (Appendix figure XV B).

The effect of SCFAs on the relative abundance of plasma PI species composed with n-6 PUFAs and n-3 PUFAs is given in Figure 3.23A and B, respectively. Males treated with SCFAs showed significantly lower relative abundance of plasma 18:0/20:4 PI (Figure 3.23A), and higher relative abundance of plasma 18:0/22:6 PI ($p<0.0001$) (Figure 3.23B), compared to their respective controls. However, females treated with SCFAs showed no effect on 18:0/20:4 or 18:0/22:6 PI,

compared to their respective controls (Figure 3.23A and B, respectively). Furthermore, male control and treatment groups had significantly higher (p<0.01) relative abundance of 16:0/20:4 PI, compared to female control and treatment groups (Figure 3.23A).

A.



B.

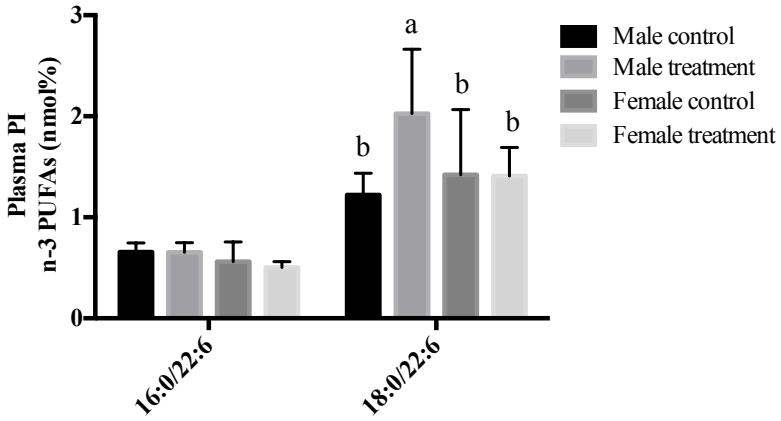


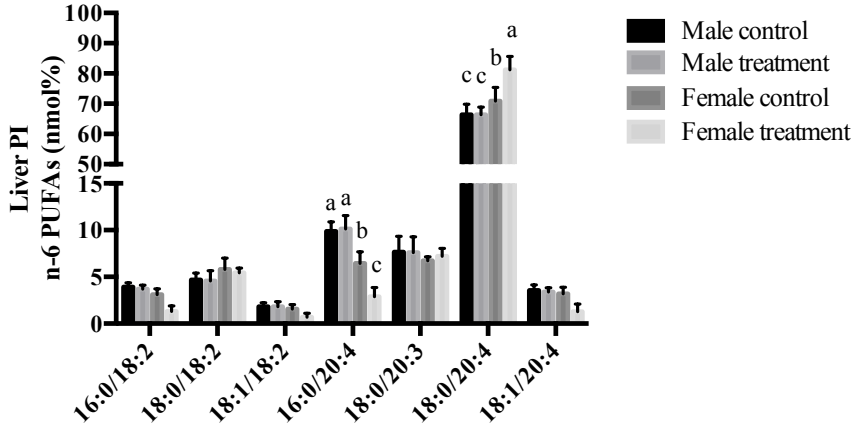
Figure 3.23: Effect of short chain fatty acids on the relative abundance of plasma PI fatty acid composed of A) n-6 PUFAs and B) n-3 PUFAs. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed as mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). PI – phosphatidylinositol, PUFAs – polyunsaturated fatty acids.

3.2.5.2. The effect of SCFAs on hepatic PI fatty acid composition

The observation chart and biplot obtained by PCA for hepatic PI is given in the Appendix (Figure XVI A and B, respectively). PCA output for hepatic PI showed no significant effect of SCFAs treatment on males, compared to its control group; MC and MT groups were found in the same PCA quadrant (Appendix figure XVI A). However, the PCA output revealed differences between female control and treatment groups as FC and FT were found in different quadrants (Appendix figure XVI A). There was no specific clustering of PI species found in either of the PCA quadrants (MC-MT, FC or FT) (Appendix figure XVI B).

The relative abundance of hepatic PI species composed with n-6 PUFAs and n-3 PUFAs is given in Figure 3.24A and B, respectively. Males treated with SCFAs showed no significant effect on the relative abundance of PI containing n-6 PUFAs and n-3 PUFAs, compared to its respective controls (Figure 3.24A and B, respectively). However, females treated with SCFAs showed significantly lower ($p<0.01$) relative abundance of hepatic 16:0/20:4 PI (Figure 3.24A), while there was a higher relative abundance of 18:0/20:4 PI ($p<0.05$) (Figure 3.24A), compared to their respective controls. The SCFAs in females revealed a significant decrease in hepatic 16:0/22:6 PI abundance (Figure 3.24B), compared to the control group.

A.



B.

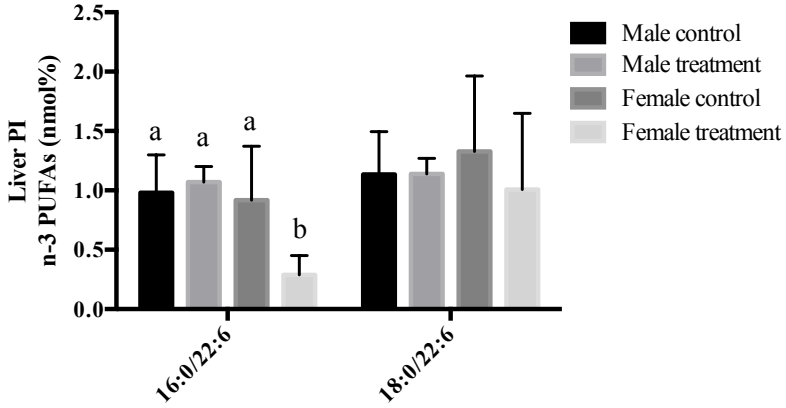


Figure 3.24: Effect of short chain fatty acids on the relative abundance of hepatic PI composed of A) n-6 PUFAs and B) n-3 PUFAs. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed as mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). PI – phosphatidylinositol, PUFAs – polyunsaturated fatty acids.

3.2.6 Effect of SCFAs on PS fatty acid composition

There was no detection and/or identification of PS species linked with plasmalogen or ether moieties, in either plasma or liver samples; therefore, the effect of SCFAs was only observed on the relative abundance of PS-diacylated species. Moreover, no lyso species were detected for either plasma or hepatic PS lipid class.

3.2.6.1. Effect of SCFAs on plasma PS fatty acid composition

The observation chart and biplot obtained by PCA for plasma PS is given in the Appendix (Figure XVII A and B, respectively). PCA output for plasma PS showed a significant effect of SCFAs treatment in both males and females, compared to their respective controls. Each of the four groups were found in different quadrants (Appendix figure XVII A). Plasma PS species composed of MUFAs (18:1 and 20:1) were clustered in the control quadrants (MC and FC), while plasma PS species composed of PUFAs (20:4 and 22:6) were clustered in the treatment quadrants (MT and FT) (Appendix figure XVII B).

There was no identification of PS species composed of SFAs in plasma samples. The effect of SCFAs on the relative abundance of plasma PS species composed with MUFAs is given in Figure 3.25A. Males and females treated with SCFAs showed significantly lower (p<0.0001) relative abundance of plasma 18:0/18:1 PS (Figure 3.25A), compared to their respective controls.

The effect of SCFAs on the relative abundance of plasma PS species composed with n-6 PUFAs and n-3 PUFAs is given in Figure 3.25B and C, respectively. There was significantly higher relative abundance of plasma 18:0/20:4 PS (p<0.0001) but lower abundance of 18:0/22:4

PS (p<0.0001) in both males and females, compared to their respective controls (Figure 3.25B). Furthermore, both males and females showed significantly lower (p<0.05), relative abundance of 22:4/22:6 PS compared to their respective controls (Figure 3.25C). Moreover, females treated with SCFAs also showed a significant decrease in the relative abundance of 18:0/22:6 PS (p<0.01), compared to its control group (Figure 3.25C). However, there was no effect of SCFAs treatment on plasma 18:1/22:6 PS in both males and females, compared to their respective controls (Figure 3.25C).

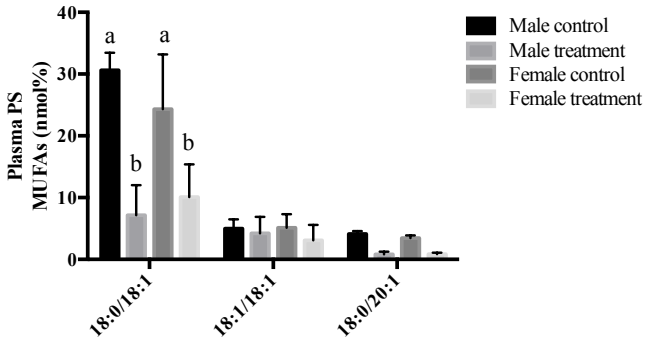
3.2.6.2. Effect of SCFAs on hepatic PS fatty acid composition

The observation chart and biplot obtained by PCA for hepatic PS is given in the Appendix (Figure XVIII A and B, respectively). PCA output for hepatic PS showed no significant effect of SCFAs treatment on males, compared to their controls; MC and MT groups were found in the same PCA quadrant (Appendix figure XVIII A). However, the PCA output revealed differences between female control and treatment groups as FC and FT were found in different quadrants. PS species composed with n-6 PUFAs (18:2 and 20:4) were clustered in the MC-MT quadrant, while PS species composed with MUFAs (18:1) and n-3 PUFAs (22:6) were clustered in the FC quadrant (Appendix figure XVIII B).

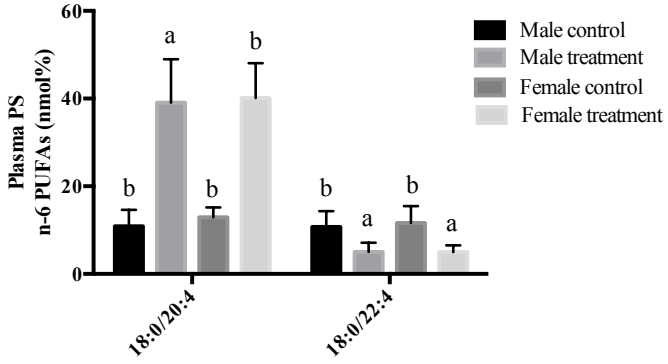
The effect of SCFAs on the relative abundance of PS species composed with MUFAs is given in Figure 3.26A. Males and females treated with SCFAs showed no significant effect on the relative abundance of plasma 18:0/18:1 PS, compared to their controls (Figure 3.26A). The effect of SCFAs on the relative abundance of PS species composed with n-6 PUFAs and n-3 PUFAs is given in Figure 3.26B and C, respectively. Males treated with SCFAs showed no effect of

treatment on hepatic n-6 PUFAs (Figure 3.26B) and n-3 PUFAs (Figure 3.26C), compared to its control group. However, females treated with SCFAs showed significantly higher (p<0.0001) relative abundance of hepatic 18:0/20:4 PS, compared to its control group (Figure 3.26B). Moreover, SCFAs treated females showed significantly lower (p<0.0001) relative abundance of hepatic 16:0/22:6, 18:0/22:6 and 18:2/22:6 PS, compared to its controls (Figure 3.26C).

A.



B.



C.

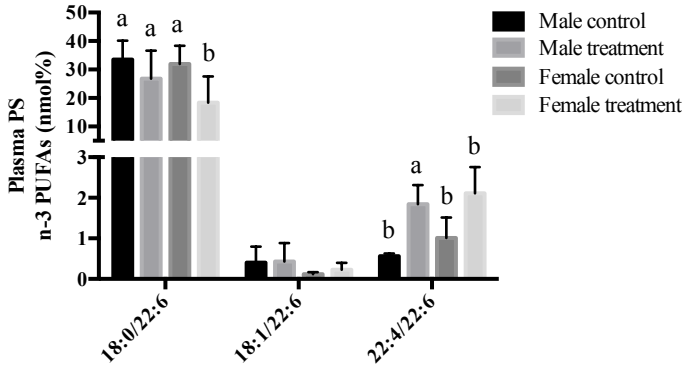
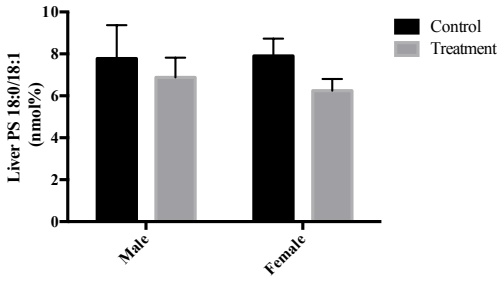
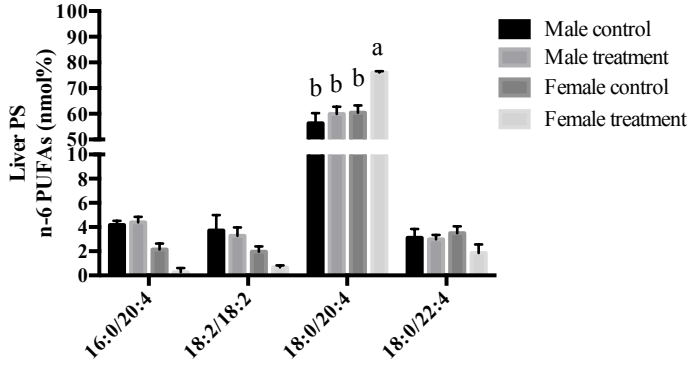


Figure 3.25: Effect of short chain fatty acids on the relative abundance of plasma PS composed of A) MUFAs, B) n-6 PUFAs and C) n-3 PUFAs. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed as mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). PS – phosphatidylserine, MUFAs – monounsaturated fatty acids, PUFAs – polyunsaturated fatty acids.

A.



B.



C.

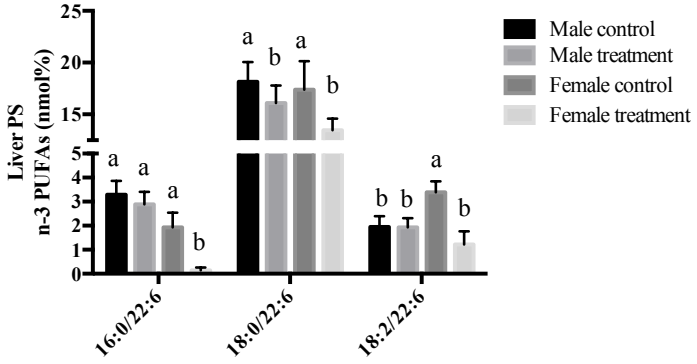


Figure 3.26: Effect of short chain fatty acids on the relative abundance of hepatic PS composed of: A) MUFAs, B) n-6 PUFAs and C) n-3 PUFAs. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed as mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). PS – phosphatidylserine, MUFAs – monounsaturated fatty acids, PUFAs – polyunsaturated fatty acids.

3.2.7. Effect of SCFAs on PA fatty acid composition

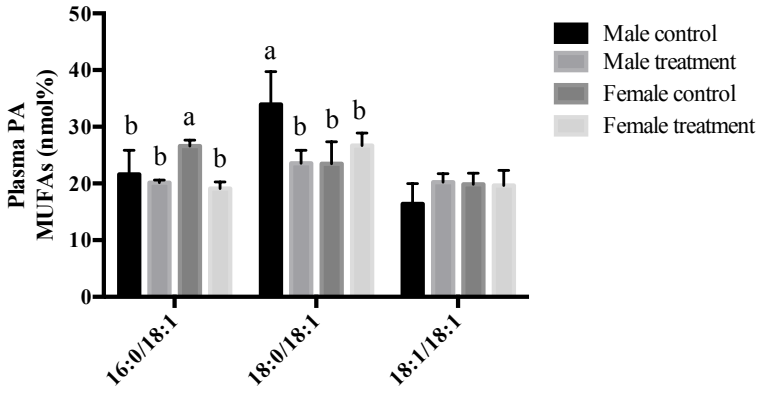
3.2.7.1. Effect of SCFAs on plasma PA fatty acid composition

The observation chart and biplot obtained by PCA for plasma PA is given in the Appendix (Figure XIX A and B respectively). PCA output for plasma PA showed a significant effect of SCFAs treatment in both males and females, compared to their controls. MC and FC groups were found in different quadrants while MT and FT were found in the same PCA quadrant (Appendix figure XIX A). PA species composed with MUFAs were clustered in the control quadrants (MC and FC), while PA species with PUFAs (18:2 and 20:4) were clustered in treatment quadrant (MT-FT) (Appendix figure XIX B).

PA species composed of SFAs and n-3 PUFAs were not identified in plasma samples. The effect of SCFAs on the relative abundance of PA species composed with MUFAs and n-6 PUFAs is given in Figure 3.27A and B, respectively. Males treated with SCFAs showed significantly lower (p<0.05) relative abundance of plasma 18:0/18:1 PA; while females treated with SCFAs showed lower relative abundance of plasma 16:0/18:1 PA; compared to their respective controls (Figure 3.27A).

Furthermore, there was significantly higher (p<0.01) relative abundance of plasma PA composed of n-6 PUFAs such as 16:0/18:2, 18:2/18:2 and 18:2/20:4 PA (Figure 3.27B) in males treated with SCFAs, compared to its controls. However, there was no effect of SCFAs on PA composed with n-6 PUFAs in females, compared to their respective controls (Figure 3.27B).

A.



B.

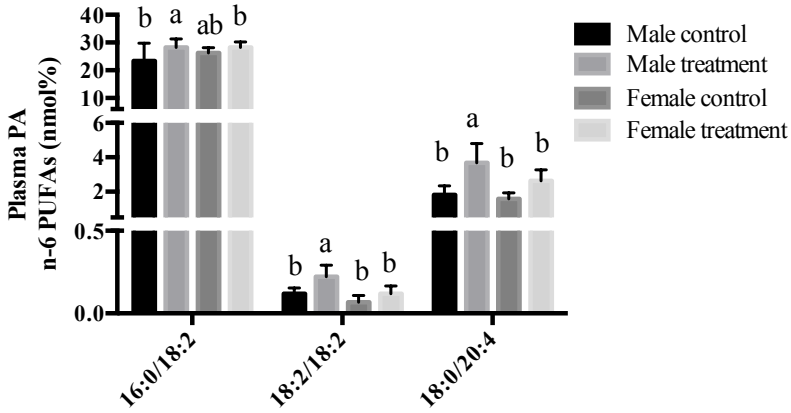


Figure 3.27: Effect of short chain fatty acids on the relative abundance of PA composed of A) MUFAs and B) n-6 PUFAs. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed in mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). PA – phosphatidic acid, MUFAs – monounsaturated fatty acids, PUFAs – polyunsaturated fatty acids.

Chapter 4 - Discussion

4.1. Effect of SCFAs on the regulation of lipid metabolic pathways

4.1.1. Effect of SCFAs on body weight, organ weight, food intake, plasma glucose and NEFA concentrations.

SCFAs have been shown to prevent weight gain, regulate appetite (Byrne et al. 2015) and improve glucose levels in rodents and in humans (den Besten et al. 2013; Chambers et al. 2015) . These beneficial effects of SCFAs are observed either indirectly (enteral route) or directly (parenteral route). In the current study, SCFAs mixture in 60:20:20 molar ratio showed no significant effect on body weight or food intake, in either males or females. Consistent with observations in this study, it was previously shown in male C57BL/6 mice that sodium butyrate when mixed in HFD at 5% w/w concentration, had no significant effect on body weight, food intake and fat content (Z. Gao et al. 2009). A recent study investigated the effect of acetate, propionate and butyrate individually, using C57BL/6 mice (sex not mentioned) fed a HFD, and reported no effect of treatment on body weight and food intake, compared to mice fed only HFD (Den Besten et al. 2015). However, in the latter study, a decrease in adipose tissue PPARγ mRNA expression and activity was reported, which stimulated fat oxidation in liver and adipose tissue, suggesting a role of SCFAs in fatty acid oxidation and energy expenditure (Den Besten et al. 2015; Z. Gao et al. 2009). In the current study, I did not observe any change in adipose tissue PPARγ mRNA expression. This discrepancy may be because in the above-mentioned study, mice were fed a HFD and then supplemented with SCFAs.

Mice on a HFD, supplemented with fermentable carbohydrates (inulin) gained less weight, compared to mice supplemented with poorly fermentable fiber (cellulose) (Frost et al. 2014). In

the same study, when acetate alone was administered I.P. in the dose of 500 mg/kg body weight instead of supplementation of dietary fibers, there was an increase in plasma acetate levels, with a significant reduction in acute food intake at one and two hours post injection. On the contrary, I found that when a mixture of SCFAs was administered by I.P. injection at the same dosage used by Frost et al. (500mg/kg); there was no change in the plasma SCFAs levels (data not shown) or food intake of either males or females. In the study by Frost et al, the decrease in food intake was suggested to be due to the action of increased plasma acetate levels, where acetate acted as an anorexic signal in gut-brain axis (Frost et al. 2014). However, I did not find an increase in plasma acetate levels (data not shown), which may explain no change in food intake or body weight in my study.

Males (control and treatment groups) had significantly higher body weight, liver weight and food intake, compared to female control and treatment groups, which was expected. SCFAs showed no effect on abdominal fat in both male and female groups; however, males treated with SCFAs had higher abdominal fat, compared to SCFAs treated females, indicating sex-specific effects. The sex-specific differences in body weight, food intake and liver weight was expected to be higher in males compared to females, due to differences in energy expenditure in both the sexes (Sugiyama and Agellon 2012).

Increased plasma glucose levels, along with increased plasma and hepatic lipid levels are associated with a higher risk of metabolic disorders like CVD, T2D and obesity (Erion, Park, and Lee 2016). In the current study, males treated with SCFAs showed a significant decrease in glucose levels, while there was no effect of SCFAs on glucose levels in females, compared to their

respective controls. It has been suggested that the effects of SCFAs on glucose metabolism depend on the ratio of propionate: butyrate, and not propionate or butyrate alone. Intraperitoneally administered acetate had no effect on blood glucose concentrations of male C57BL/6 mice (Frost et al. 2014), emphasizing that a combination of SCFAs, and not acetate alone, is required to influence blood glucose concentrations as observed in the current study. The decrease in plasma glucose levels by SCFAs has been shown to be due to activation of a SCFA receptor, known as FFAR 2 (den Besten et al. 2013), which should be investigated in the future. The effects of SCFAs on glucose concentrations were found to be sex-specific. The sex-specific differences in plasma glucose levels could be due to the role of estrogen in maintaining glucose homeostasis in females (Sugiyama and Agellon 2012).

4.1.2. Effect of SCFAs on cholesterol metabolism

4.1.2.1. Effect of SCFAs on plasma and hepatic cholesterol levels

In the current study, SCFA treatment caused a significant decrease in plasma TC and FC concentrations in males, compared to their respective controls. However, no significant differences were observed in plasma ChE levels in males treated with SCFAs, suggesting that the decrease in plasma TC levels is due to a decrease in FC levels. On the other hand, females showed no effect of SCFAs treatment on plasma TC, FC and ChE levels. Furthermore, males treated with SCFAs also revealed a decrease in hepatic TC, compared to its control group. However, SCFAs showed no significant differences in hepatic FC and ChE levels in both males and females, compared to their respective controls. Previous studies have shown that dietary fibers and its metabolites, SCFAs, are capable of reducing plasma cholesterol levels (Hara et al. 1998; Fushimi et al. 2006).

SCFA-fed rats had lower plasma cholesterol levels, compared to rats fed fiber free diet (Hara et al. 1998). These authors found that the decrease in cholesterol levels by SCFA is not due to a decrease in cholesterol synthesis (Hara et al. 1999), which is similar to my findings where I did not find a significant effect of SCFAs on the mRNA expression of HMGCR. However, another group have shown that dietary acetate reduced serum cholesterol levels, which was due to a decrease in HMG-CoA enzyme concentration (Fushimi et al. 2006). These authors found that acetic acid treatment also increased fecal bile content; however, there was no change in CYP7A1 activity or mRNA levels. Another similar study conducted on male hamsters showed that feeding HCD containing acetate, propionate or butyrate, decreased plasma TC, with a concomitant increase in fecal bile acids (Zhao et al. 2017). This group suggested that the decrease in plasma TC levels was due to upregulation of genes such as SREBP2, LDLR and CYP7A1, and concluded that SCFAs with 2-4 carbons (acetate, propionate and butyrate) are hypocholesterolemic. I did not measure fecal bile acids; however, the mRNA expression of CYP7A1 remained unchanged in my study.

Dietary propionate has been shown to decrease the TC synthesis rate by reduction in the enzyme activity of HMGCR and HMGCS (den Besten et al. 2013). However, I did not find an effect of SCFAs on HMGCR mRNA expression. HMGCR is regulated at the transcriptional and posttranscriptional levels (Feingold and Grunfeld 2015; Michael W King 2016), and SCFAs may impact HMGCR enzyme activity. It is also possible that the decrease in plasma FC levels is due to an increase in LCAT activity. LCAT is involved in the hydrolysis of FA at *sn*-2 position of phospholipids, which then transfers the hydrolyzed FA to cholesterol to yield ChE (Sorci-Thomas, Bhat, and Thomas 2009). ChE is the storage form of cholesterol, and excess accumulation of

cholesterol is associated with a higher risk of heart diseases (Viollet et al. 2009). I did not find any change in plasma and hepatic levels of ChE, suggesting that the decrease in FC might be responsible for an overall decrease in TC levels in males treated with SCFAs.

I observed sex-specific effects on the regulation of cholesterol metabolism; however, no studies to date have investigated the sex-specific effects of SCFAs on cholesterol metabolism. My findings revealed that males treated with SCFAs had lower plasma and hepatic TC levels, while SCFAs-treated females had no effect of treatment. In females, higher levels of estrogen are responsible for the reduced hepatic lipogenesis and increased lipolysis in adipocytes which leads to decreased *de-novo* synthesis of lipids (D'Eon et al. 2005; H. Gao et al. 2006; Bryzgalova et al. 2008). However, Suligyan and Agellon (2012) suggested that the differences in sex-specific lipid metabolism is not solely dependent on estrogens and androgens, but other important components like metabolic fate of nutrient intake also contributes to this synergy.

4.1.2.2. Effect of SCFAs on plasma and hepatic ChE fatty acid composition

The fatty acid composition of plasma and hepatic ChE are associated with insulin resistance and blood glucose levels (Lewis-Barned et al. 2000; Rosqvist et al. 2017). For example, a clinical trial carried out on Finnish population found that diabetic subjects had higher levels of serum ChE – 18:3 (ALA), 20:3 (ETA) and 20:4 (AA), compared to non-diabetic subjects (Salomaa et al. 1990). This clinical trial also showed differences in plasma and hepatic ChE composition. In the current study, I found that males had no effect of SCFAs on the plasma or hepatic ChE fatty acid composition. However, females treated with SCFAs showed a significant decrease in the relative abundance of plasma 20:4 ChE, and revealed a significant increase in plasma 22:6 ChE, compared

to its control group. It is known that 20:4 ChE acts as a substrate for oxidizing agents, such as 12 or 15-lipoxygenase; thus, the metabolites generated by 20:4 ChE are associated with atherosclerosis (Harkewicz et al. 2008). On the other hand, n-3 PUFAs such as 20:5 (EPA) and 22:6 (DHA), lowers the propensity for AA metabolites (Lands 2015) that are inflammatory**.** My findings support that SCFAs reduce the incorporation of inflammatory 20:4 ChE, while increasing the incorporation of 22:6 that is anti-inflammatory. Others have shown that butyrate (1g/kg), when administered in db/db mice via I.P. route, reduced the mRNA expression of inflammatory cytokines (IL-1, IL-6 and TNF-α) in adipose tissue (Wang X., He G., Peng Y., Zhong W., 2015). In the current study, SCFAs caused a higher abundance of 22:6 ChE in females, which may be associated with a reduction in inflammatory cytokines.

The cholesterol esterification reaction is catalyzed via LCAT and ACAT2 enzymes in plasma and liver, respectively (Michael W King 2016). The increase in LCAT activity might be responsible for the decrease in plasma 20:4 ChE abundance relative to ChE 22:6, which was increased. I propose a pathway (Figure 4.1) for the sex-specific effects of SCFAs on cholesterol metabolism, where LCAT may decrease plasma TC and FC levels in males, and 20:4 ChE abundance in females.

It was interesting to note in this study that the fatty acids identified with ChE were of n-6 and n-3 PUFA classes. Studies have shown that the serum ChE fatty acid composition is different in rats and other species (Law, Field, and George 1980)**.** Rat plasma ChE is comprised of ~50% AA, while rabbits have a very low ChE - AA content. On the other hand, humans tend to have mainly 18:0 ChE and only 5-10% of AA in ChE (Law, Field, and George 1980). In the current

study, the relative abundance of ChE 20:4 was ~80%; which is higher than 20:4 ChE content suggested for rat plasma in the literature. It is possible that the property of unsaturated ChE to exhibit dipole moment in ESI, might make them look artificially higher in abundance compared to equimolar amount of saturated ChE (16:0 and 18:0), which were not identified in this study (Bowden et al. 2011).

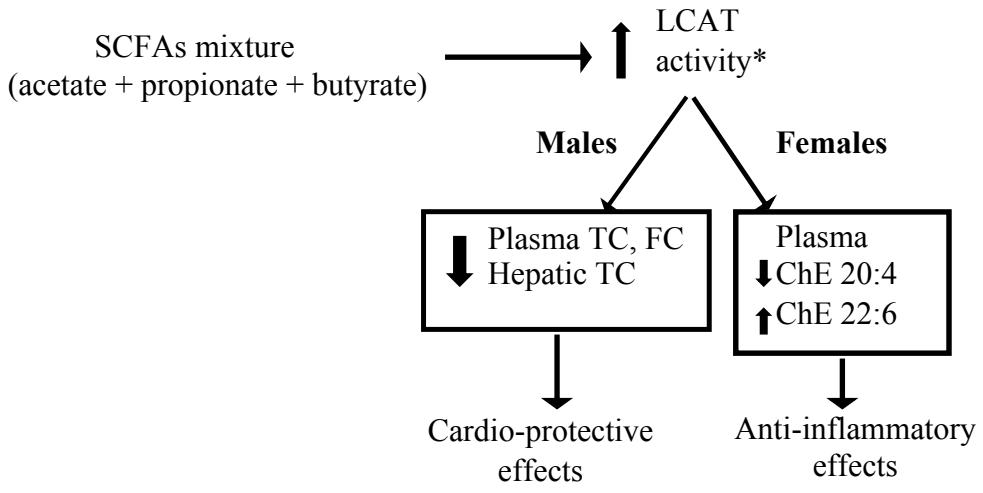


Figure 4.1 - Proposed pathway on the sex-specific effects of SCFAs on cholesterol metabolism. An increase in LCAT activity may be involved in decreasing plasma and hepatic cholesterol levels in males, while in females, it may decrease the abundance of ChE 20:4 and increase the abundance of ChE 22:6 via independent pathways regulated via sex-hormones. . SCFAs – short chain fatty acids, TC – total cholesterol, FC – free cholesterol, ChE – cholesteryl esters, LCAT – lecithin-cholesterol acyltransferase (*indicates experiment not performed in this study)

4.1.3. Effect of SCFAs on TG metabolism

Mammals obtain their energy in the form of fats either from dietary TG or *de-novo* synthesized TG. In the current study, all animals were fed a standard chow diet, thus, the fatty acid composition of dietary TG of control and treatment groups was similar, suggesting that the effects of SCFAs on TG metabolism are likely due to changes in *de-novo* synthesis.

4.1.3.1. Effect of SCFAs on plasma and hepatic TG levels

An increase in TG levels is associated with hyperlipidemia, insulin resistance, obesity, vascular diseases, non-alcoholic fatty liver disease and other metabolic diseases (Mu and Porsgaard 2005). In the current study, the SCFA mixture reduced plasma and hepatic TG levels in males, compared to its controls; while SCFA treated females showed no effect on TG levels in either plasma or liver. Studies have shown that mice and rats fed a HFD supplemented with propionate and butyrate, are protected against development of obesity and insulin resistance (Canfora, Jocken, and Blaak 2015; Lin et al. 2012). The effect of SCFAs on TG metabolism has been suggested to be due to an increase in FAO in multiple tissues (den Besten et al. 2013), and a decrease in fat storage in adipose tissue (Den Besten et al. 2015). In the current study, I found that SCFAs reduced the mRNA expression of hepatic ACC1 in both males and females, compared to their respective controls. Thus, the decrease in plasma and hepatic TG levels in males is likely due to inhibition of ACC1, the rate limiting enzyme in fatty acid synthesis (Mao J. et al. 2008).

Previous studies have shown that SCFAs inhibit storage of fat in adipose tissue in mice fed a HFD (Kimura et al. 2013). Moreover, SCFAs reduced lipolysis in adipose tissue (Den Besten et al. 2015). In the current study, there were no changes in adipose tissue TG levels. Furthermore,

there was no effect of SCFAs on the mRNA expression of PPARγ, which was consistent with no change in plasma NEFA levels. This discrepancy may be because the majority of the studies on SCFAs treatment used animal models that were fed a HFD, while animals in the current study were fed a standard laboratory chow diet.

Acetate gets incorporated into LCFAs in the liver, and is then utilized for the formation of complex lipids during the process of *de-novo* lipogenesis and cholesterol synthesis (den Besten et al. 2013). On the other hand, propionate and butyrate, have been shown to inhibit these processes. It has been suggested that a combination of SCFAs may negate the effects of individual SCFA on lipid metabolism (Morrison and Preston 2016). As in the current study, a combination of acetate, propionate and butyrate was used, this may also explain the differences in the effects observed on TG metabolism in liver and adipose tissue, compared to previously published studies that studied the effects of individual SCFAs on TG metabolism (Lin et al. 2012, Kimura et al. 2013, Den Besten et al. 2015).

4.1.3.2. Effect of SCFAs on plasma and hepatic TG fatty acid composition

The alternation in the positional distribution of fatty acids in TG species can occur either via switching of positions within the TG molecule or between two different TG molecules (Karupaiah and Sundram 2007). Due to the tendency of TG molecules to retain the fatty acid at *sn*-2 position, plasma and hepatic TG species with SFAs (16:0 and 18:0), MUFAs (16:1), n-6 PUFAs (20:4) and n-3 PUFAs (22:6) at *sn*-2 position were analyzed and compared. I found that males showed no effect of SCFAs treatment on the relative abundance of plasma and hepatic TG

species, compared to its control group. However, SCFAs treated females showed decreased relative abundance of TG species composed of SFAs (16:0 and 18:0) at all three *sn*- positions.

There was no effect of SCFAs on TG species composed of MUFAs, n-6 or n-3 PUFAs, in either males or females, compared to their respective controls. A study conducted on hamsters concluded that diets rich in C18:0 and devoid of cholesterol, had lowest fat digestibility and greatest steroid excretion (Imaizumi K., Kuroiwa, Sugano M., 1993). TG composed of C16:0 at *sn*-2 position in palm oil showed higher levels of plasma TG in newborn piglets, compared to that composed of *sn*-2 C18:1 (Innis SM, Quinlan P., 1993). Similarly, TG composed of C16:0 at *sn*-2 position in lard resulted in higher TG levels in rat plasma (Lopez-lopoz A. et al, Early Human Dev, 2001). Positional differences in the SFAs have also been hypothesized to modulate the transport of n-6 and n-3 PUFAs (Innis SM and Dyer R, J nutr 1997). Thus, SCFA mediated changes in the fatty acid composition of TG may impact the fatty acid composition of other lipids in circulation. It is well known that SFAs are associated with CVD (Mensink R., 2016). I found that SCFAs-treated males showed decreased plasma and hepatic TG levels. Even though SCFAs treated females showed no change in total TG levels, the relative abundance of TG species composed of 16:0 and 18:0 was significantly decreased, compared to its control group. Thus, SCFA mediated alterations in TG fatty acyl species may be of importance in CVD. Females may have cardio-protective effects due to high levels of estrogen compared to males; however, decrease in plasma TG levels in males and SFAs relative abundance in females suggests sex-specific effects of SCFAs. I have summarized my findings by proposing a pathway (Figure 4.2) to explain the sex-specific effects of SCFAs on TG metabolism.

It was also observed that there was no significant difference in the relative abundance of hepatic TG species composed of SFAs, MUFAs, n-6 PUFAs or n-3 PUFAs. This indicates that SCFAs did not serve as substrate for TG synthesis, or alternately the esterification of fatty acids. The endogenous synthesis of TG occurs mainly in liver, adipose tissue and intestine (Rui 2014). In this study, the route of administration being intraperitoneal, I expected changes in the fatty acid synthesis pathway in liver. I found that SCFAs treatment showed a significant decrease in hepatic ACC1 mRNA expression, however, there was no change in hepatic TG species fatty acid composition. Interestingly, I found alternations in plasma TG species, suggesting that SCFAs might have an effect on the packaging and secretion of lipoproteins (such as VLDL). Lipids packaged for release in the blood stream and distribution to peripheral tissues are acted upon by lipases (lipoprotein lipase, hepatic lipase) (Rui 2014); it is likely SCFAs impact lipases, which should be investigated in the future.

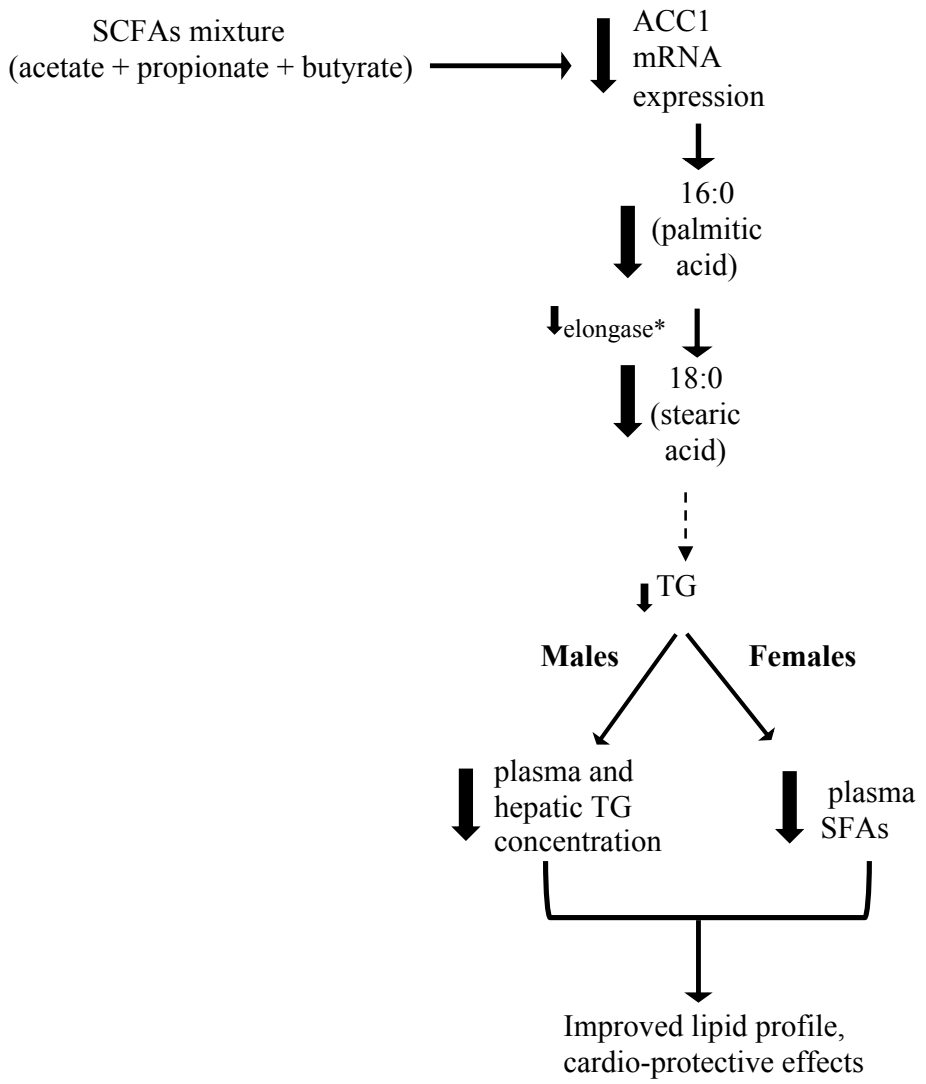


Figure 4.2 - Proposed pathway showing sex-specific effect of SCFAs on triacylglycerol metabolism. It is proposed that a decrease in ACC mRNA expression will lead to a decrease in 16:0 and 18:0 by reduced activity of elongase. This may in turn be responsible for a decrease in plasma and hepatic TG levels in males, and plasma TG composed of SFAs in females. SCFAs – short chain fatty acids, TG – triacylglycerols, SFA – saturated fatty acids, ACC – acetyl CoA-carboxylase. (* indicates experiment not performed in this study)

4.1.4. Effect of SCFAs on fatty acyl composition of phospholipids

The positional distribution of fatty acids at the *sn*-1 and *sn*-2 positions of the glycerol moiety in the structure of PLs is important in metabolic regulation (Beerman C et al., 2005). The fatty acid composition of PLs is influenced by processes such as phospholipid degradation and phospholipid remodeling. Phospholipases act on PLs to generate lysoPLs, which are potent bioactive lipids (Balgoma D, et al., 2010). I used lipidomics analysis to detect plasma and hepatic fatty acid composition of PC, LPC, PE, LPE, PS, PI and plasma PA.

4.1.4.1. Effect of SCFAs on PC fatty acid composition

The primary structure of PC contains C16:0 or C18:0 at the *sn*-1 position, and C18:1, C18:2 or C18:3 at the *sn*-2 position. With the action of enzymes - phospholipase A1 and A2; these primary fatty acids are released; the addition of newly synthesized fatty acid/s makes the secondary structure of PC. These PC lipid species with two acyl chains are more commonly known as diacylated-PC (Beerman C et al., 2005).

4.1.4.1.1. Effect of SCFAs on plasma and hepatic diacylated PC fatty acid composition

In the current study, SCFA treatment decreased the relative abundance of PC containing C16:0 and C16:1 at the *sn*-2 position, and increased the abundance of n-3 PUFAs such as C22:5 and C22:6 at the *sn*-2 position in males (irrespective of C16:0 or C18:0 at the *sn*-1 position), compared to its controls. These findings suggest that SCFAs may have an important role in the incorporation of n-3 PUFAs in plasma PC. Similarly, SCFAs increased the relative abundance of 18:0/22:6 PC in the females compared to its controls, further suggesting a role of SCFAs in remodeling PC with increased abundance of n-3 PUFA. N-3 PUFAs are generally considered anti-

inflammatory and protect against heart disease (Ratnayake and Galli 2009). Contrary to plasma, there was no effect of SCFAs treatment on hepatic PC species composed of SFAs (16:0 or 18:0) or PUFAs (18:2, 20:4 or 22:6). A recent study designed to investigate an early onset marker for the side-effects of tamoxifen (a breast cancer drug) found that plasma AA (20:4) containing PC species was reduced in Sprague-Dawley rats (K. Saito et al. 2017). Thus, alterations in PC fatty acyl composition appears to be of significance.

I found that SCFAs treatment reduced the mRNA expression of hepatic ACC1 in males, compared to control group. Thus, the decrease in plasma 18:0/16:0 PC could be due to decreased fatty acid synthesis in the liver. The increase in 16:0/18:2, 16:0/22:6, 18:0/22:5 and 18:0/22:6 PC could be due to the effect of SCFAs on the regulation of elongation and desaturation enzymes**.** A recent study by Chalil A et al. (2018) suggested that an increase in plasma 16:0/22:6 PC levels is due to increased PEMT expression, and PEMT activity along with increased FADS protein levels. Although no direct comparison can be made with the current study, it would be interesting to look into hepatic PEMT and other enzymes involved in PUFA synthesis because SCFAs showed a significant increase in plasma PC composed of n-3 PUFAs irrespective of C16:0 or C18:0 in the lipid structure.

It was also observed that male control and treatment groups had lower relative abundance of plasma and hepatic 18:0/20:4 and 18:0/22:6 PC, compared to female control and treatment groups. Moreover, males (both control and treatment groups) had higher abundance of hepatic 16:0/20:4 PC, compared to female groups. This sex-specific change is similar to a previous report where male rats had lower AA (20:4) and DHA (22:6) content in plasma PLs, compared to females

(K. Saito et al. 2017). This sex-specific change in plasma and hepatic AA and DHA was suggested to be due to a higher expression of liver desaturases in female rats, which replete their DHA status more readily than males (Kitson et al. 2012). The increase in 16:0/20:4, and a decrease in 18:0/20:4 in in this study points towards alterations in the elongation enzymes between males and females.

4.1.4.1.2. Effect of SCFAs on plasma and hepatic LPC fatty acid composition

LPC is the most abundant lysoPL, generated as a result of the transacylation reaction catalyzed by LCAT enzyme, or due to cleavage of ester bond by PLA_1 or PLA_2 enzymes. LPC species composed of SFAs (16:0 and 18:0), MUFAs (18:1), PUFAs (18:2, 20:4 and 22:6) have been found in human blood. Studies have confirmed that depending on their specific structure, LPCs performs distinct biological roles in metabolism (Rytczak P., Drzazga A., 2013; Kim Y.L., Im Y.J., 2017; Rao S.P., Reiderer M., 2013). In the current study, males treated with SCFAs had higher relative abundance of hepatic LPC – 16:0e and 18:0p. As discussed above, SCFAs treatment caused a decrease in the relative abundance of plasma diacylated PC species composed of SFAs (16:0 and 18:0) in males. This suggests a higher conversion of diacylated PC to LPC with the help of LCAT or PLA enzyme.

Recent studies have showed that LPCs with saturated acyl chain such as 16:0 LPC modulates the immune response; whereas polyunsaturated acyl LPCs, such as 20:4 LPC, and 22:6 LPC, may have anti-inflammatory action **(**Hung N.D., Kim M.R., 2009**;** Jin M.C., Hung N.D., et al, 2012). In addition to the involvement of PC species in inflammatory processes; these PLs have also been suggested to have beneficial effects in the therapy of Alzheimer's and Schizophrenia like neurodegenerative diseases, which are characterized by impaired synthesis of PC and LPC,

and significantly lower levels of plasma LPCs; respectively (Grimm et al. 2011; Orešič et al. 2012). LPCs are found to be associated with secretion of enzymes involved with absorption and transport of lipids (Drarzga A., 2014)**.** LPC also delivers long chain PUFA, specifically DHA to the brain (Grimm et al. 2011). I found that SCFAs caused a higher relative abundance of plasma 18:0/22:5 and 18:0/22:6 PC, and hepatic 20:4 LPC, suggesting transport of these PC species in the central circulation and possibly to the brain.

However, I did not observe any significant change in the relative abundance of brain PC composed of n-3 PUFAs (22:5 and 22:6; Appendix figure XX), suggesting that the SCFA mediated changes in plasma PC and hepatic LPC profile do not affect brain PC fatty acids. This could be because of the short duration of the current study (seven days) to observe an effect on brain fatty acids.

There was a significantly lower abundance of plasma and hepatic LPC – 18:0 and higher abundance of 18:2 LPC in males (both control and treatment groups), compared to females. Males also had a lower relative abundance of plasma and hepatic diacylated 18:0/20:4 and 18:0/22:6 PC, compared to females. This suggests that females might tend to retain the PUFAs in PC structure while in males, PC species with PUFAs are converted to LPC.

4.1.4.2. Effect of SCFAs on PE fatty acid composition

PE is generated mainly by the CDP-ethanolamine or PSD pathway in which PS is decarboxylated to PE (van der Veen et al. 2017). The biological functions of PE include, autophagy of cells, folding of membrane proteins as lipid chaperone, immune response and also as precursor

for other lipids such as anandamide, glycosylphosphatidylinositol anchors and PC, which helps in various signaling pathways (Patel D and Witt S.N., 2017). Similar to PC and LPC, the fatty acyl chain/s on the PE and LPE structures have their own distinct biological roles. In addition to PE-diacylated and LPE, recently PlsPE have been actively researched for their significant contribution in membrane fusion and possess oxidants scavenging properties (Moukarzel et al. 2016)**.** However, the biological significance of PlsPE composed of varied fatty acyl chains is still poorly understood.

In the current study, SCFAs increased the total abundance of plasma PE-diacylated species relative to total PlsPE, which was decreased in plasma. The lipid species responsible for an increase of PE-diacylated abundance were – 18:0/18:2, 18:0/20:4 and 16:0/22:6 PE, and those species responsible for a decrease of PlsPE were – 18:0p/18:1, 18:0p/20:4, 16:0p/22:6 and 18:0p/22:6 PE. Furthermore, males did not show any effect of SCFAs on hepatic PE-diacylated or PlsPE species; but females showed decreased hepatic PE abundance relative to hepatic PlsPE species. These findings indicate that SCFAs modulates plasma PE composition, and the alternations in fatty acid composition could attributed to PE biosynthesis in liver in females but not in males.

4.1.4.2.1. Effect of SCFAs on plasma and hepatic diacylated PE fatty acid composition

In the current study, SCFAs caused a significantly higher relative abundance of plasma 18:0/18:2 and -18:0/20:4 PE in both males and females, compared to their respective controls. Interestingly, AA containing PE have been found to have stimulatory role in ferroptotic cell death, and has been suggested for treatment of cancer (Kagan V.E., Mao G., 2017)**.** Moreover, SCFAs treatment showed increased abundance of 16:0/22:6 PE only in males compared to its control,

suggesting that SCFAs may have anti-inflammatory effects due to increased incorporation of n-3 PUFA. Furthermore, SCFAs decreased the abundance of hepatic 18:0/22:5 PE relative to that of 18:0/22:6 PE in females, compared to its controls. This could be due to a higher conversion of DHA from EPA (Kitson et al. 2012), suggesting that SCFAs replete DHA content in PE, similar to PC species in SCFA treated females.

Treatment with SCFAs also revealed sex-specific effects where male control and treatment groups showed higher relative abundance of hepatic 16:0/18:1 PE, compared to female control and treatment groups. This is consistent with another study, where female rats had lower levels of hepatic MUFAs (16:1 and 18:1) compared to male rats' due to higher activity of Δ9 desaturase (Rankovic S., Popovic T., 2017).

4.1.4.2.2. Effect of SCFAs on plasma and hepatic PlsPE fatty acid composition

PlsPE contribute upto 85% of the total PE abundance in mammalian tissues, which is not the case with any other vinyl-ether linked PLs. The brain has the highest amount of PlsPE, and liver is found to have the least abundance of PlsPE. However, PlsPE acts as reservoir for LC-PUFAs at its *sn*-2 position (Moukarzel et al. 2016). In the current study, SCFAs decreased the total plasma PlsPE abundance relative to PE-diacylated abundance in both males and females. PE-diacylated levels have been shown to be tightly regulated in case of PlsPE deficiency to keep total PE and PUFA levels constant (Dorninger et al. 2015) . This group also found that the compensation was majorly favored by PE composed of 20:4 and not PE 22:6. I found that SCFAs significantly increased plasma and hepatic 18:0/20:4 PE, while plasma and hepatic 18:0p/20:4 and -18:0p/22:6 PE were significantly decreased.

The production of both PE-diacylated and PlsPE by Kennedy pathway involves few common steps of biosynthesis (van der Veen et al. 2017), for example, in the last step of PE biosynthesis pathway, if the CDP-ethanolamine couples to DAG; the end-product is diacylated PE whereas coupling to alkylacylglycerol generates PlsPE. This step is regulated by a rate-limiting enzyme known as ethanolaminephosphotransferase. The adaptation to maintain total PE levels depends on the levels of CDP-ethanolamine generated by CTP: phosphoethanolamine cytidyltransferase (ECT) (van der Veen et al. 2017). Findings in this study suggest that SCFAs treatment likely modulates hepatic ECT activity in females as there was a significant effect of SCFAs on plasma and hepatic PE and PlsPE composition in females, but this was not observed in males.

4.1.4.2.3. Effect of SCFAs on plasma and hepatic LPE fatty acid composition

LPE is not one of the major lyso-glycero phospholipids, and does not have a significant biological effect on immune-response, eicosanoid production or transport of PUFAs (Li et al. 2016). I found that SCFAs increased plasma 18:0 LPE in males, and decreased hepatic 16:0 and 18:0 LPE in females compared to the respective controls. A decrease in SFAs containing LPEs indicates that SCFAs are likely decreasing the hydrolysis of PE, and there is less conversion of PE-diacylated and PlsPE to its lyso form. These findings further suggests that SCFAs may downregulate the production of pro-inflammatory eicosanoids, which is induced by palmitic acid in lyso PLs (Dennis and Norris 2015). Furthermore, SCFAs may help in maintaining the cell integrity and membrane fluidity by maintaining the total PE levels (PE diacylated + PlsPE) by not converting these to lyso species.

4.1.4.3. Effect of SCFAs on plasma and hepatic PS fatty acid composition

The biosynthesis of PS involves base-exchange reactions of serine for choline and ethanolamine in PC and PE, respectively (Cajka and Fiehn 2014). PC and PE are synthesized by the decarboxylation of PS lipid species (D. E. Vance and Vance 2008); thus, the fatty acid composition of PS species is quite similar to PC and PE where the *sn*-1 position is usually occupied with 18:0 and the *sn*-2 by PUFAs such as 18:2, 20:4 or 22:6.

I found that SCFAs treatment decreased the relative abundance of PS plasma MUFA (18:0/18:1), but increased PUFAs (18:0/20:4 and -22:4/22:6) in both males and females, compared to its respective controls. Consistent to other lipid classes, males showed no effect of SCFAs treatment on hepatic PS composition, while in females, there was a decrease in the relative abundance of hepatic 16:0/22:6 and 18:2/22:6 PS, but 18:0/20:4 PS increased. The decrease in DHA containing PS species, and an increase in AA containing PS species is unlike the changes in PC and PE species that showed an increase in DHA relative to AA. The interconversion of PLs due to Land's cycle (phospholipid remodeling) might be the reason for the differences in fatty acid composition of PC, PE and PS (Okuno et al. 2018). This indicates that SCFAs may have effects on acyl transferases (such as LPCAT3), which controls the transfer of acyl chains between PLs (Rong et al. 2015).

4.1.4.4. Effect of SCFAs on plasma and hepatic PI fatty acid composition

PI is a membrane lipid that occurs with varied number of phosphate molecules esterified to hydroxyl group of inositol, more commonly known as polyphosphoinositides (PIP_2 and PIP_3)

(Irvine 2016). These polyphosphoinosities are important lipids involved as transducers of signals for cell growth and differentiation (Falkenburger et al. 2010).

It was interesting to note that the SCFAs treatment of males decreased the abundance of plasma PI - 18:0/20:4, relative to 18:0/22:6 PI which was increased, compared to controls. The alterations in plasma fatty acyl species suggest an effect of SCFAs on lipoproteins as there was no effect of SCFAs on hepatic PI. An increase in the relative abundance of plasma DHA after SCFA treatment is consistent with plasma PC and PE, emphasizing that SCFAs likely have anti-inflammatory effects. On the other hand, females showed alternations in the hepatic PE, LPE, PS and PI species, suggesting changes in the activity of elongase and desaturase enzymes (Kitson, Stroud, and Stark 2010).

Males had significantly higher relative abundance of plasma 16:0/20:4 PI, compared to females. Females tend to have higher abundance of 20:4 and 22:6 due to increased desaturase activity (Kitson et al. 2012). However, an increased level of plasma 16:0/20:4 PI in males indicates the effects of SCFAs observed on PI composition is due to the action of phospholipases, and not hepatic desaturases (Beerman C et al., 2005). Moreover, it is interesting to observe that PI species containing C16:0 at the *sn*-1 position was higher in males than females, whereas those containing C18:0 at the *sn*-1 position were lower than female groups. These results are consistent with a study by Marks et al. (2013) where they show that female Sprague-Dawley rats have a decreased abundance of 16:0, and an increased abundance of 18:0 in liver phospholipids, compared to male rats (Marks, Kitson, and Stark 2013). This group suggested that sex-differences are due to an increased expression of elongase 6 enzyme in females, which is mediated by sex-hormones.

4.1.4.5. Effect of SCFAs on plasma PA fatty acid composition

PA is generated by the phosphorylation of the glycerol moiety and addition of two acyl chains during TG biosynthesis. Once formed, the phosphate group at the *sn*-3 position is esterified by an alcohol group (from choline, ethanolamine or serine) to generate PC, PE and PS, respectively (van der Veen et al. 2017). Thus, PA acts as the precursor for the biosynthesis of all the other PLs (D. E. Vance and Vance 2008). The alternations observed in the fatty acid composition of other PLs can be attributed to changes in the PA fatty acid composition. In the current study, SCFAs treatment showed a significant decrease in the relative abundance of plasma 16:0/18:1 PA but increase in plasma 16:0/18:2, 18:2/18:2, and 18:0/20:4 PA (n-6 PUFAs) in males, compared to its controls. On the other hand, females treated with SCFAs only showed a significant decrease in the relative abundance of plasma 18:0/18:1 PA, but no effect on PA containing n-6 PUFAs, compared to its controls. Considering that PA acts as precursor for synthesis of other PLs, it is possible that the decrease in plasma MUFAs in plasma PlsPE and PS could be due to a decrease in plasma PA - 18:1 and an increase in 18:2 and 20:4 species in PC, PE and PS lipids, is due to an increase in plasma PA - 18:2 and 20:4. This is because PA acts as a precursor for the synthesis of other PLs via the CDP-choline/ethanolamine or PSD pathway (Hatch and Choy 2004; D. E. Vance and Vance 2008). I have tried to propose the phospholipid remodeling alterations in MUFAs and PUFAs compositions by SCFAs treatment depicted in Figure 4.3.

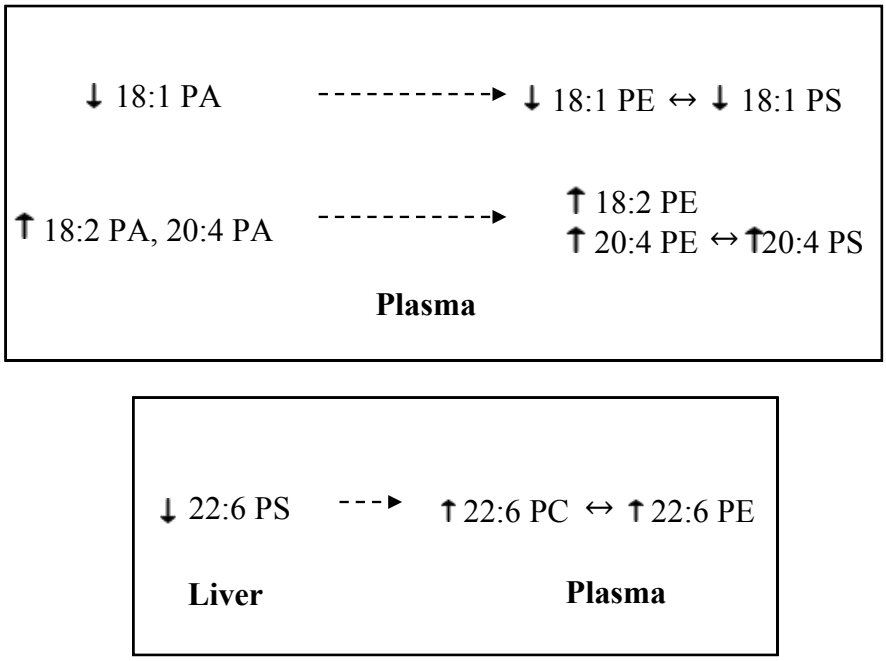


Figure 4.3 Proposed phospholipid remodeling by SCFA treatment. A decrease in PA abundance may decrease the abundance of PE and PS, and a decrease in 22:6 PS might relatively increase the abundance of PC and PE composed of DHA. PA – phosphatidic acid, PC – phosphatidylcholine, PE – phosphatidylethanolamine and PS – phosphatidylserine.

Chapter 5 - Conclusion and Future Directions

In summary, males treated with SCFAs showed a significant decrease in plasma and hepatic TC and TG levels, plasma glucose levels, plasma SFAs (18:0/16:0 PC, 18:0p/18:1 PE) and MUFAs (18:0/16:1 PC, 18:0/18:1 PS, 18:0/18:1 PA), with a relative increase in the abundance of PUFAs (16:0/18:2, 18:0/20:4, 16:0/22:6, 18:0/22:5, 18:0/22:6 - PC; 18:0/18:2, 18:0/20:4, 16:0/22:6 - PE; 18:0/20:4, 18:0/22:6 - PS; 18:0/22:6 PI; 16:0/18:2, 18:2/18:2, 18:0/20:4 - PA); compared to their respective controls. However, there was no effect of SCFAs on the relative abundance of hepatic phospholipids except for an increase in 18:1 LPC and a decrease in 18:0/22:6 PS, compared to respective controls. This indicates that the biosynthesis of LCFAs is not affected by SCFAs in males. The changes in plasma lipidomic profile suggests that SCFAs have a significant effect on the process of phospholipid remodeling in circulation.

Females treated with SCFAs showed a similar effect on plasma PLs, where MUFAs (18:0/18:1 PS, 16:0/18:1 PA) abundance was significantly reduced, relative to an increased abundance of PUFAs (18:0/22:6 PC; 18:0/18:2 and 18:0/20:4 PE; 18:0/20:4 and 22:4/22:6 PS), compared to its controls. Furthermore, SCFA treated females also showed an effect on hepatic PLs, where a significant decrease was observed in the abundance of SFAs (16:0 LPC) and MUFAs (18:1 LPC; PE composed of 16:0/18:1, 16:0p/18:1 and 18:0p/18:1), with relative increase in the abundance of PUFAs (20:4 LPC; 18:0/20:4 and 18:0/22:6 PE; 18:0/20:4 PS; 18:0/20:4 PI); compared to their respective controls. It was also observed that there was decrease in the relative abundance of plasma and hepatic PS composed of 22:6 (16:0/22:6, 18:0/22:6, 18:2/22:6 PS), hepatic 16:0/20:4 and 16:0/22:6 PI. This suggests that unlike males, SCFAs affect hepatic metabolism of the females. The alternations in hepatic profile of PLs by the SCFAs mixture can

be attributed to changes in hepatic ACC1 which might have an effect on other enzymes involved with biosynthesis (SCD, D5D, D6D, ELOVL) and transfer (LCAT, LPCAT) of PUFAs.

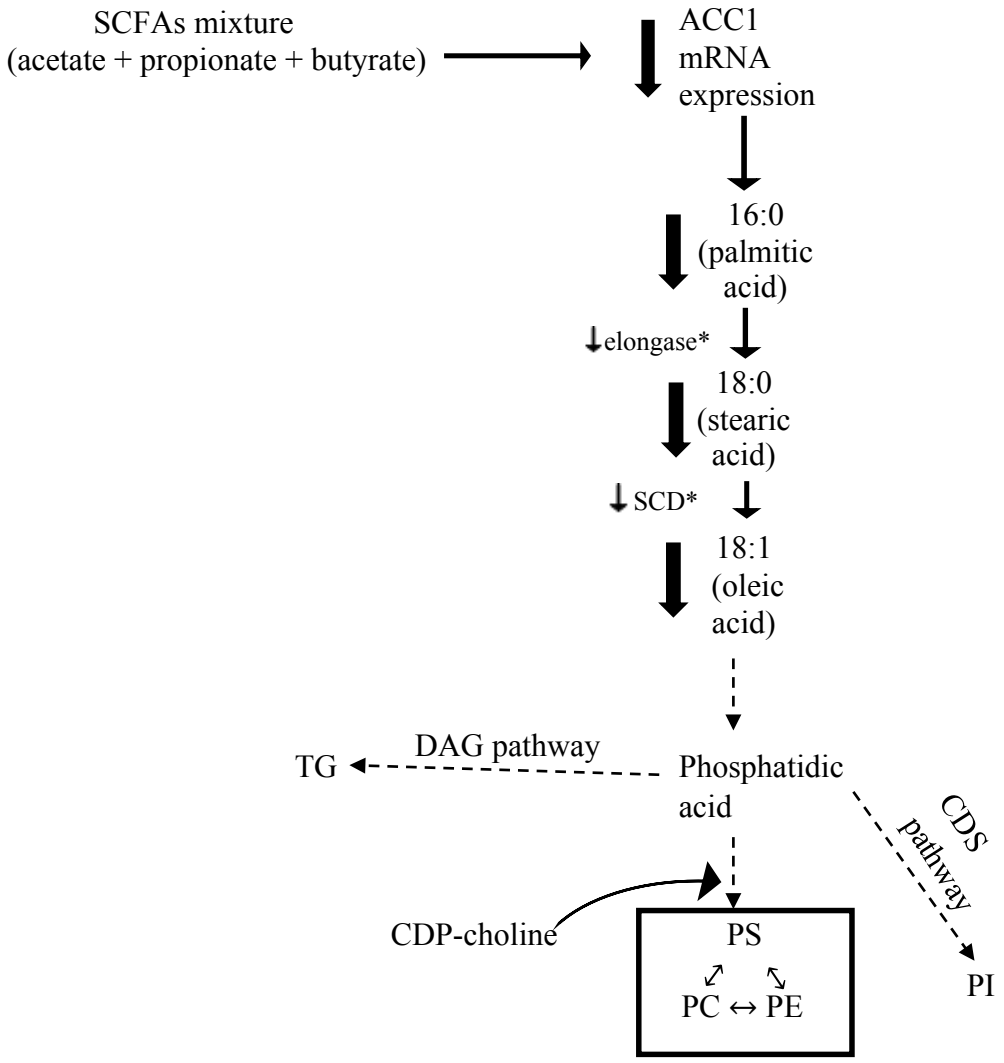


Figure 5.1 Proposed pathway for phospholipid metabolism in female rats treated with SCFAs. ACC1 – acetyl CoA carboxylase, CDS – cytidine diphosphate synthase, CDP – cytidine diphosphate, DAG – diacylglycerol, PA – phosphatidic acid, PC – phosphatidylcholine, PE – phosphatidylethanolamine, PI – phosphoinositol, PS – phosphatidylserine, SCD – stearoyl CoA desaturase, SCFAs – short chain fatty acids and TG – triacylglycerols.

A diversity in the composition of lipid molecular species is due to remodeling of PUFAs into cellular PLs. One of the most researched phospholipid remodeling pathway is the Lands cycle which is responsible for changes in membrane composition by the remodeling of AA amongst various phospholipids (Hatch and Choy 2004). The Lands cycle involves the action of two enzyme classes - acyl CoA synthases and acyl transferases such as LPCAT3/MBOAT5 and LPCAT1/MBOAT7 to generate CoA esters of PUFAs, which further incorporates AA into PC and PI species (Okuno et al. 2018). In the current study, the increase in hepatic 20:4 LPC could be responsible for the increase in the relative abundance of 18:0/20:4 species in hepatic PE and PS, and a decrease in hepatic 18:0/20:4 PI, indicating a higher activity of LPCAT3 and a lower activity of LPCAT1 in females treated with SCFAs. Enrichment of AA by LPCAT3 in membrane lipids have shown an efficient transfer of TG species by reducing the accumulation of TG in hepatocytes and enterocytes and promoting clustering of TG in high-density lipoproteins (Hashidate-Yoshida et al. 2015).

In addition to phospholipid remodeling, SCFAs might also have an effect on LCAT and lipase enzyme activities in females. This is because females treated with SCFAs showed a significant decrease in the abundance of plasma 20:4 ChE relative to hepatic 20:4 LPC; and also, showed a decreased SFAs (18:0/16:0/18:0, 18:0/18:0/18:0) in plasma TG species; compared to respective controls. Thus, future studies should involve investigating the activity of enzymes involved in lipid remodeling, elongation and desaturation. It was also interesting to find that all the PLs species containing 18:0 at the *sn*-1 position increased in abundance, compared to 16:0 at the *sn*-1 position.

It is clearly evident that SCFAs have sex-specific effects on lipid metabolism and the lipidomic profile. SCFAs decreased the levels of plasma and hepatic TC, ChE, TG and glucose in males, but there was no effect in females, compared to their respective controls. However, females showed more prominent effects of SCFAs on plasma and hepatic lipidomics profile. The decreased abundance of arachidonyl cholesteryl ester, TG composed of SFAs (18:0 and 16:0) and PLs composed of MUFAs (18:1) with relatively increased abundance of PUFAs (20:4 and 22:6) in plasma and liver indicates the role of SCFAs in inflammatory processes. Overall, my findings suggest that SCFAs have beneficial effects in both sexes; however, the effects may involve different pathways in males and females.

BIBLIOGRAPHY

Alard, Jeanne et al. 2015. "Beneficial Metabolic Effects of Selected Probiotics on Diet-Induced Obesity and Insulin Resistance in Mice Are Associated with Improvement of Dysbiotic Gut Microbiota." *Environmental microbiology* 18(33): 1–30.

Alberti, K. George M.M., Paul Zimmet, and Jonathan Shaw. 2005. "The Metabolic Syndrome - A New Worldwide Definition." *Lancet* 366(9491): 1059–62.

Aoyama, Michiko, Joji Kotani, and Makoto Usami. 2010. "Butyrate and Propionate Induced Activated or Non-Activated Neutrophil Apoptosis via HDAC Inhibitor Activity but without Activating GPR-41/GPR-43 Pathways." *Nutrition* 26(6): 653–61.

Arpaia, Nicholas et al. 2014. "HHS Public Access." 504(7480): 451–55.

Ballard, F John. 1974. "Production and Utilization of Acetate in Mammals." : 401–11.

Barber, Michael C., Nigel T. Price, and Maureen T. Travers. 2005. "Structure and Regulation of Acetyl-CoA Carboxylase Genes of Metazoa." *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*.

Basu, R et al. 2006. "Effects of Age and Sex on Postprandial Glucose Metabolism: Differences in Glucose Turnover, Insulin Secretion, Insulin Action, and Hepatic Insulin Extraction." *Diabetes* 55(7): 2001–14.

Berg, Jeremy M, John L Tymoczko, and Lubert Stryer. 2002. "Glycolysis Is an Energy-Conversion Pathway in Many Organisms." In *Biochemistry*, , section 16.1.

Bergman, E N. 1990. "Energy Contributions of Volatile Fatty Acids from the Gastrointestinal Tract in Various Species." *Physiological Reviews* 70(2): 567 LP-590.

den Besten, Gijs et al. 2013. “The Role of Short-Chain Fatty Acids in the Interplay between Diet, Gut Microbiota, and Host Energy Metabolism.” *Journal of Lipid Research* 54(9): 2325–40.

Den Besten, Gijs et al. 2015. “Short-Chain Fatty Acids Protect against High-Fat Diet-Induced Obesity via a Pparg-Dependent Switch from Lipogenesis to Fat Oxidation.” *Diabetes* 64(7): 2398–2408.

Binder, Henry J. 2010. “Role of Colonic Short-Chain Fatty Acid Transport in Diarrhea.” *Annual Review of Physiology* 72: 297–313.

Bligh, E.G. and Dyer, W.J. 1959. “A Raoid Method of Total Lipid Exytaction and Purification.” *Canadian Journal of Biochemistry and Physiology* 37(8): 911–17.

Bloemen, Johanne G. et al. 2009. “Short Chain Fatty Acids Exchange across the Gut and Liver in Humans Measured at Surgery.” *Clinical Nutrition* 28(6): 657–61.

Boulangé, Claire L. et al. 2016. “Impact of the Gut Microbiota on Inflammation, Obesity, and Metabolic Disease.” *Genome Medicine* 8(1): 1–12.

Bowden, John A. et al. 2011. “Electrospray Ionization Tandem Mass Spectrometry of Sodiated Adducts of Cholesteryl Esters.” *Lipids*.

Braverman, Nancy E., and Ann B. Moser. 2012. “Functions of Plasmalogen Lipids in Health and Disease.” *Biochimica et Biophysica Acta - Molecular Basis of Disease* 1822(9): 1442–52.

Broniec, Agnieszka et al. 2011. “Interactions of Plasmalogens and Their Diacyl Analogs with Singlet Oxygen in Selected Model Systems.” *Free Radical Biology and Medicine* 50(7): 892–98.

Bryzgalova, G. et al. 2008. “Mechanisms of Antidiabetogenic and Body Weight-Lowering

Effects of Estrogen in High-Fat Diet-Fed Mice." *AJP: Endocrinology and Metabolism* 295(4): E904–12.

Byrne, C S, E S Chambers, D J Morrison, and G Frost. 2015. "The Role of Short Chain Fatty Acids in Appetite Regulation and Energy Homeostasis." *International journal of obesity (2005)* 39(9): 1331–38.

Cajka, Tomas, and Oliver Fiehn. 2014. "Comprehensive Analysis of Lipids in Biological Systems by Liquid Chromatography-Mass Spectrometry." *TrAC - Trends in Analytical Chemistry* 61: 192–206.

Canfora, Emanuel E., Johan W. Jocken, and Ellen E. Blaak. 2015. "Short-Chain Fatty Acids in Control of Body Weight and Insulin Sensitivity." *Nature Reviews. Endocrinology* 11(10): 577–91.

Chambers, E S et al. 2015. "Effects of Targeted Delivery of Propionate to the Human Colon on Appetite Regulation, Body Weight Maintenance and Adiposity in Overweight Adults." *Gut* 64(11): 1744–54.

Clausen, M R, and P B Mortensen. 1995. "Kinetic Studies on Colonocyte Metabolism of Short Chain Fatty Acids and Glucose in Ulcerative Colitis." *Gut* 37(5): 684–89.

Clemente, Jose C., Luke K. Ursell, Laura Wegener Parfrey, and Rob Knight. 2012. "The Impact of the Gut Microbiota on Human Health: An Integrative View." *Cell* 148(6): 1258–70.

Conlon, Michael A., and Anthony R. Bird. 2015. "The Impact of Diet and Lifestyle on Gut Microbiota and Human Health." *Nutrients* 7(1): 17–44.

Cordain, Loren et al. 2005. "Origins and Evolution of the Western Diet : Health Implications for

The." (June).

Corsetti, J P et al. 2000. "Effect of Dietary Fat on the Development of Non-Insulin Dependent Diabetes Mellitus in Obese Zucker Diabetic Fatty Male and Female Rats." *Atherosclerosis* 148(2): 231–41.

Cuche, G, J C Cuber, and C H Malbert. 2000. "Ileal Short-Chain Fatty Acids Inhibit Gastric Motility by a Humoral Pathway." *Am J Physiol Gastrointest Liver Physiol* 279: 925–30.

Cummings, J H et al. 1987. "Short Chain Fatty Acids in Human Large Intestine, Portal, Hepatic and Venous Blood." *Gut* 28(10): 1221–27.

D'Eon, Tara M. et al. 2005. "Estrogen Regulation of Adiposity and Fuel Partitioning: Evidence of Genomic and Non-Genomic Regulation of Lipogenic and Oxidative Pathways." *Journal of Biological Chemistry* 280(43): 35983–91.

David, Lawrence A. et al. 2014. "Diet Rapidly and Reproducibly Alters the Human Gut Microbiome." *Nature* 505(7484): 559–63.

Demigné, C et al. 1995. "Effect of Propionate on Fatty Acid and Cholesterol Synthesis and on Acetate Metabolism in Isolated Rat Hepatocytes." *The British journal of nutrition* 74(2): 209–19.

Dennis, Edward A., and Paul C. Norris. 2015. "Eicosanoid Storm in Infection and Inflammation." *Nature Reviews Immunology*.

Dorninger, Fabian et al. 2015. "Homeostasis of Phospholipids - The Level of Phosphatidylethanolamine Tightly Adapts to Changes in Ethanolamine Plasmalogens." *Biochimica et biophysica acta*.

Duarte, Joao A. G. et al. 2014. “A High-Fat Diet Suppresses de Novo Lipogenesis and Desaturation but Not Elongation and Triglyceride Synthesis in Mice.” *Journal of Lipid Research* 55(12): 2541–53.

Duncan, Sylvia H., Petra Louis, and Harry J. Flint. 2004. “Lactate-Utilizing Bacteria, Isolated from Human Feces, That Produce Butyrate as a Major Fermentation Product.” *Applied and Environmental Microbiology* 70(10): 5810–17.

Eaton, S. Boyd. 2006. “The Ancestral Human Diet: What Was It and Should It Be a Paradigm for Contemporary Nutrition?” *Proceedings of the Nutrition Society* 65(01): 1–6.

Edge, Leading. 2016. “Review From Dietary Fiber to Host Physiology : Short-Chain Fatty Acids as Key Bacterial Metabolites.”

Falkenburger, Björn H. et al. 2010. “Phosphoinositides: Lipid Regulators of Membrane Proteins.” In *Journal of Physiology*,.

Feingold, K R, and C Grunfeld. 2015. “Endotext: Introduction to Lipids and Lipoproteins.” *NCBI Bookshelf*.

Field, F. Jeffrey, Nathan T.P. Kam, and Satya N. Mathur. 1990. “Regulation of Cholesterol Metabolism in the Intestine.” *Gastroenterology*.

Folch, Jordi, M Lees, and G H Sloane Stanley. 1957. “A Simple Method for the Isolation and Purification of Total Lipids from Animal Animal Tissues.” *The Journal of Biological Chemistry* 226: 497–509.

Fouhy, Fiona et al. 2012. “Knowledge , Knowledge Gaps and the Use of High-Throughput Sequencing to Address These Gaps © 2012 Landes Bioscience . Do Not Distribute . © 2012

Landes Bioscience .” (June): 203–20.

Freeland, Kristin R., and Thomas M.S. Wolever. 2010. “Acute Effects of Intravenous and Rectal Acetate on Glucagon-like Peptide-1, Peptide YY, Ghrelin, Adiponectin and Tumour Necrosis Factor-α.” *British Journal of Nutrition* 103(3): 460–66.

Frias, Juan P. et al. 2001. “Decreased Susceptibility to Fatty Acid-Induced Peripheral Tissue Insulin Resistance in Women.” *Diabetes* 50(6): 1344–50.

Frost, Gary et al. 2014. “The Short-Chain Fatty Acid Acetate Reduces Appetite via a Central Homeostatic Mechanism.” *Nature Communications*.

Fushimi, Takashi et al. 2006. “Dietary Acetic Acid Reduces Serum Cholesterol and Triacylglycerols in Rats Fed a Cholesterol-Rich Diet.” *The British journal of nutrition* 95(5): 916–24.

Gao, Hui et al. 2006. “Long-Term Administration of Estradiol Decreases Expression of Hepatic Lipogenic Genes and Improves Insulin Sensitivity in Ob/Ob Mice: A Possible Mechanism Is through Direct Regulation of Signal Transducer and Activator of Transcription 3.” *Molecular Endocrinology* 20(6): 1287–99.

Gao, Zhanguo et al. 2009. “Butyrate Improves Insulin Sensitivity and Increases Energy Expenditure in Mice.” *Diabetes* 58(7): 1509–17.

Georgiadi, Anastasia, and Sander Kersten. 2012. “Mechanisms of Gene Regulation by Fatty Acids 1 , 2.” (2): 127–34.

Grimm, Marcus O. W. et al. 2011. “Intracellular APP Domain Regulates Serine-Palmitoyl-CoA Transferase Expression and Is Affected in Alzheimer’s Disease.” *International Journal of*

Alzheimer's Disease 2011: 1–8.

Hadjiagapiou, C et al. 2000. "Mechanism(s) of Butyrate Transport in Caco-2 Cells: Role of Monocarboxylate Transporter 1." *American journal of physiology. Gastrointestinal and liver physiology* 279(4): G775–80.

Hale, P J, J V Wright, and M Nattrass. 1985. "Differences in Insulin Sensitivity between Normal Men and Women." *Metabolism: clinical and experimental* 34(12): 1133–38.

Han, Xianlin, and Richard W. Gross. 1990. "Plasmenylcholine and Phosphatidylcholine Membrane Bilayers Possess Distinct Conformational Motifs." *Biochemistry* 29(20): 4992–96.

Han and Richard W. Gross. 2005. "Shotgun Lipidomics: Electrospray Ionization Mass Spectrometric Analysis and Quantitation of Cellular Lipidomes Directly from Crude Extracts of Biological Samples." *Mass Spectrometry Reviews* 24(3): 367–412.

Hara, H, S Haga, Y Aoyama, and S Kiriyama. 1999. "Short-Chain Fatty Acids Suppress Cholesterol Synthesis in Rat Liver and Intestine." *The Journal of nutrition* 129(5): 942–48.

Hara, H, S Haga, T Kasai, and S Kiriyama. 1998. "Fermentation Products of Sugar-Beet Fiber by Cecal Bacteria Lower Plasma Cholesterol Concentration in Rats." *J Nutr*.

Harkewicz, Richard et al. 2008. "Cholesteryl Ester Hydroperoxides Are Biologically Active Components of Minimally Oxidized Low Density Lipoprotein." *The Journal of biological chemistry*.

Hashidate-Yoshida, Tomomi et al. 2015. "Fatty Acyl-Chain Remodeling by LPCAT3 Enriches Arachidonate in Phospholipid Membranes and Regulates Triglyceride Transport." *eLife*

2015(4): 1–75.

Hatch, Grant M., and Patrick C. Choy. 2004. “Phospholipid Biosynthesis.” *Advances in Molecular and Cell Biology*.

Hernandez, Teri L. et al. 2011. “Fat Redistribution Following Suction Lipectomy: Defense of Body Fat and Patterns of Restoration.” *Obesity* 19(7): 1388–95.

Hijova, E., and A. Chmelarova. 2007. “Short Chain Fatty Acids and Colonic Health.” *Bratislavsk?? lek??rske listy* 108(8): 354–58.

Hijova, E. 2007. “Short Chain Fatty Acids and Colonic Health.” *Bratisl Lek Listy* 108(8): 354–58.

Hishikawa, D. et al. 2008. “Discovery of a Lysophospholipid Acyltransferase Family Essential for Membrane Asymmetry and Diversity.” *Proceedings of the National Academy of Sciences* 105(8): 2830–35.

Holscher, Hannah D. 2017. “Dietary Fi Ber and Prebiotics and the Gastrointestinal Microbiota.” 8(2): 172–84.

Horrocks, Lloyd A., and Mukut Sharma. 1982. “Plasmalogens and O-Alkyl Glycerophospholipids.” *New Comprehensive Biochemistry* 4(C): 51–93.

Irvine, Robin F. 2016. “A Short History of Inositol Lipids.” *Journal of Lipid Research*.

Innis SM al., 1993. "Saturated fatty acid chain length and positional distribution in infant formula: effects on growth and plasma lipids and ketones in piglets". Am J Clin Nutr 57(3):382-90.

Karupaiah, Tilakavati, and Kalyana Sundram. 2007. “Effects of Stereospecific Positioning of

Fatty Acids in Triacylglycerol Structures in Native and Randomized Fats: A Review of Their Nutritional Implications." *Nutrition and Metabolism*.

Kimura, Ikuo et al. 2013. "The Gut Microbiota Suppresses Insulin-Mediated Fat Accumulation via the Short-Chain Fatty Acid Receptor GPR43." *Nature Communications* 4.

Kitson, Alex P., Tracy L. Smith, Kristin A. Marks, and Ken D. Stark. 2012. "Tissue-Specific Sex Differences in Docosahexaenoic Acid and Δ6-Desaturase in Rats Fed a Standard Chow Diet." *Applied Physiology, Nutrition, and Metabolism*.

Kitson, Alex P., Chad K. Stroud, and Ken D. Stark. 2010. "Elevated Production of Docosahexaenoic Acid in Females: Potential Molecular Mechanisms." *Lipids*.

Koliada, Alexander et al. 2017. "Association between Body Mass Index and Firmicutes/Bacteroidetes Ratio in an Adult Ukrainian Population." *BMC Microbiology* 17(1): 4–9.

Lands, Bill. 2015. "Omega-3 PUFAs Lower the Propensity for Arachidonic Acid Cascade Overreactions." *BioMed Research International*.

Larsen, Nadja, and FK Vogensen. 2010. "Gut Microbiota in Human Adults with Type 2 Diabetes Differs from Non-Diabetic Adults." *PloS one* 5(2): e9085.

Law, M D, Henry Field, and The George. 1980. "Of." 235(7): 6–9.

LeBlanc, Jean Guy et al. 2017. "Beneficial Effects on Host Energy Metabolism of Short-Chain Fatty Acids and Vitamins Produced by Commensal and Probiotic Bacteria." *Microbial Cell Factories* 16(1).

Lehner, Richard, and A. Kuksis. 1996. "Biosynthesis of Triacylglycerols." *Progress in Lipid*

Research 35(2): 169–201.

Lewis-Barned, N. J. et al. 2000. "Plasma Cholesteryl Ester Fatty Acid Composition, Insulin Sensitivity, the Menopause and Hormone Replacement Therapy." *Journal of Endocrinology*.

Li, Ya-feng et al. 2016. "Atherosclerosis." 21(1): 70–88.

Lin, Hua V. et al. 2012. "Butyrate and Propionate Protect against Diet-Induced Obesity and Regulate Gut Hormones via Free Fatty Acid Receptor 3-Independent Mechanisms." *PLoS ONE* 7(4).

Link, Jenny C, Xuqi Chen, Arthur P Arnold, and K Reue. 2013. "Metabolic Impact of Sex Chromosomes." *Mini-Review Adipocyte* 2(2): 74–79.

Livak, K J, and T D Schmittgen. 2001. "Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-ΔΔCT Method." *Methods* 25: 402–8.

Lu, Yuanyuan et al. 2016. "Short Chain Fatty Acids Prevent High-Fat-Diet-Induced Obesity in Mice by Regulating G Protein-Coupled Receptors and Gut Microbiota." *Scientific Reports* 6(1): 37589.

Macotela, Yazmin, Jeremie Boucher, Thien T. Tran, and C. Ronald Kahn. 2009. "Sex and Depot Differences in Adipocyte Insulin Sensitivity and Glucose." *Diabetes* 58(4): 803–12.

Marks, Kristin A., Alex P. Kitson, and Ken D. Stark. 2013. "Hepatic and Plasma Sex Differences in Saturated and Monounsaturated Fatty Acids Are Associated with Differences in Expression of Elongase 6, but Not Stearoyl-CoA Desaturase in Sprague-Dawley Rats." *Genes and Nutrition*.

Martínez-Uña, Maite et al. 2015. "S-Adenosylmethionine Increases Circulating Very-Low Density Lipoprotein Clearance in Non-Alcoholic Fatty Liver Disease." *Journal of Hepatology* 62(3): 673–81.

Mcneil, N I, J H Cummings, and W P T James. 1978. "Short Chain Fatty Acid Absorption by the Human Large Intestine." *Gut* 19: 819–22.

Michael W King. 2016. "Cholesterol: Synthesis, Metabolism, Regulation." *The Medical Biochemistry Page*.

Miller, Terry L., and Meyer J. Wolin. 1996. "Pathways of Acetate, Propionate, and Butyrate Formation by the Human Fecal Microbial Flora." *Applied and Environmental Microbiology* 62(5): 1589–92.

Millet, S. et al. 2010. "Prediction of in Vivo Short-Chain Fatty Acid Production in Hindgut Fermenting Mammals: Problems and Pitfalls." *Critical Reviews in Food Science and Nutrition* 50(7): 605–19.

Mollica, Maria Pina et al. 2017. "Butyrate Regulates Liver Mitochondrial Function, Efficiency, and Dynamic, in Insulin Resistant Obese Mice." *Diabetes* 66(February): db160924.

Moon, Young-Ah, Robert E. Hammer, and Jay D. Horton. 2009. "Deletion of ELOVL5 Leads to Fatty Liver through Activation of SREBP-1c in Mice." *Journal of Lipid Research* 50(3): 412–23.

Morrison, Douglas J., and Tom Preston. 2016. "Formation of Short Chain Fatty Acids by the Gut Microbiota and Their Impact on Human Metabolism." *Gut Microbes* 7(3): 189–200.

Moukarzel, S. et al. 2016. "Human Milk Plasmalogens Are Highly Enriched in Long-Chain

PUFAs." *Journal of Nutrition*.

Mu, Huiling, and Trine Porsgaard. 2005. "The Metabolism of Structured Triacylglycerols." *Progress in Lipid Research* 44(6): 430–48.

Mudgil, Deepak, and Sheweta Barak. 2013. "Composition, Properties and Health Benefits of Indigestible Carbohydrate Polymers as Dietary Fiber: A Review." *International Journal of Biological Macromolecules* 61: 1–6.

Ohira, Hideo, Wao Tsutsui, and Yoshio Fujioka. 2017. "Are Short Chain Fatty Acids in Gut Microbiota Defensive Players for Inflammation and Atherosclerosis?" *Journal of Atherosclerosis and Thrombosis* 24(7): 660–72. https://www.jstage.jst.go.jp/article/jat/24/7/24_RV17006/_article.

Okuno, T. et al. 2018. "Altered Eicosanoid Production and Phospholipids Remodeling during Cell Culture." *Journal of Lipid Research*.

Opazo, Maria C et al. 2018. "Intestinal Microbiota Influences Non-Intestinal Related Autoimmune Diseases." 9(March): 1–20.

Orešič, Matej et al. 2012. "Phospholipids and Insulin Resistance in Psychosis: A Lipidomics Study of Twin Pairs Discordant for Schizophrenia." *Genome Medicine* 4(1).

Parsons, Kevin J., W. James Cooper, and R. Craig Albertson. 2009. "Limits of Principal Components Analysis for Producing a Common Trait Space: Implications for Inferring Selection, Contingency, and Chance in Evolution." *PLoS ONE* 4(11).

Pradas, Irene et al. 2018. "Lipidomics Reveals a Tissue-Specific Fingerprint." *Frontiers in Physiology*.

Pryde, Susan E. et al. 2002. “The Microbiology of Butyrate Formation in the Human Colon.” *FEMS Microbiology Letters* 217(2): 133–39.

Ragsdale, Stephen W., and Elizabeth Pierce. 2008. “Acetogenesis and the Wood-Ljungdahl Pathway of CO2fixation.” *Biochimica et Biophysica Acta - Proteins and Proteomics* 1784(12): 1873–98.

Ratnayake, W. M.Nimal, and Claudio Galli. 2009. “Fat and Fatty Acid Terminology, Methods of Analysis and Fat Digestion and Metabolism: A Background Review Paper.” *Annals of Nutrition and Metabolism* 55(1–3): 8–43.

Remely, M et al. 2014. “Effects of Short Chain Fatty Acid Producing Bacteria on Epigenetic Regulation of FFAR3 in Type 2 Diabetes and Obesity.” *Gene* 537(1): 85–92.

Rey, Federico E. et al. 2010. “Dissecting the in Vivo Metabolic Potential of Two Human Gut Acetogens.” *Journal of Biological Chemistry* 285(29): 22082–90.

Rodrı, Juan Miguel et al. 2015. “The Composition of the Gut Microbiota throughout Life, with an Emphasis on Early Life.” 1: 1–17.

Rong, Xin et al. 2015. “Lpcat3-Dependent Production of Arachidonoyl Phospholipids Is a Key Determinant of Triglyceride Secretion.” *eLife* 2015(4).

Rosqvist, Fredrik et al. 2017. “Fatty Acid Composition in Serum Cholesterol Esters and Phospholipids Is Linked to Visceral and Subcutaneous Adipose Tissue Content in Elderly Individuals: A Cross-Sectional Study.” *Lipids in Health and Disease* 16(1).

Rui, Liangyou. 2014. “Energy Metabolism in the Liver.” *Comprehensive Physiology*.

Saito, Isao. 2012. “Epidemiological Evidence of Type 2 Diabetes Mellitus, Metabolic Syndrome,

and Cardiovascular Disease in Japan." *Circulation journal : official journal of the Japanese Circulation Society* 76(5): 1066–73.

Saito, Kosuke et al. 2017. "Arachidonic Acid-Containing Phosphatidylcholine Characterized by Consolidated Plasma and Liver Lipidomics as an Early Onset Marker for Tamoxifen-Induced Hepatic Phospholipidosis." *Journal of Applied Toxicology*.

Sakakibara, Shoji et al. 2006. "Acetic Acid Activates Hepatic AMPK and Reduces Hyperglycemia in Diabetic KK-A(y) Mice." *Biochemical and Biophysical Research Communications* 344(2): 597–604.

Salomaa, V et al. 1990. "Fatty Acid Composition of Serum Cholesterol Esters in Different Degrees of Glucose Intolerance: A Population-Based Study." *Metabolism: clinical and experimental*.

Schönfeld, Peter, and Lech Wojtczak. 2016. 57 Journal of Lipid Research *Short- and Medium-Chain Fatty Acids in Energy Metabolism: The Cellular Perspective*.

Scorletti, E, and C D Byrne. 2013. "Omega-3 Fatty Acids, Hepatic Lipid Metabolism, and Nonalcoholic Fatty Liver Disease." *Annual Review of Nutrition* 33: 231–48. http://www.ncbi.nlm.nih.gov/pubmed/23862644.

Snijder, M. B. et al. 2005. "Low Subcutaneous Thigh Fat Is a Risk Factor for Unfavourable Glucose and Lipid Levels, Independently of High Abdominal Fat. The Health ABC Study." *Diabetologia* 48(2): 301–8.

Soeters, Maarten R. et al. 2007. "Gender-Related Differences in the Metabolic Response to Fasting." *Journal of Clinical Endocrinology and Metabolism* 92(9): 3646–52.

Sorci-Thomas, Mary G., Shaila Bhat, and Michael J. Thomas. 2009. "Activation of Lecithin: Cholesterol Acyltransferase by HDL ApoA-I Central Helices." *Future Lipidology*.

Stables, MJ, and DW Gilroy. 2011. "Old and New Generation Lipid Mediators in Acute Inflammation and Resolution." *progress in lipid research* 50(1): 35–51.

Sugiyama, Michael G., and Luis B. Agellon. 2012. "Sex Differences in Lipid Metabolism and Metabolic Disease Risk." *Biochemistry and Cell Biology* 90(2): 124–41. http://www.nrcresearchpress.com/doi/10.1139/o11-067.

The Liver Meeting 2014 Abstracts, 60(1). et al. 2007. "Prevalence of Nonalcoholic Fatty Liver Disease and Its Association with Cardiovascular Disease among Type 2 Diabetic Patients." *Diabetes care*.

Tlc-fid, Iatroscan, Iatroscan Tlc-fid, Iatroscan Tlc-fid, and Iatron Laboratories. 2003. "Quantitation of Lipid Classes by Thin-Layer Chromatography with Flame Ionization." : 1–14.

Topping, David L, and Peter M Clifton. 2001. "Short-Chain Fatty Acids and Human Colonic Function: Roles of Resistant Starch and Nonstarch Polysaccharides." *Physiological Reviews* 81(3): 1031 LP-1064.

Trevaskis, James L., Emily A. Meyer, Jose E. Galgani, and Andrew A. Butler. 2008. "Counterintuitive Effects of Double-Heterozygous Null Melanocortin-4 Receptor and Leptin Genes on Diet-Induced Obesity and Insulin Resistance in C57BL/6J Mice." *Endocrinology* 149(1): 174–84.

Turnbaugh, Peter et al. 2005. "Obesity Alters Gut Microbial Ecology." 015671.

De Vadder, Filipe et al. 2014. “Microbiota-Generated Metabolites Promote Metabolic Benefits via Gut-Brain Neural Circuits.” *Cell* 156(1–2): 84–96.

VAGUE, J. 1956. “The Degree of Masculine Differentiation of Obesities: A Factor Determining Predisposition to Diabetes, Atherosclerosis, Gout, and Uric Calculous Disease.” *The American journal of clinical nutrition* 4(1): 20–34.

Vance, Dennis E., and Jean E. Vance. 2008. “Phospholipid Biosynthesis in Eukaryotes.” In *Biochemistry of Lipids, Lipoproteins and Membranes*,.

Vance, Jean E. 1990. “Lipoproteins Secreted by Cultured Rat Hepatocytes Contain the Antioxidant 1-Alk-1-Enyl-2-Acylglycerophosphoethanolamine.” *Biochimica et Biophysica Acta (BBA)/Lipids and Lipid Metabolism* 1045(2): 128–34.

Varlamov, O, and C L Bethea. 2015. “Sex-Specific Differences in Lipid and Glucose Metabolism.” *Frontiers in …*.

Varlamov, Oleg, Cynthia L. Bethea, and Charles T. Roberts. 2014. “Sex-Specific Differences in Lipid and Glucose Metabolism.” *Frontiers in Endocrinology* 5(DEC).

Vásquez, Valeria, Michael Krieg, Dean Lockhead, and Miriam B. Goodman. 2014. “Phospholipids That Contain Polyunsaturated Fatty Acids Enhance Neuronal Cell Mechanics and Touch Sensation.” *Cell Reports*.

van der Veen, Jelske N. et al. 2017. “The Critical Role of Phosphatidylcholine and Phosphatidylethanolamine Metabolism in Health and Disease.” *Biochimica et Biophysica Acta - Biomembranes* 1859(9): 1558–72.

Van Der Veen, Jelske N., Susanne Lingrell, and Dennis E. Vance. 2012. “The Membrane Lipid

Phosphatidylcholine Is an Unexpected Source of Triacylglycerol in the Liver." *Journal of Biological Chemistry* 287(28): 23418–26.

Vidal, Natalia P. et al. 2018. "The Use of Natural Media Amendments to Produce Kale Enhanced with Functional Lipids in Controlled Environment Production System." *Scientific Reports* 8(1): 14771.

Vidyasagar, Sadasivan et al. 2019. "Role of Short-Chain Fatty Acids in Colonic HCO 3 Secretion." 06520: 1217–26.

Vinolo, Marco A R et al. 2011. "Suppressive Effect of Short-Chain Fatty Acids on Production of Proinflammatory Mediators by Neutrophils." *Journal of Nutritional Biochemistry*.

Viollet, B. et al. 2009. "AMP-Activated Protein Kinase in the Regulation of Hepatic Energy Metabolism: From Physiology to Therapeutic Perspectives." In *Acta Physiologica*,.

Vrieze, A. et al. 2010. "The Environment within: How Gut Microbiota May Influence Metabolism and Body Composition." *Diabetologia* 53(4): 606–13.

Wang, Yun et al. 2006. "Regulation of Hepatic Fatty Acid Elongase and Desaturase Expression in Diabetes and Obesity." *Journal of Lipid Research* 47(9): 2028–41.

Watson, J. Throck., and O. David. Sparkman. 2007. 4. Edition, John Wiley and Sons Chichester *Introduction to Mass Spectrometry: Instrumentation, Applications and Strategies for Data Interpretation.*

Whitman, W. B., D. C. Coleman, and W. J. Wiebe. 1998. "Prokaryotes: The Unseen Majority." *Proceedings of the National Academy of Sciences* 95(12): 6578–83.

Wong, Julia M.W. et al. 2006. "Colonic Health: Fermentation and Short Chain Fatty Acids." In

Journal of Clinical Gastroenterology, , 235–43.

X., Han, Holtzman D.M., and McKeel Jr. D.W. 2001. "Plasmalogen Deficiency in Early Alzheimer's Disease Subjects and in Animal Models: Molecular Characterization Using Electrospray Ionization Mass Spectrometry." *Journal of Neurochemistry* 77(4): 1168–80.

Yamashita, Hiromi et al. 2007. "Improvement of Obesity and Glucose Tolerance by Acetate in Type 2 Diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) Rats." *Bioscience, Biotechnology, and Biochemistry* 71(5): 1236–43.

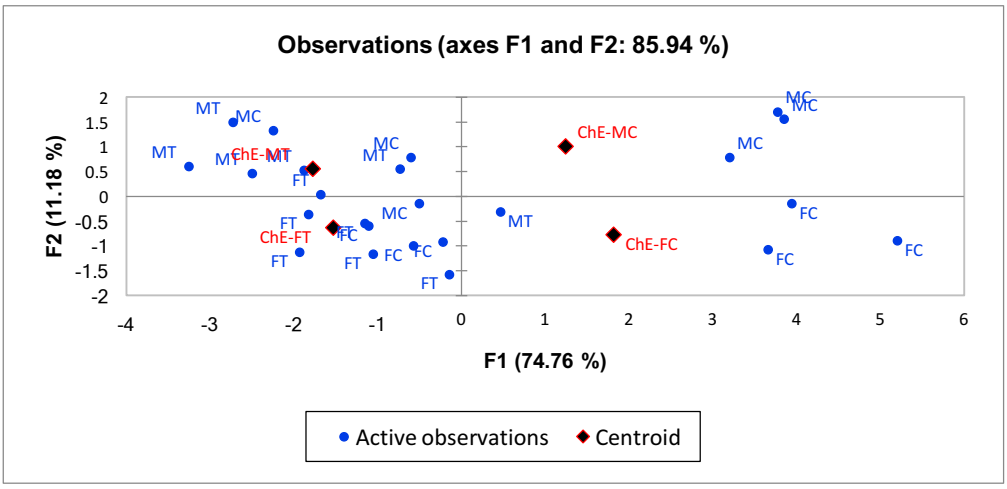
Zhao, Yimin et al. 2017. "Structure-Specific Effects of Short-Chain Fatty Acids on Plasma Cholesterol Concentration in Male Syrian Hamsters." *Journal of Agricultural and Food Chemistry*.

Zhou, J. et al. 2008. "Dietary Resistant Starch Upregulates Total GLP-1 and PYY in a Sustained Day-Long Manner through Fermentation in Rodents." *AJP: Endocrinology and Metabolism* 295(5): E1160–66.

Zoetendal A.D.L. Akkermans, W.M. Akkermans van-Vliet, J.A.G.M. de Visser, and W.M. de Vos., E G. 2001. "The Host Genotype Affects the Bacterial Community in the Human Gastrointestinal Tract." *Microbial Ecol. Health Dis.* 13(3): 129–34.

APPENDIX

A.



B.

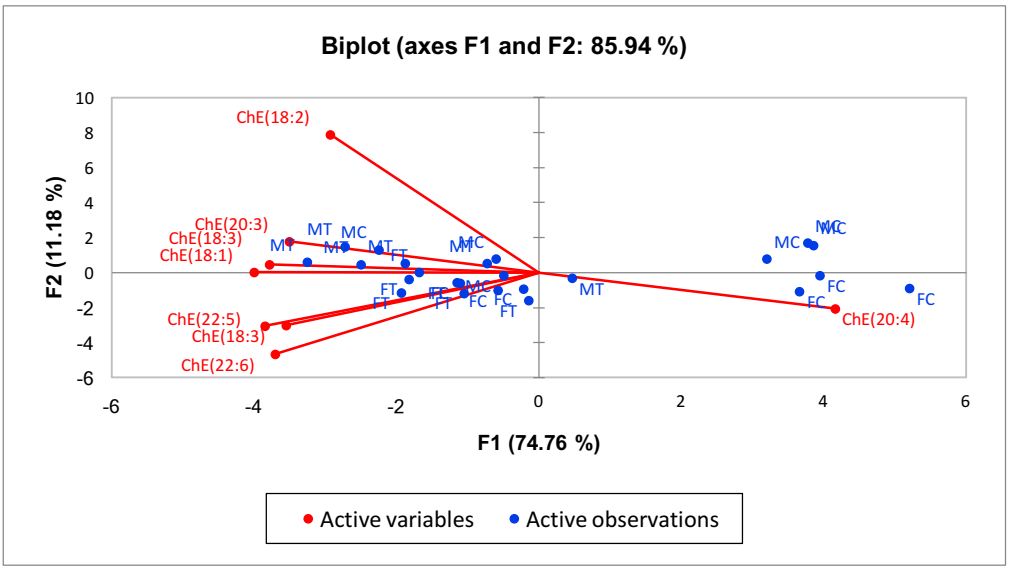
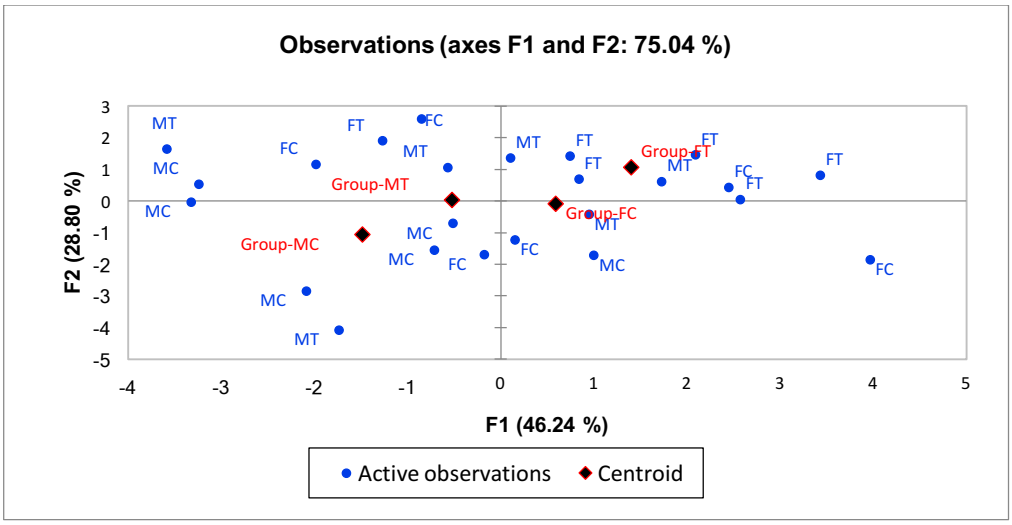


Figure I: Plasma ChE PCA output. A) observation chart and B) biplot showing clustering of ChE molecular species with SCFA treatments and control. Data were analyzed using multi-variate analysis. ChE – cholesteryl ester, MC – male control, MT – male treatment, FC – female control, FT – female treatment, PCA – principal component analysis.

A.



B.

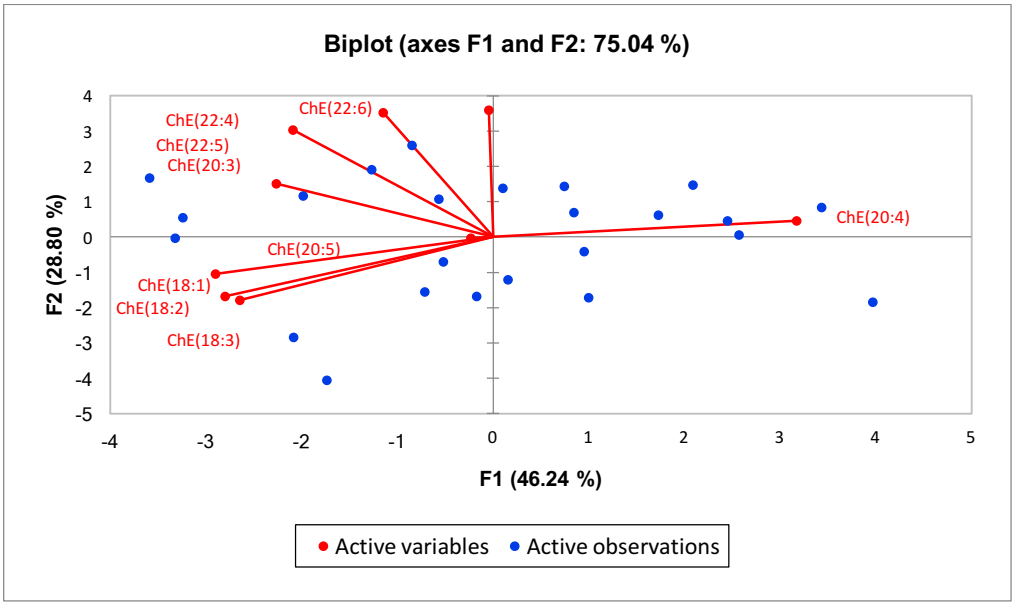
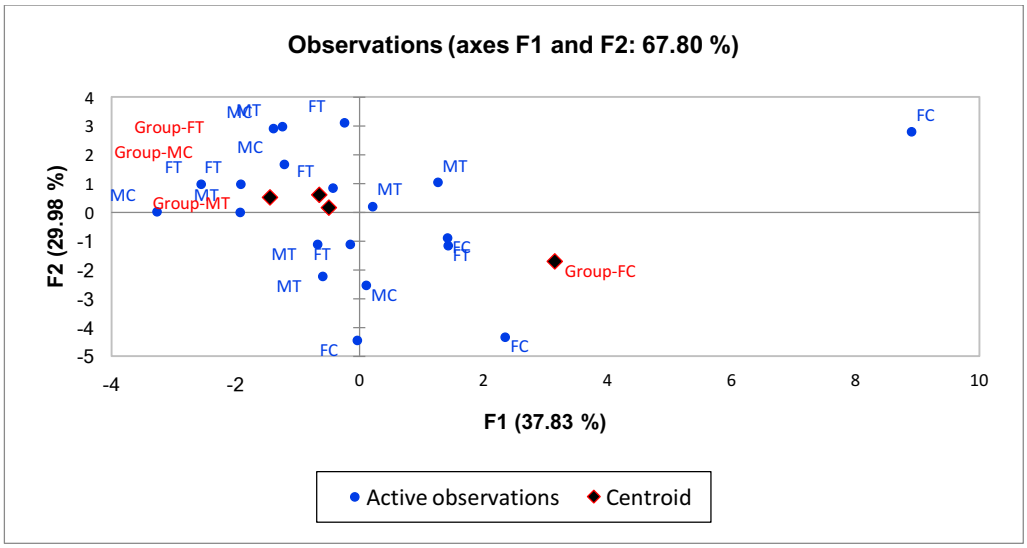


Figure II: Hepatic ChE PCA output. A) observation chart and B) biplot showing clustering of the ChE molecular species with SCFA treatments and control. Data were analyzed using multi-variate analysis. ChE – cholesteryl ester, MC – male control, MT – male treatment, FC – female control, FT – female treatment, PCA – principal component analysis.

A.



B.

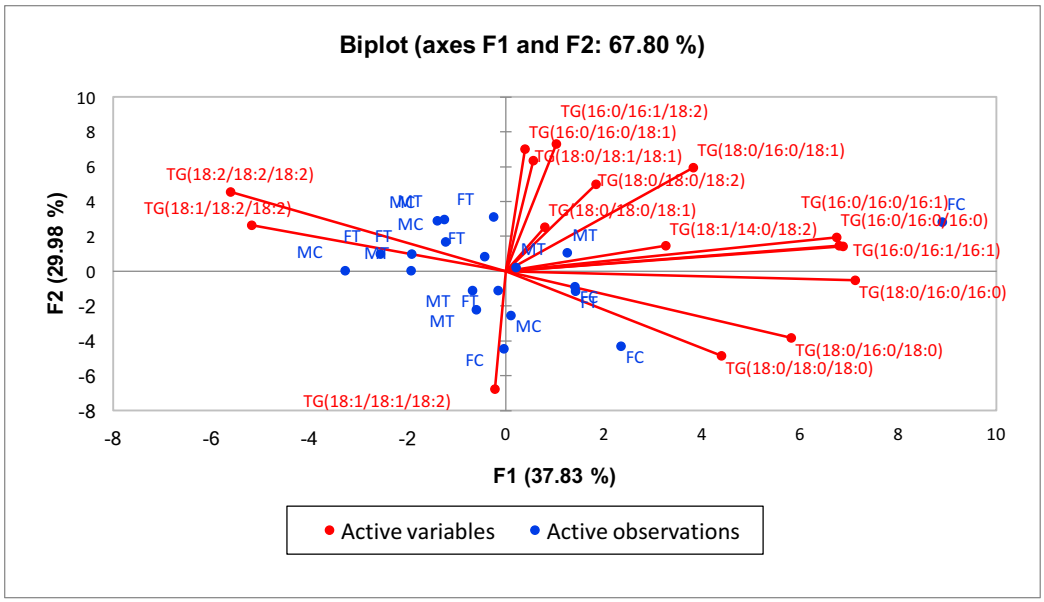
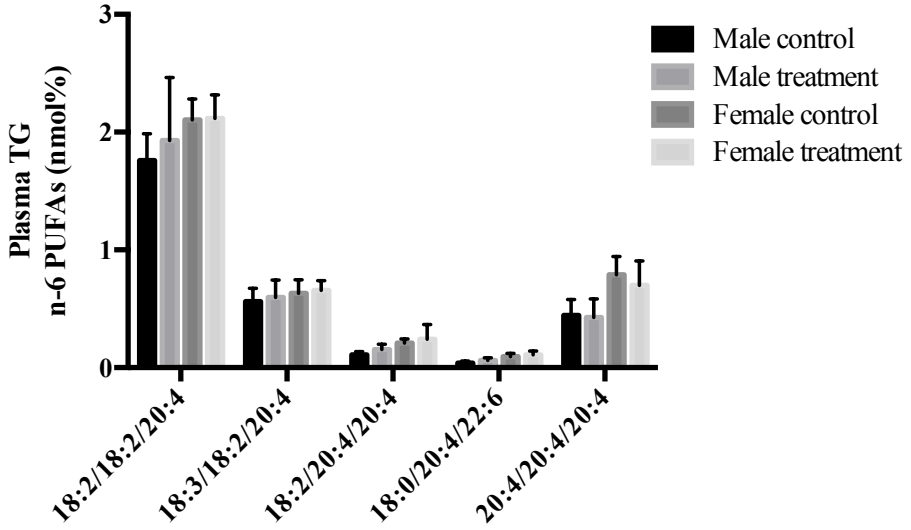
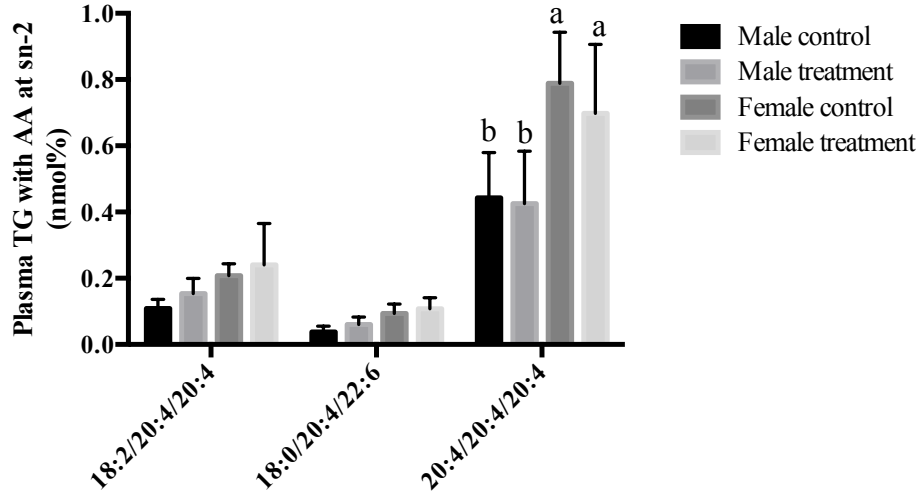


Figure III: Effect of short chain fatty acids on plasma TG composition. A) observation chart and B) biplot showing clustering of the TG molecular species with SCFA treatments and control. Data were analyzed using multi-variate analysis. TG – triacylglycerols. MC – male control, MT – male treatment, FC – female control, FT – female treatment.

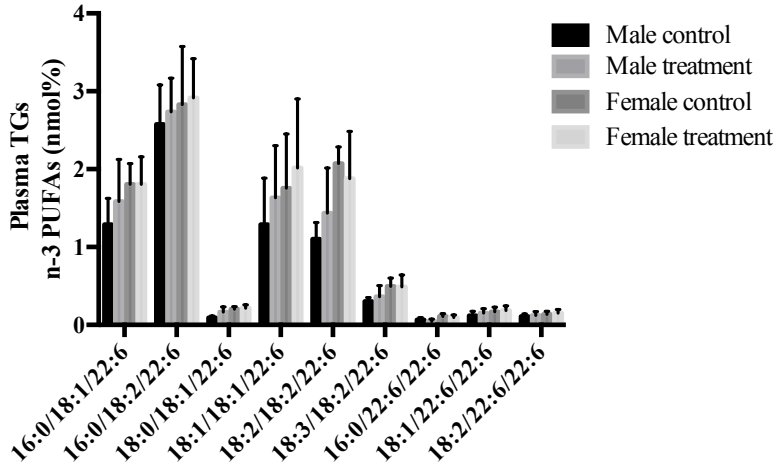
A.


Plasma TG
n-6 PUFAs (nmol%)
3
2
1
0
18:2/18:2/20:4
18:3/18:2/20:4
18:2/20:4/20:4
18:0/20:4/22:6
20:4/20:4/20:4
Male control
Male treatment
Female control
Female treatment

B.


Plasma TG with AA at sn-2
(nmol%)
1.0
0.8
0.6
0.4
0.2
0.0
b
b
a
a
18:2/20:4/20:4
18:0/20:4/22:6
20:4/20:4/20:4
Male control
Male treatment
Female control
Female treatment

C.



D.

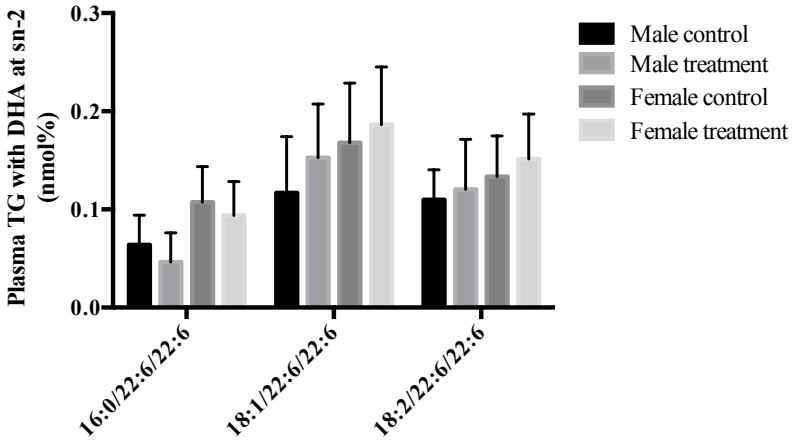
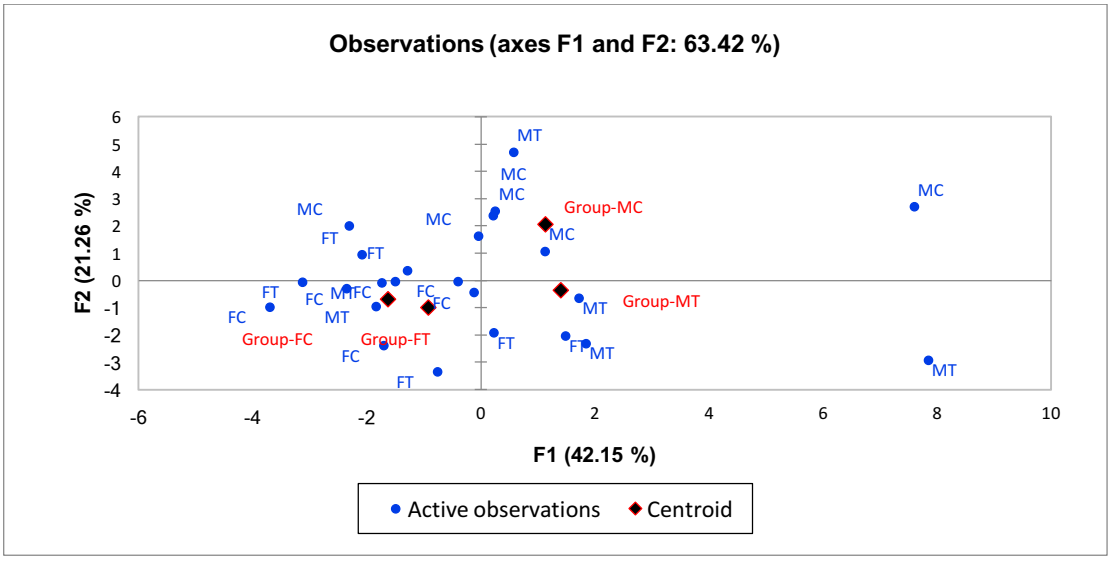


Figure IV - Effect of short chain fatty acids on the relative abundance of hepatic TG composed of: A) n-6 PUFAs B) AA at *sn*-2 position C) n-3 PUFAs and D) DHA at *sn*-2 position. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed as mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). AA – arachidonic acid, DHA – docosahexaenoic acid, TG – triacylglycerols, PUFAs – polyunsaturated fatty acids.

A.



B.

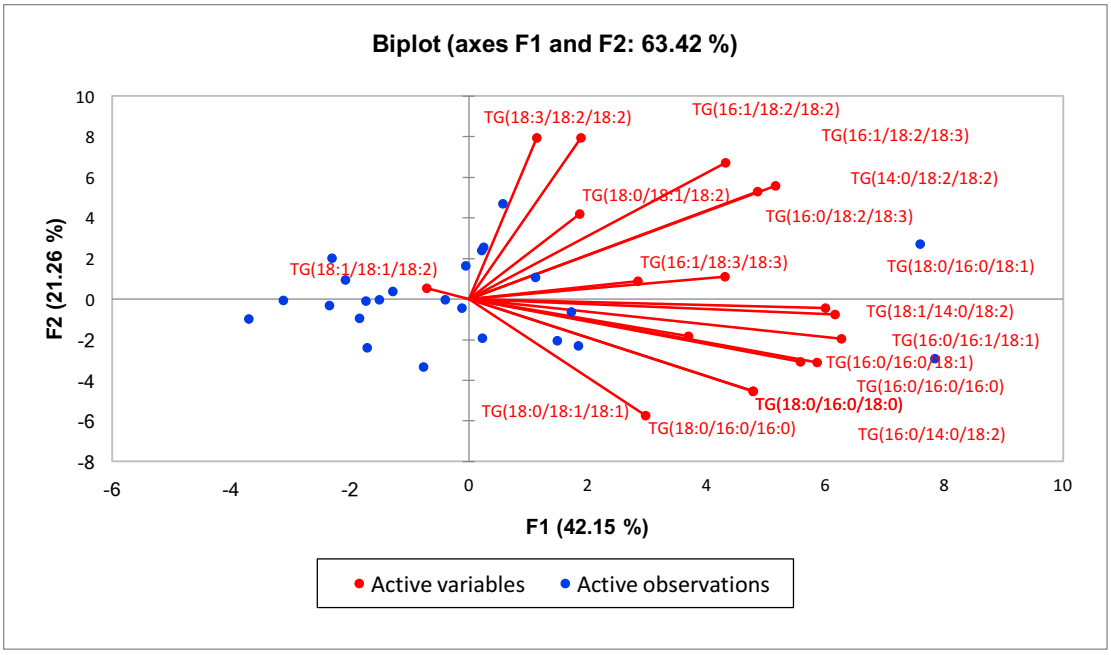
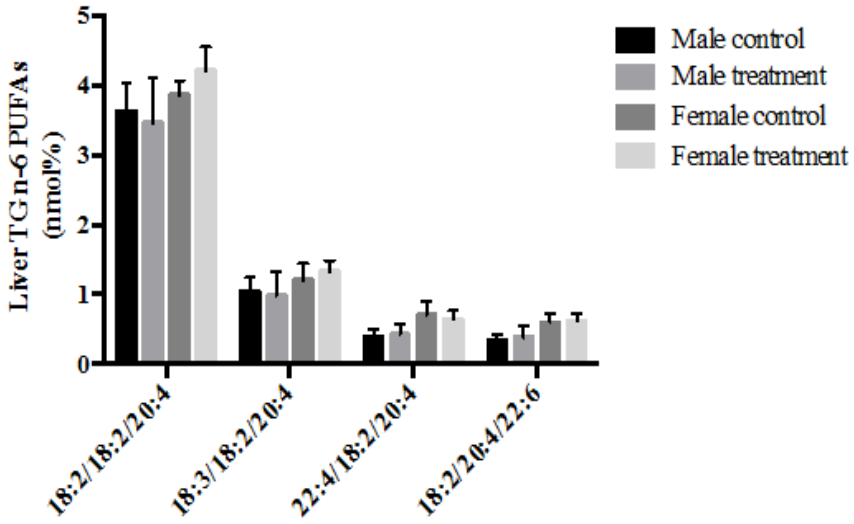


Figure V: Hepatic TG PCA output. A) observation chart and B) biplot showing clustering of TG molecular species with SCFA treatment and control. Data were analyzed using multi-variate analysis. TG - triacylglycerols, MC – male control, MT – male treatment, FC – female control, FT – female treatment, PCA – principal component analysis.

A.



B.

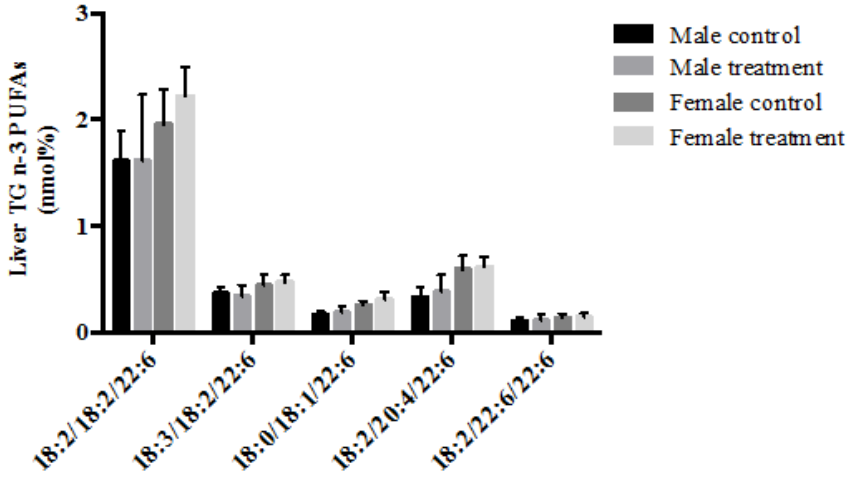
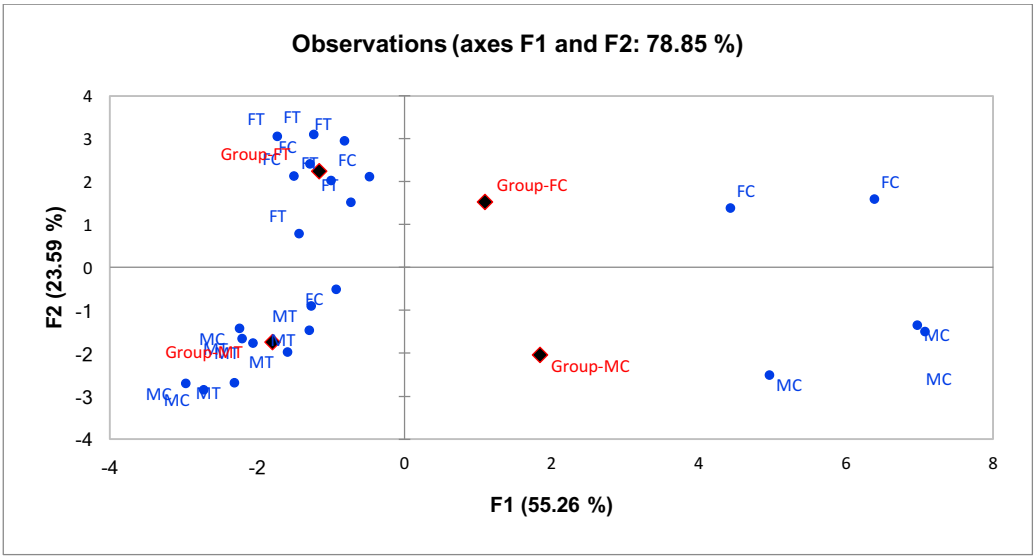


Figure VI - Effect of short chain fatty acids on the relative abundance of hepatic TG composed of: A) n-6 PUFAs and B) n-3 PUFAs. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. All data are expressed as mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). TG – triacylglycerols, PUFAs – poly unsaturated fatty acids.

A.



B.

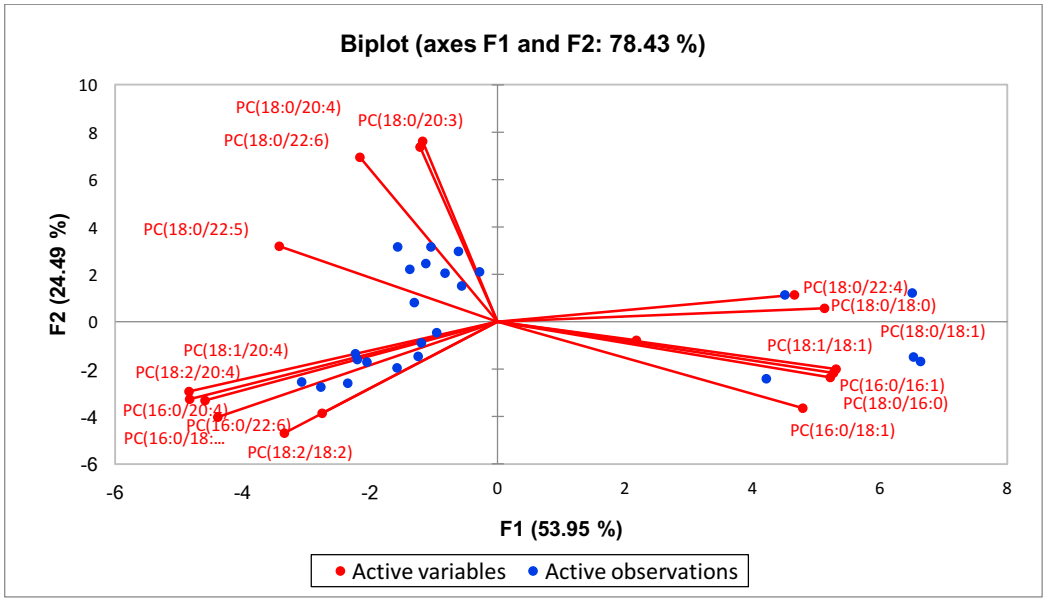
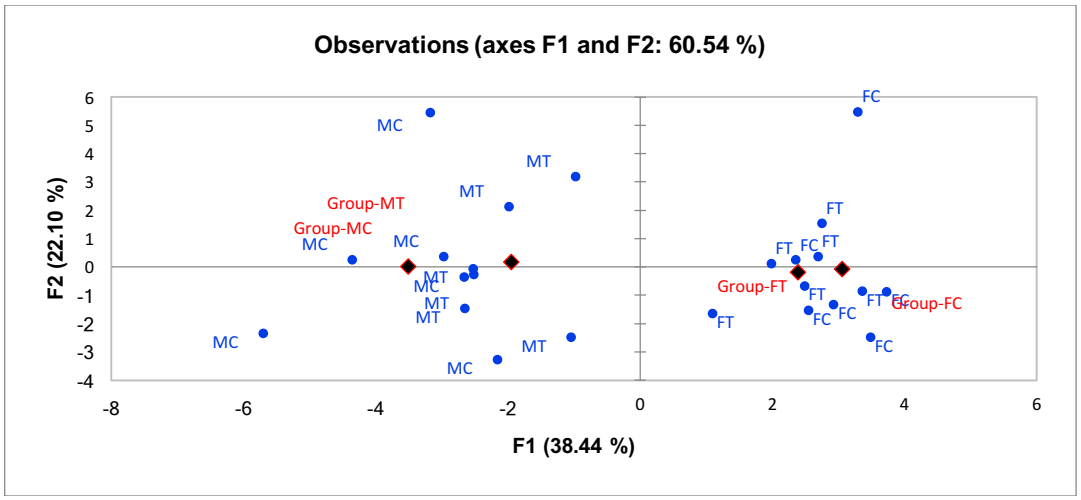


Figure VII: Plasma PC-diacylated PCA output. A) observation chart and B) biplot showing clustering of PC species with SCFA treatment and control. Data were analyzed using multi-variant analysis. PC – phosphatidylcholine, PCA – principal component analysis, MC – male control, MT – male treatment, FC – female control, FT – female treatment.

A.



B.

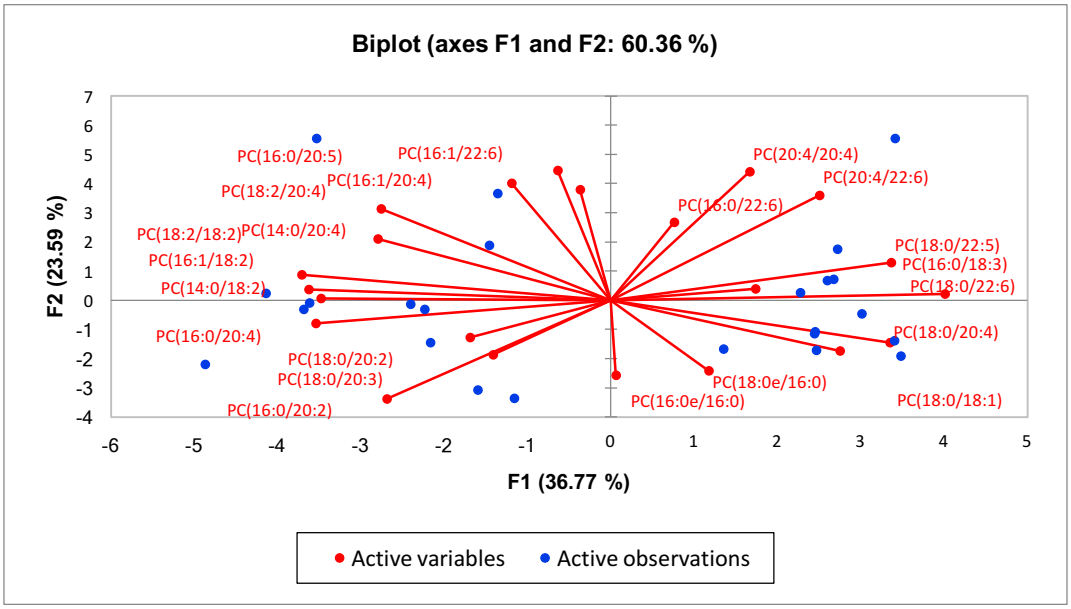
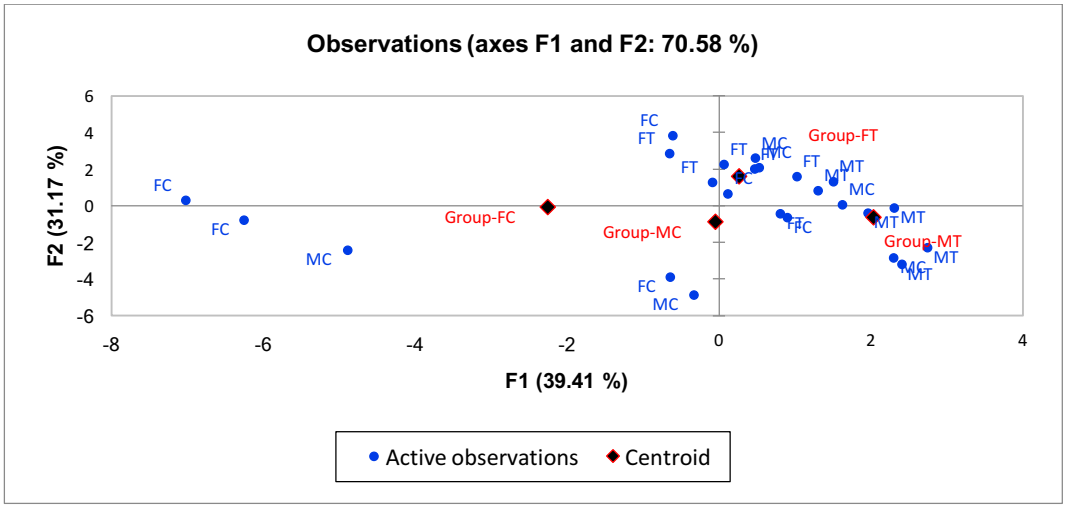


Figure VIII: Hepatic PC-diacylated PCA output. A) observation chart and B) biplot showing clustering of PC molecular species with SCFA treatment and control. Data were analyzed using multi-variate analysis. PC – phosphatidylcholine, PCA – principal component analysis, MC – male control, MT – male treatment, FC – female control, FT – female treatment.

A.



B.

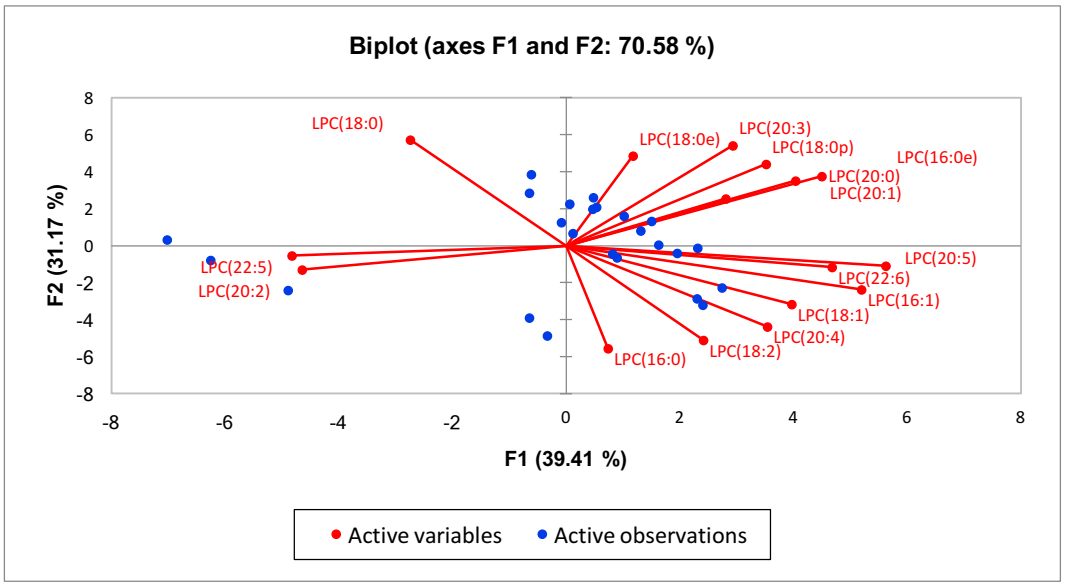
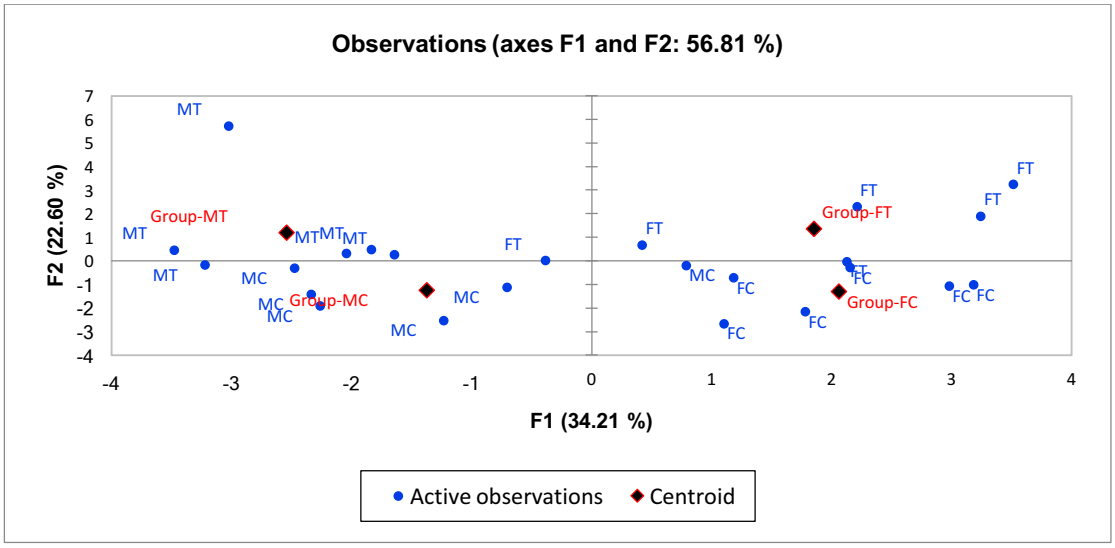


Figure IX: Plasma LPC PCA output. A) observation chart and B) biplot showing clustering of LPC molecular species with SCFA treatment and control. Data were analyzed using multi-variate analysis. LPC – lyso phosphatidylcholine, PCA – principal component analysis, MC – male control, MT – male treatment, FC – female control, FT – female treatment.

A.



B.

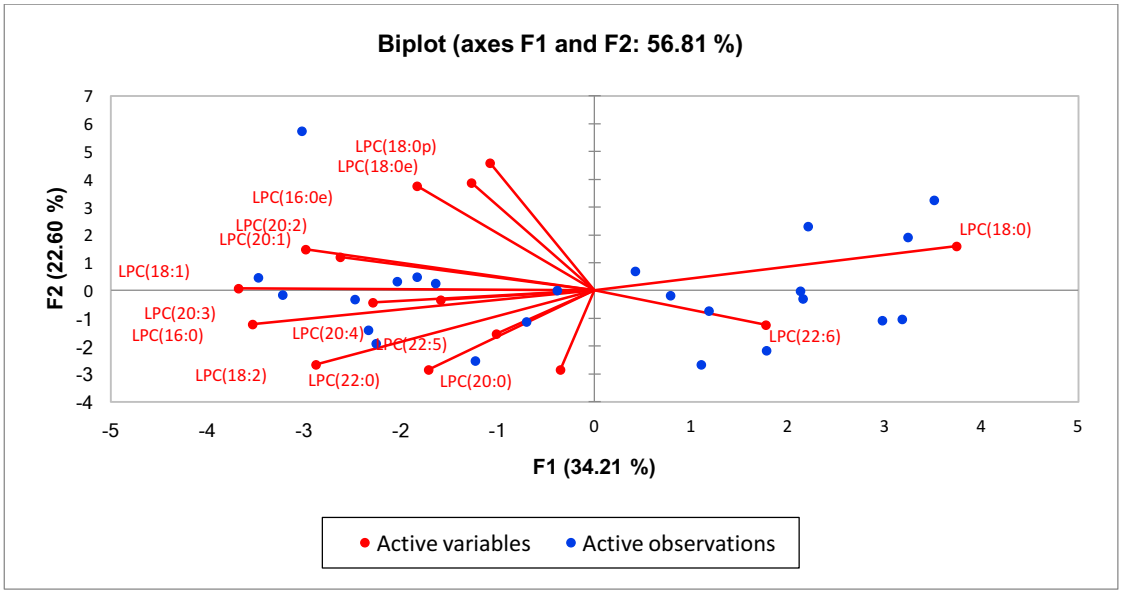
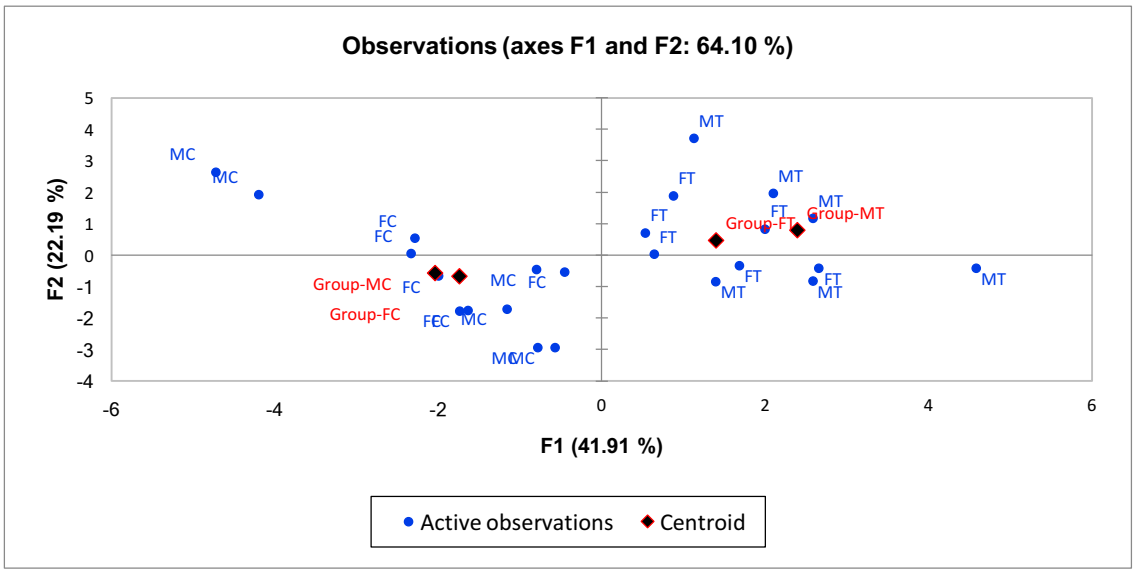


Figure X: Hepatic LPC PCA output. A) observation chart and B) biplot showing clustering of PC molecular species with SCFA treatment and control. Data were analyzed using multi-variate analysis. LPC – lyso phosphatidylcholine, PCA – principal component analysis, MC – male control, MT – male treatment, FC – female control, FT – female treatment.

A.



B.

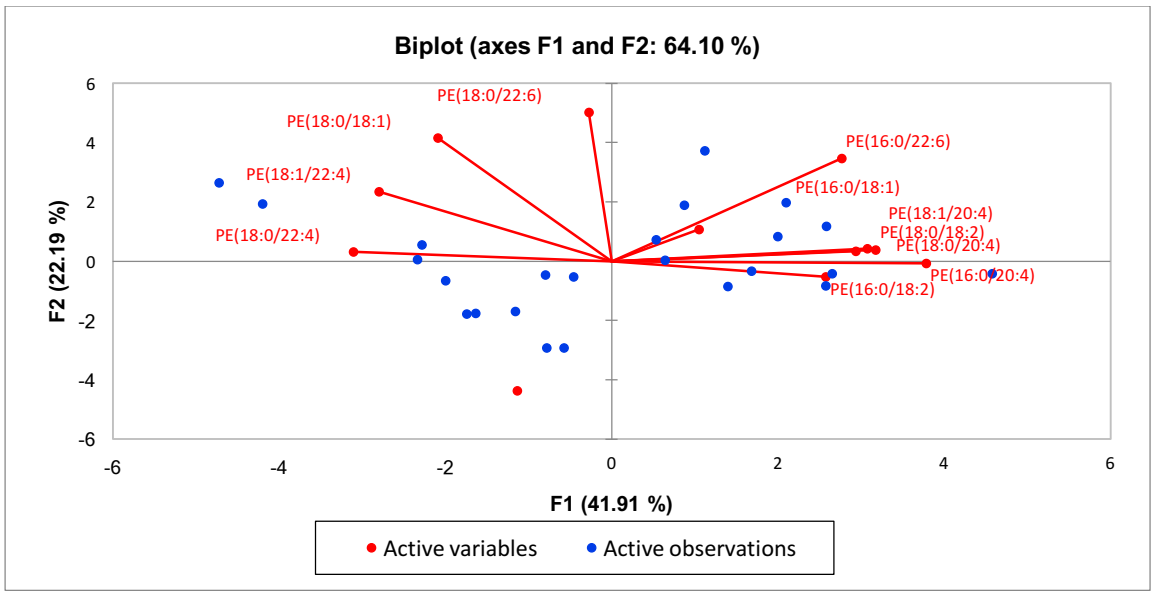
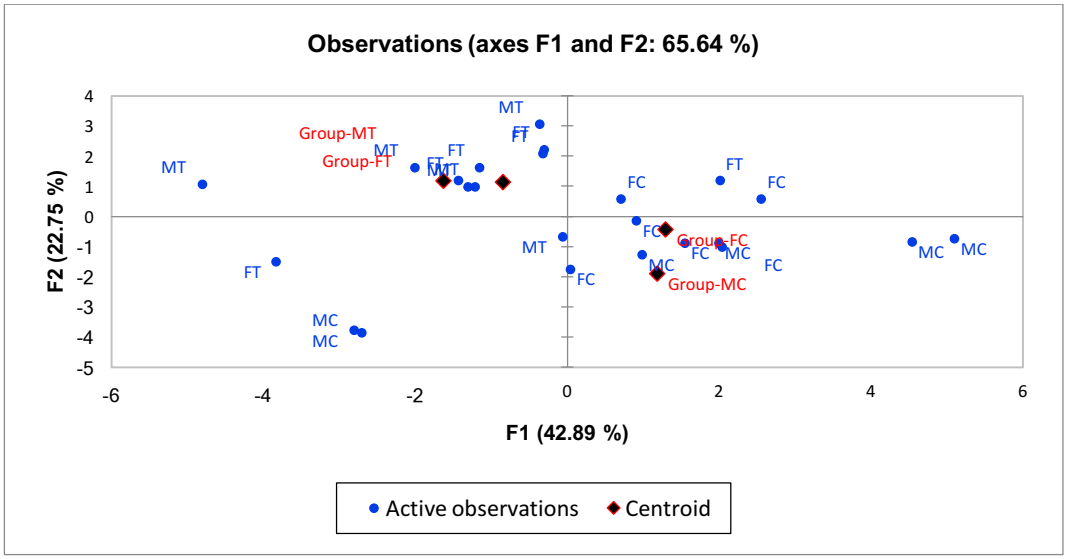


Figure XI: Plasma PE-diacylated PCA output. A) observation chart and B) biplot showing clustering of PE molecular species with SCFA treatment and control. Data were analyzed using multi-variate analysis. PE – phosphatidylethanolamine, PCA – principal component analysis, MC – male control, MT – male treatment, FC – female control, FT – female treatment.

A.



B.

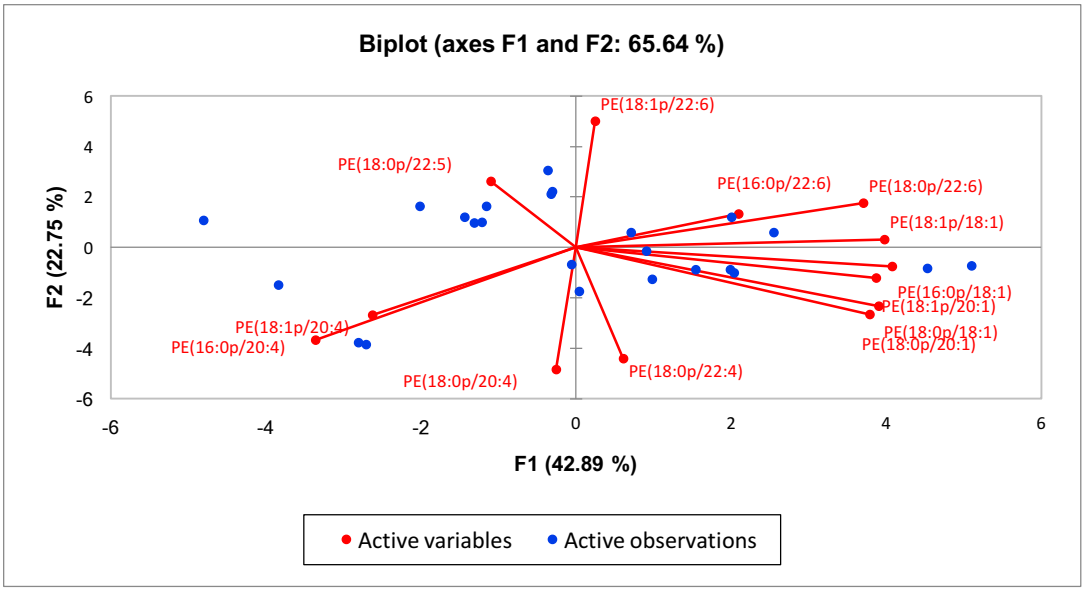
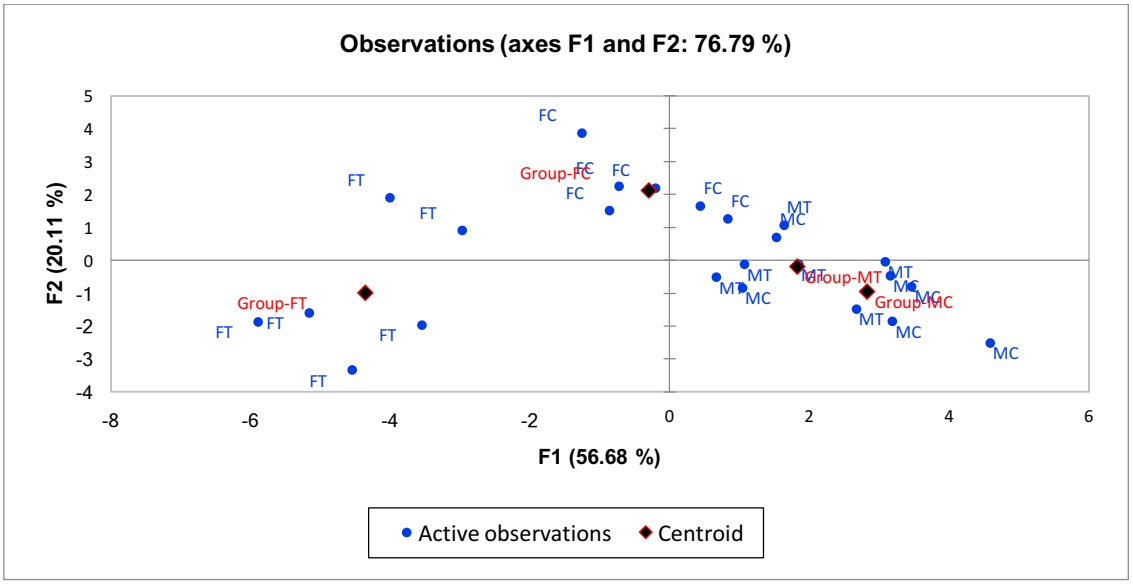


Figure XII: Plasma PlsPE PCA output. A) observation chart and B) biplot showing clustering of PE plasmalogen molecular species with SCFA treatment and control. Data were analyzed using multi-variate analysis. PlsPE – phosphatidylethanolamine plasmalogen, PCA – principal component analysis, MC – male control, MT – male treatment, FC – female control, FT – female treatment.

A.



B.

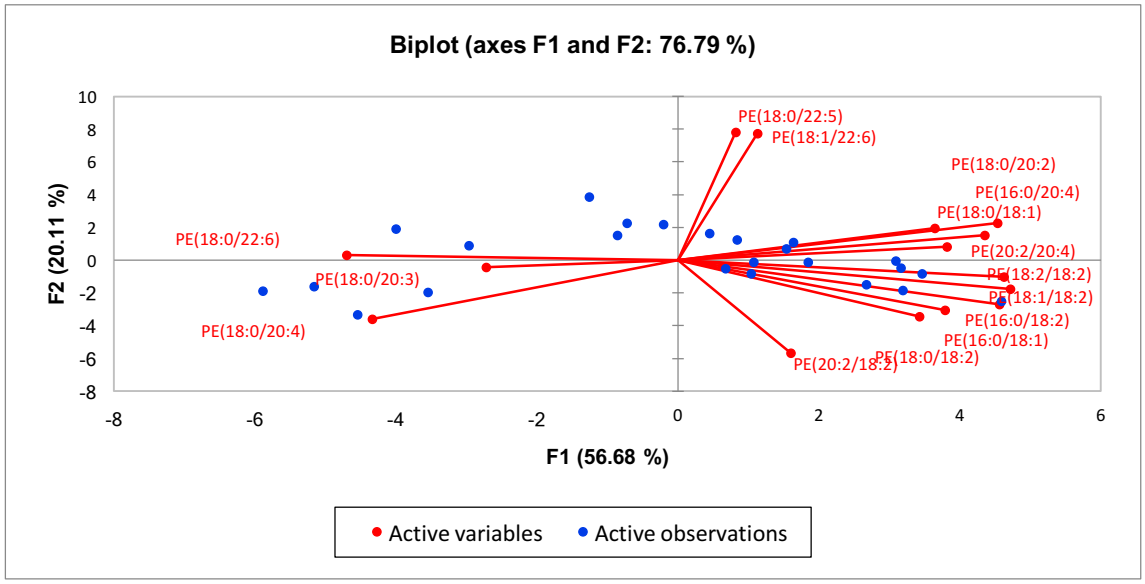
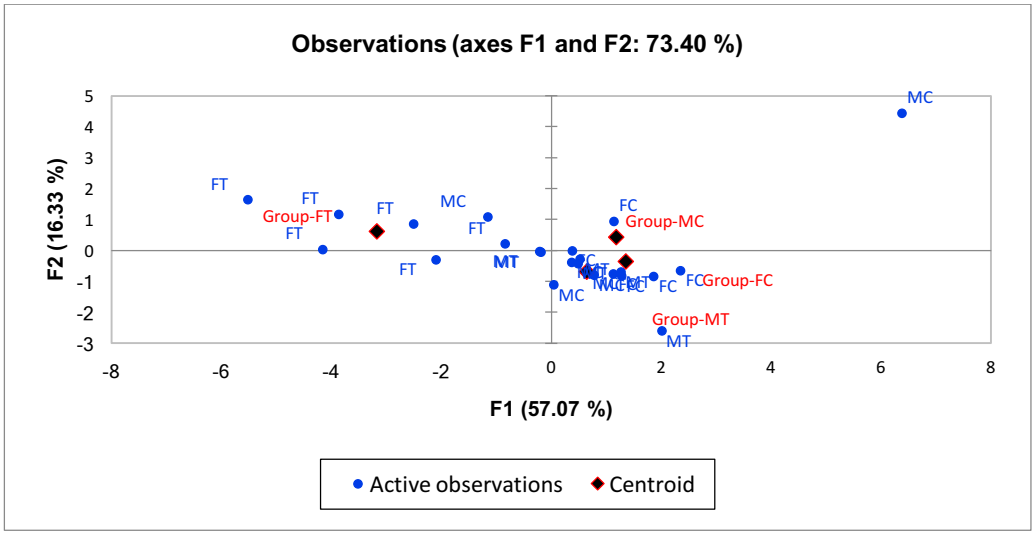


Figure XIII: Hepatic PE-diacylated PCA output. A) observation chart and B) biplot showing clustering of PE molecular species with SCFA treatment and control. Data were analyzed using multi-variant analysis. PC – phosphatidylethanolamine, MC – male control, MT – male treatment, FC – female control, FT – female treatment.

A.



B.

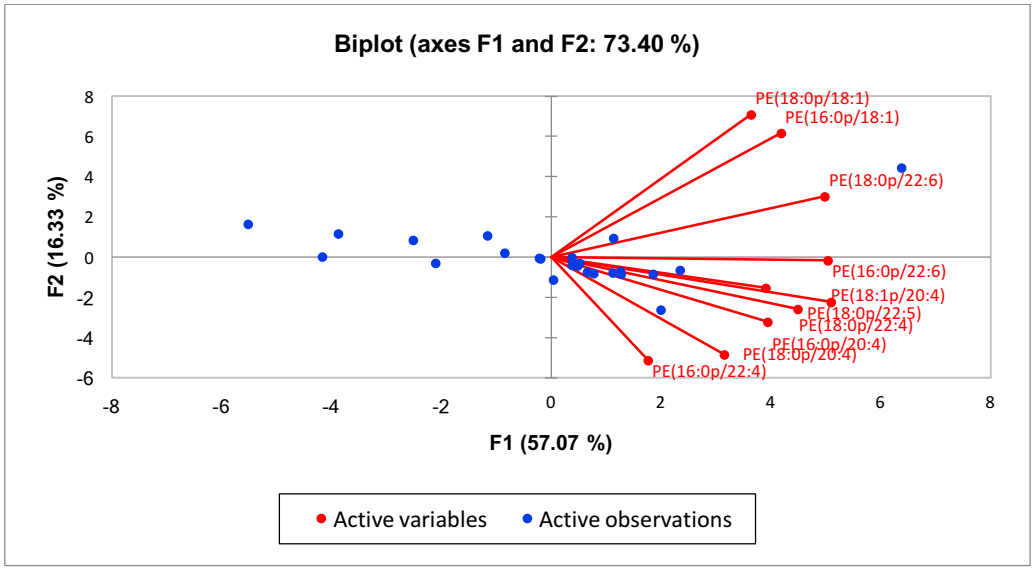
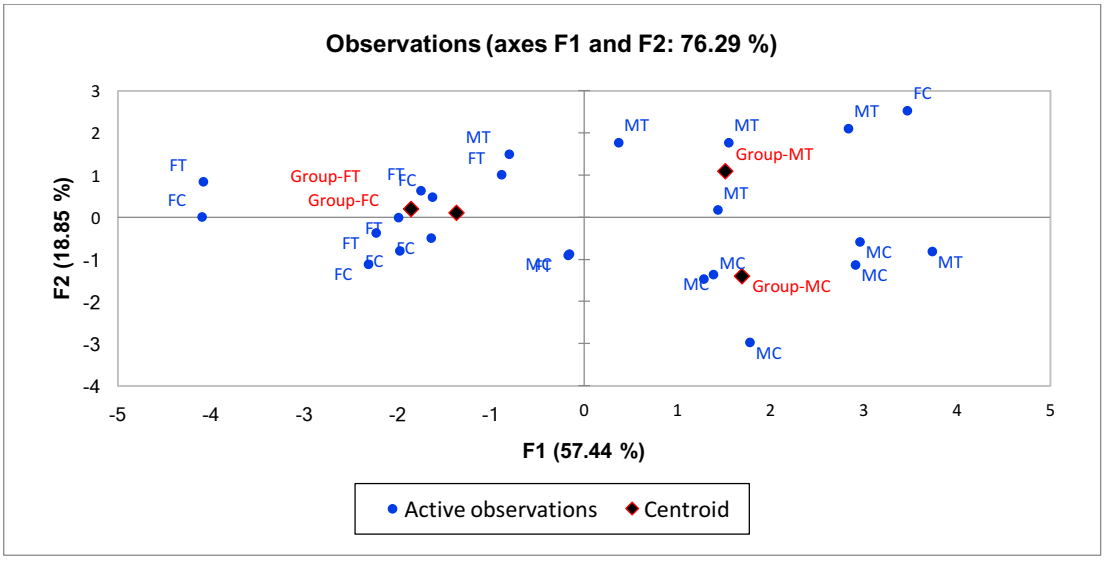


Figure XIV: Hepatic PlsPE PCA output. A) observation chart and B) biplot showing clustering of PE plasmalogen molecular species with SCFA treatment and control. Data were analyzed using multi-variate analysis. PlsPE – phosphatidylethanolamine plasmalogen, PCA – principal component analysis, MC – male control, MT – male treatment, FC – female control, FT – female treatment.

A.



B.

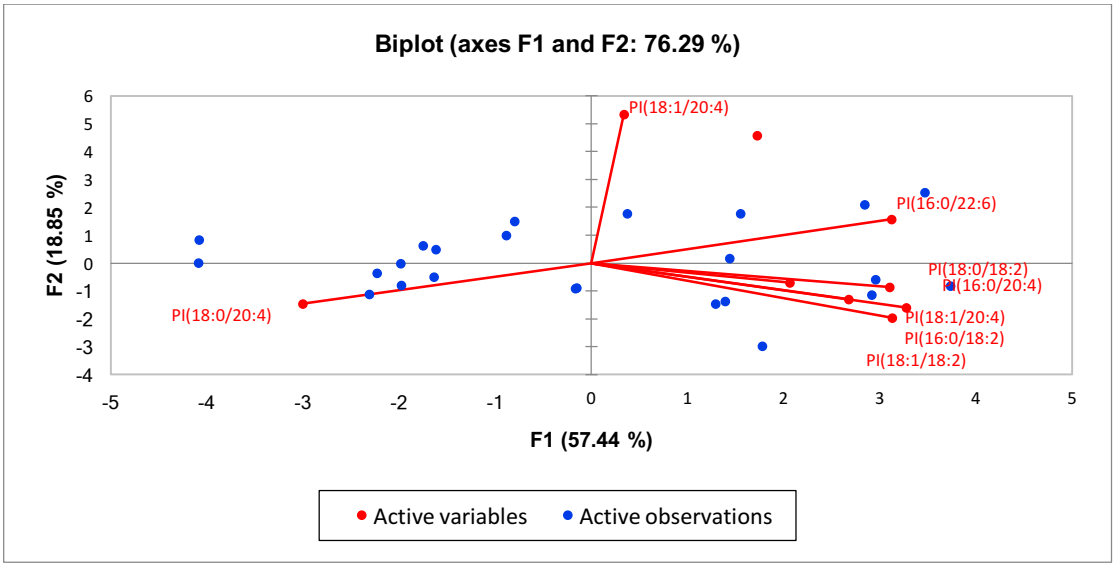
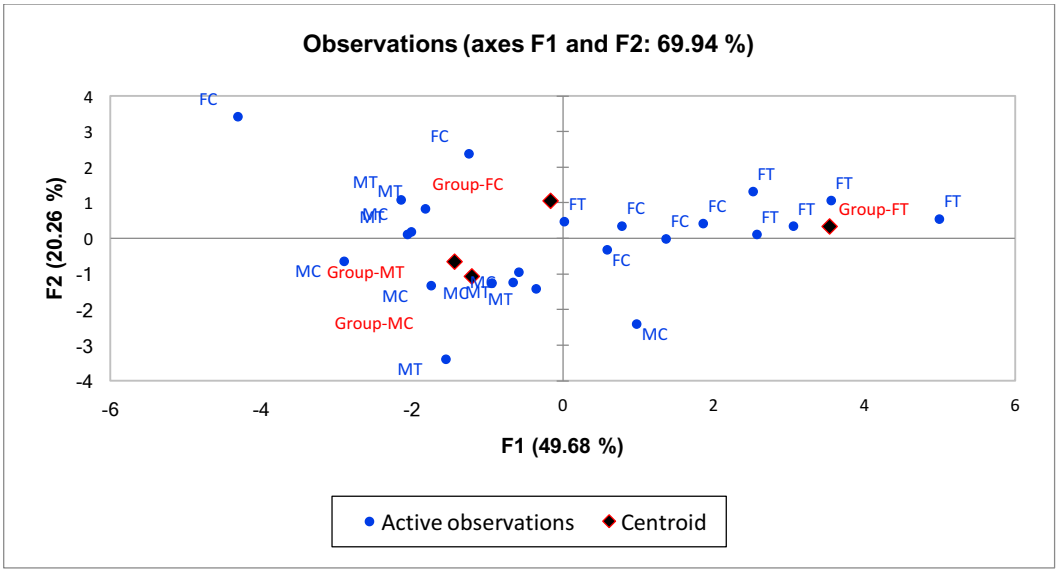


Figure XV: Plasma PI PCA output. A) observation chart and B) biplot showing clustering of PI molecular species with SCFA treatment and control. Data were analyzed using multi-variant analysis. PI – phosphatidylinositol, PCA – principal component analysis, MC – male control, MT – male treatment, FC – female control, FT – female treatment.

A.



B.

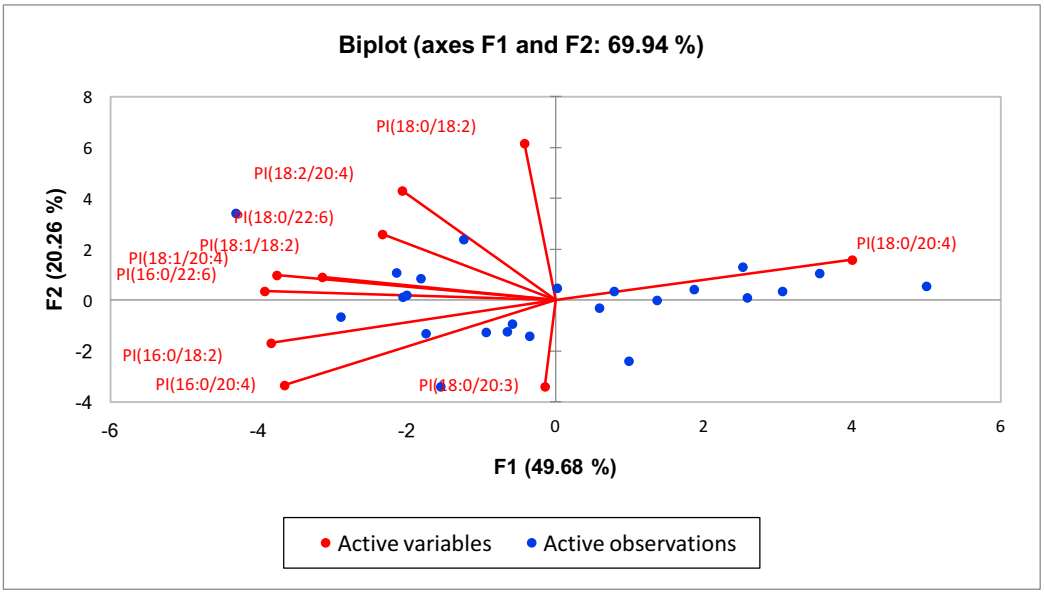
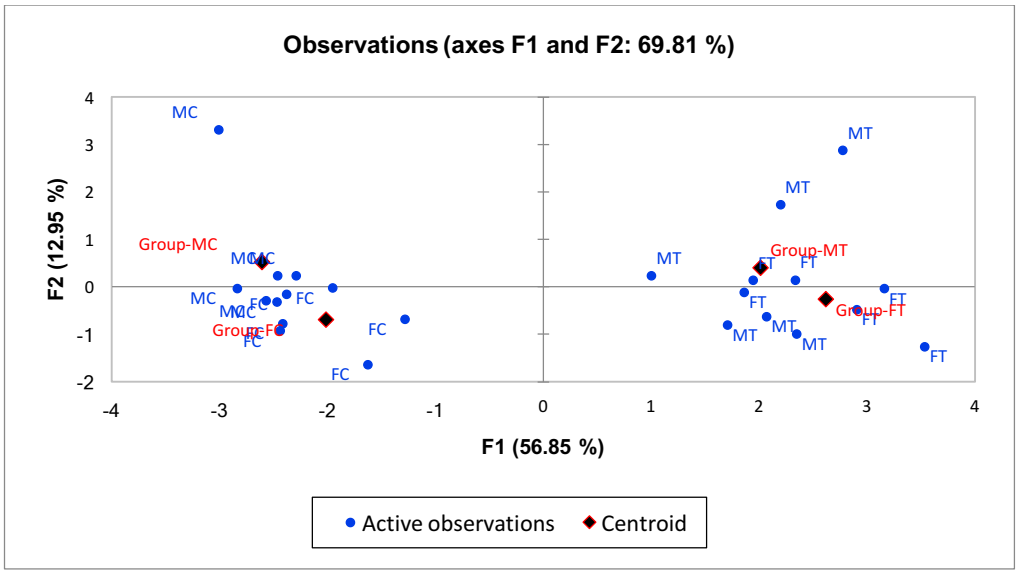


Figure XVI: Hepatic PI PCA output. A) observation chart and B) biplot showing clustering of PI molecular species with SCFA treatment and control. Data were analyzed using multi-variate analysis. PI – phosphatidylinositol, MC – male control, MT – male treatment, FC – female control, FT – female treatment.

A.



B.

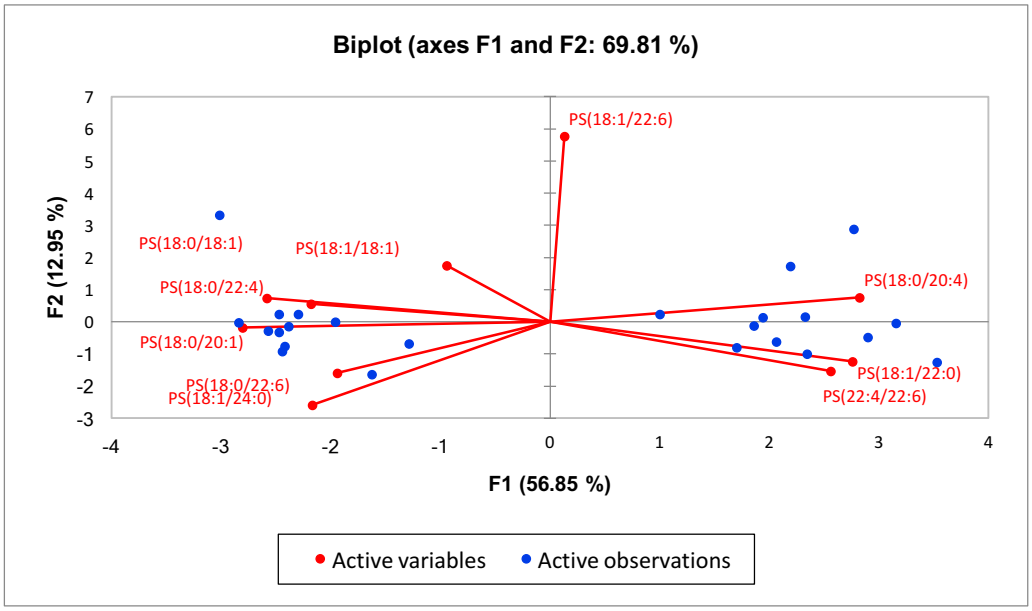
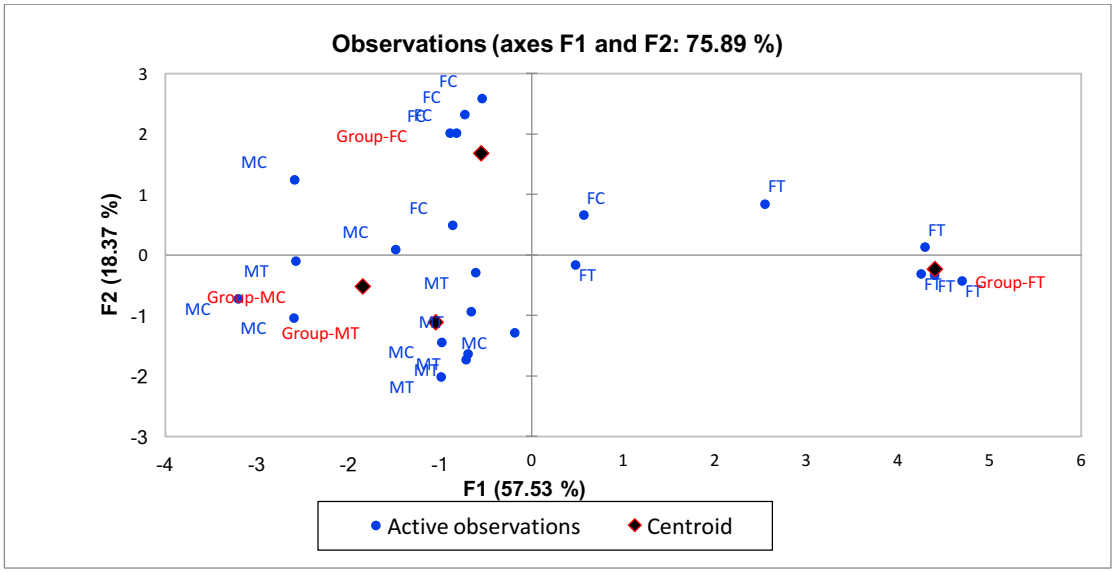


Figure XVII: Plasma PS PCA output. A) observation chart and B) biplot showing clustering of PS molecular species with SCFA treatment and control. Data were analyzed using multi-variate analysis. PS – phosphatidylserine, PCA – principal component analysis, MC – male control, MT – male treatment, FC – female control, FT – female treatment

A.



B.

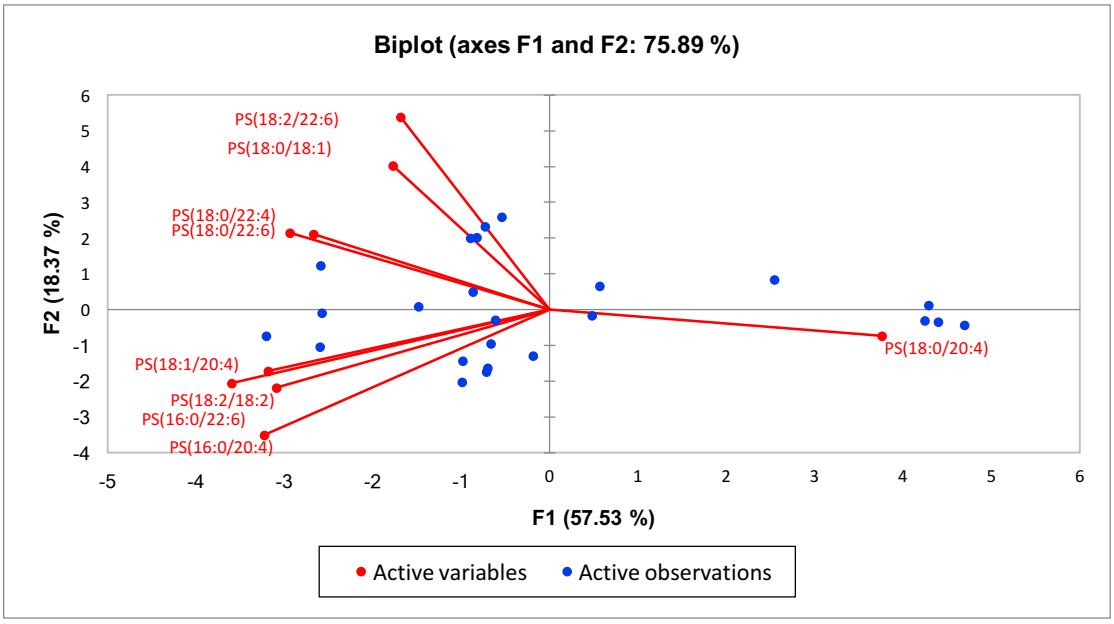
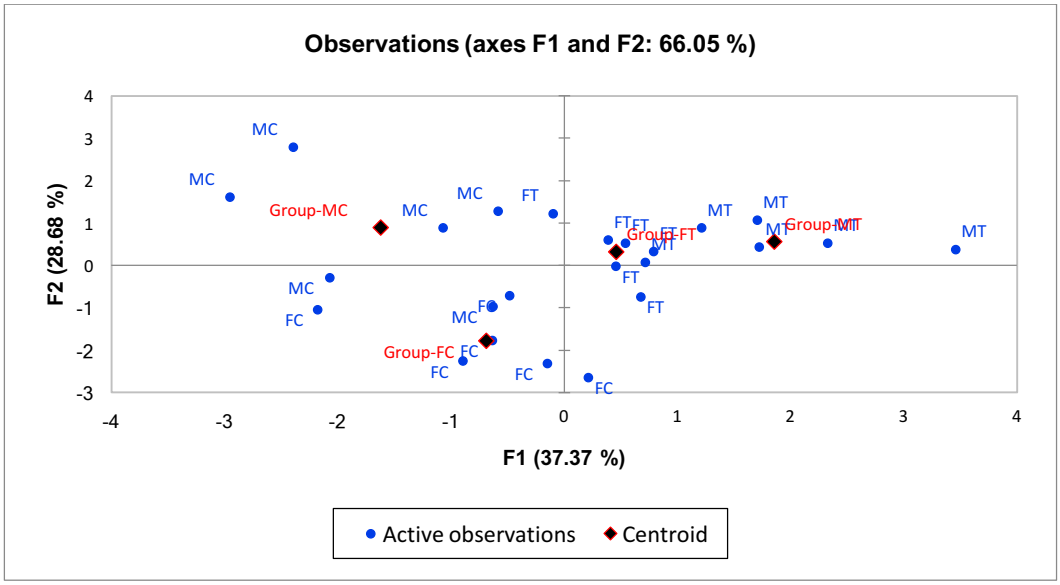


Figure XVIII: Hepatic PS PCA output. A) observation chart and B) biplot showing clustering of PC molecular species with SCFA treatment and control. Data were analyzed using multi-variate analysis. PS – phosphatidylserine, PCA – principal component analysis, MC – male control, MT – male treatment, FC – female control, FT – female treatment.

A.



B.

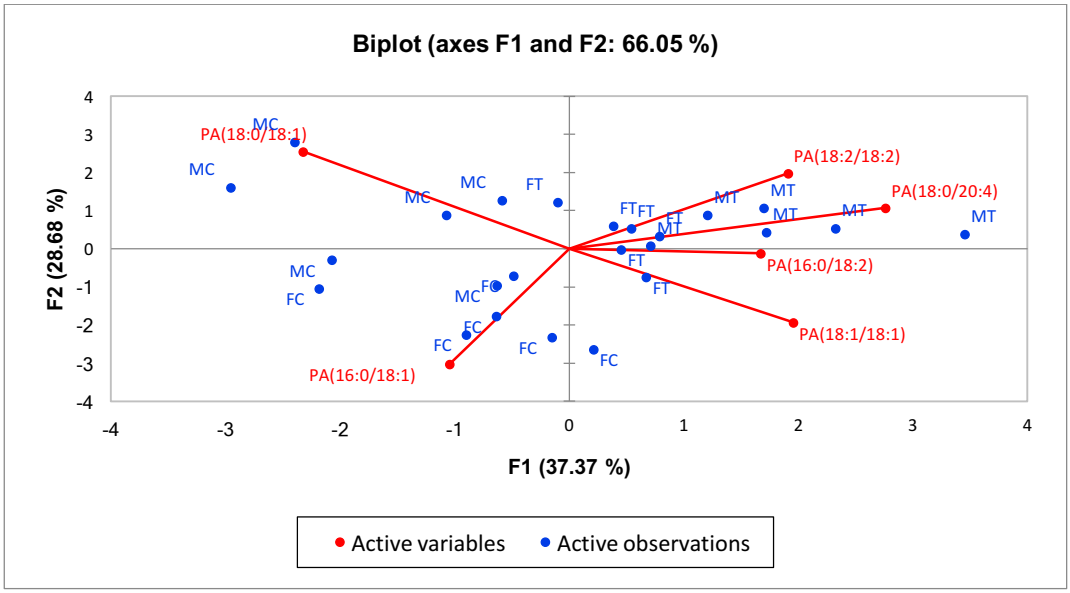


Figure XIX: Plasma PA PCA output. A) observation chart and B) biplot showing clustering of PA molecular species with SCFA treatment and control. Data were analyzed using multi-variate analysis. PA – phosphatidic acid, PCA – principal component analysis, MC – male control, MT – male treatment, FC – female control, FT – female treatment.

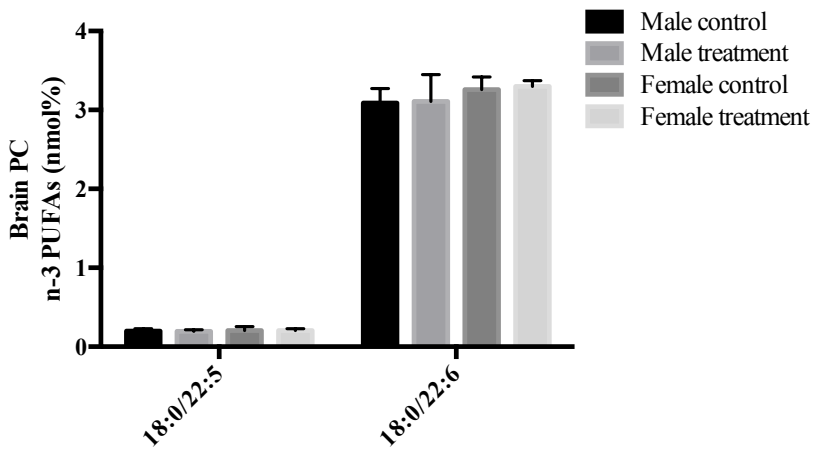


Figure XX – Effect of short chain fatty acids on brain PC with n-3 PUFAs. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test. Data are expressed as mean ± SD. Different superscripts indicate significant difference amongst groups. $P < 0.05$ was considered significant (n=6). PC – phosphatidylcholine, PUFAs – polyunsaturated fatty acids.