FATTY ACID DESATURASE 3 (FADS3) INFLUENCE ON LONG CHAIN POLYUNSATURATED FATTY ACID BIOSYNTHESIS

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FATTY ACID DESATURASE 3 (FADS3) INFLUENCE ON LONG CHAIN POLYUNSATURATED FATTY ACID BIOSYNTHESIS

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The fatty acid desaturase gene cluster (11q12-13.1) codes for proteins that mediate the biosynthesis of long chain polyunsaturated fatty acids (LCPUFA). Of the three members of the family, FADS1 and FADS2 code for enzymes that insert double bonds in specific positions in fatty acids. *FADS3* is the third member of the *FADS* gene cluster and its function remains elusive. Here, we generated the first *Fads3* knockout (KO) mouse with an aim to characterize its metabolic phenotype and clues to *in vivo* function.

Chapter 1 reviews contemporary knowledge of the LCPUFA biosynthetic pathway. Recent data show that LCPUFA synthesis may be regulated at all steps of the pathway, including the elongation steps. Genetic polymorphisms and epigenetics are now known to influence overall flux through the biosynthetic pathway. Alternative transcripts also impact fatty acid levels.

Chapter 2 describes studies on the first Fads3 knockout mice. No differences in overt phenotypes (survival, fertility, growth rate) were observed. Brain docosahexaenoic acid (DHA) of postnatal day 1 (P1) KO mice were lower than WT. P1 KO liver *Fads1* and *Fads2* mRNA were downregulated whereas expression of elongation of very long chain gene 2 (*Elovl2*) and

Elovl5 were upregulated compared to age-matched WT. *Fads3* enhances liver-mediated DHA synthesis to support brain DHA accretion before and during the brain growth spurt.

Chapter 3 describes discovery of a ninth alternative transcript, *Fads3AT9*, in WT mice. Eleven WT tissues were analyzed for the classical transcript (*Fads3*CS) and AT9 expression, as well as in cultured adipocytes. Surprisingly, *Fads3AT9* abundance was 10 fold greater than *Fads3CS* in pancreas and 20:4n-6, the immediate desaturation product of the *Fads1* coded Δ5-desaturase, was highest in pancreas. The findings suggest that *Fads3AT9* may play a role in the regulation and/or biosynthesis of LCPUFA from precursors.

Chapter 4 describes a comparison of fatty acid profiles in high oleic (HO) and high linoleic fed animals. WT and Fads3 KO mice both had higher heart and brain omega-3 LCPUFA with HO diets than high linoleic. In summary, *Fads3* regulates LCPUFA biosynthesis via modulation of desaturation and elongation and its *AT9* may also play roles in LCPUFA biosynthesis.

BIOGRAPHICAL SKETCH

Jiyao Zhang was born in Xi'an, China in 1987. Her hometown is a historical city located in the northwest of China and famous for the Silk Road, the Terracotta Army of Emperor Qin Shi Huang and many local foods. She earned her bachelor's degree in Food Safety and Quality, Northwest A&F University, 2009. The more she studied about Food Science, the better she knew that Food Science and Nutrition Science were different and she wanted to explore mechanisms of the impact of nutrients on health. Jiyao continued her master's program in Human Nutrition at Florida State University where she investigated the function of blueberry polyphenols from 2009-2011. In the fall of 2011 she was fortunate to be recruited to the Division of Nutritional Sciences at Cornell University. After five years in graduate school tending a mouse colony daily, she characterized the function of fatty acid desaturase 3 (*Fads3*) using the first global *Fads3* knockout mouse. In the future, she expected to continue exploring the role of nutrients in prevention and/or treatment of chronic diseases. She hopes to put "personalized nutrition" into practice, designing diets that are targeted to person's health needs.

This dissertation is dedicated to

my dear parents, Wei Zhang, Fangli Zhang,

my husband, Xiaohu Liu

and my son, Alexander Z. Liu

for their endless love and support.

这篇论文谨献给我最亲爱的

父亲张炜，母亲张芳丽，

先生刘小虎

和我的儿子刘奕希。

感谢他们对我无尽的关爱和支持！

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CHAPTER 1

DESATURASE AND ELONGASE LIMITING ENDOGENOUS LONG CHAIN POLYUNSATURATED FATTY ACID BIOSYNTHESIS[1]

1.1 INTRODUCTION

Omega3 (ω3 or n-3) and omega6 (ω6 or n-6) long chain polyunsaturated fatty acids (LCPUFA) are ubiquitous in mammalian tissue. They are key nutrients critical for growth and development, are bioactive cellular components of membrane phospholipids, serve as substrates for signaling molecules and act as direct modulators of gene expression [1, 2]. The degree of unsaturation of the biological membranes is modulated by the action of the desaturation and elongation enzymes mediating fatty acid biosynthesis and metabolism. In most organisms *endogenous* synthesis of LCPUFA from PUFA precursors is possible, but the transformations and efficiencies are specific to cell types and species. In humans, genetic variants within genes encoding for desaturation and elongation enzymes were shown to be associated with LCPUFA levels and complex disease phenotypes. Here, we present recent information gained from studies related to desaturases and elongases limiting endogenous LCPUFA synthesis.

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[1] Zhang JY, Kothapalli KS, Brenna JT. Desaturase and elongase-limiting endogenous long-chain polyunsaturated fatty acid biosynthesis. Curr Opin Clin Nutr Metab Care. 2016 Mar;19(2):103-10. doi:10.1097/MCO.0000000000000254. PMID: 26828581

1.2 LCPUFA BIOSYNTHESIS

LCPUFA are endogenously biosynthesized from 18:3n-3 and 18:2n-6 PUFA precursors by position-specific desaturation and carbon chain-elongation reactions, as shown in Figure 1.1. The two PUFA series n-3 and n-6 compete for the same enzymes in the LCPUFA biosynthetic pathway, originally worked out in rodents based on tissue composition resulting from diets rich in 18:3n-3 (alpha-linolenic acid) or 18:2n-6 (linoleic acid). The Δ6-desaturase (fatty acid desaturase 2, FADS2) metabolizes both 18:3n-3 and 18:2n-6, resulting in the synthesis of 6,9,12,15-18:4 and 6,9,12-18:3, respectively. This initial Δ6-desaturation step is widely regarded as rate limiting for LCPUFA endogenous biosynthesis based on biochemical studies, however recent data indicate that other steps in the pathway can limit LCPUFA levels and complex phenotypes. Both 20:5n-3 (eicosapentaenoic acid, EPA) and 20:4n-6 (arachidonic acid) can be further elongated and desaturated to yield 22:6n-3 (docosahexaenoic acid, DHA) and 22:5n-6, respectively. The final steps were long thought to be by direct Δ4-desaturation via 22:5n-3→22:6n-3. Biochemical data in rat liver developed in the 1990s established an alternative coupled microsomal-peroxisomal pathway via 22:5n-3→24:5n-3→24:6n-3→22:6n-3, where the last step is one round of β-oxidation in the peroxisomes [3]. Molecular studies since 2001 established that Δ4-desaturase (FADS2) is the final step in marine microorganisms (e.g.*Thraustochytrium),* marine teleost fish, and mammals, and very recently in humans [3].

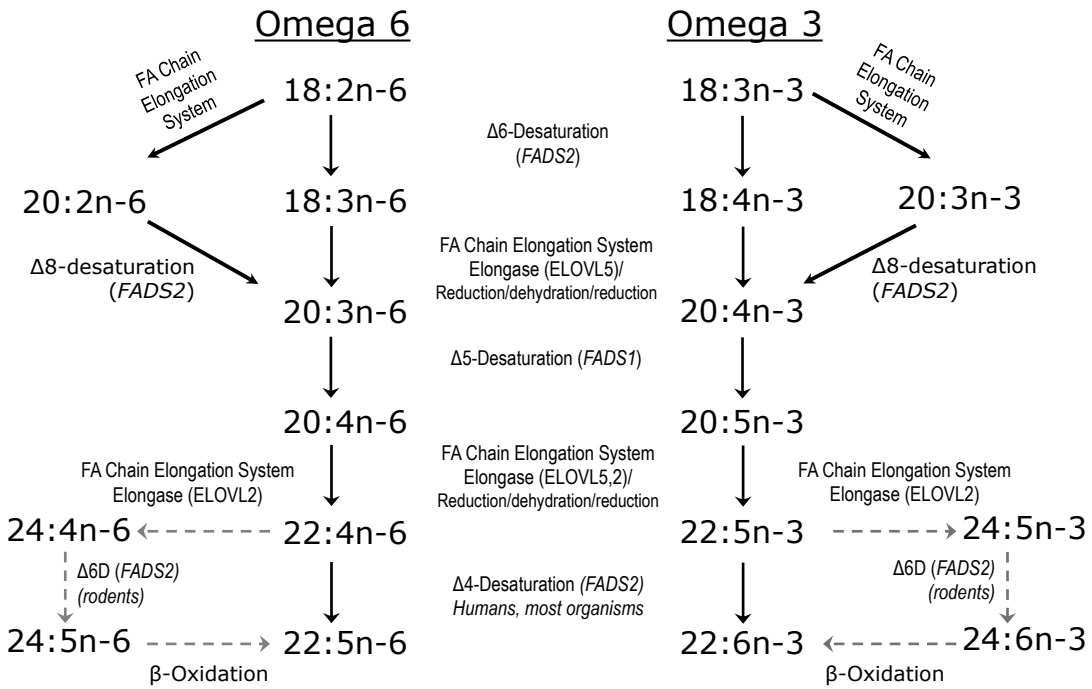

Figure 1.1. LCPUFA Biosynthesis Pathway. The omega 6 (n-6) and omega 3 (n-3) fatty acids are substrates in competition for the same sets of FADS and ELOVL catalyzing desaturation and elongation, respectively. Elongation is mediated by a four enzyme coupled system; the first, rate limiting enzyme is the “elongase”.

1.3 DESATURASES

Desaturase enzymes perform dehydrogenation reactions and introduce a stereospecific double bond between defined carbons of fatty acyl chains. They have evolved independently twice; the Acyl-acyl carrier protein (ACP) desaturases are soluble enzymes found in the plant plastid and most wide-spread membrane-bound desaturase enzymes found in prokaryotes and eukaryotes [4]. In humans, membrane-bound PUFA desaturases known as “front-end” desaturases introduce a nascent double bond between an existing double bond usually located between the carboxyl group and the 9^{th} carbon atoms from the terminal methyl (n-9) [5]. Front-end desaturation proceeds at the Δ4, Δ5, Δ6 and Δ8 positions and is responsible for endogenous biosynthesis of LCPUFA [3, 6, 7].

FADS1 (Δ5-desaturase), *FADS2* (Δ6-desaturase/Δ8-desaturase/Δ4-desaturase) and *FADS3* are located as a cluster within 100 kb region on the long arm of human chromosome 11 (HSA11q12-13.1), whereas, mouse *Fads* homologs with similar structural organization are localized to chromosome 19 [8, 9]. All three genes have evolved by gene duplication events, share 12 exons and 11 introns, and contain well conserved cytochrome *b5* domain and three histidine repeats (HXXXH, HXXHH and QXXHH).

1.3.1 FADS2 (Δ6, Δ8, Δ4-DESATURASE)

FADS2 (OMIM#606149) in humans spans 39.1 kb of genomic DNA, encoding a 444-amino acid protein with a molecular mass of 52.3 kDa [8]. *FADS2* is a

trifunctional even carbon numbered desaturase and acts on at least eleven known fatty acid substrates. In addition to humans, *Fads2* has been cloned from mouse, rat and *Caenorhabditis elegans* [10]. It introduces a double bond at the Δ6 position (between carbons 6 and 7) by acting on at least six substrates (18:2n-6 → 18:3n-6, 18:3n-3 → 18:4n-3, 24:5n-3 → 24:6n-3, 24:4n-6 → 24:5n-6, 16:0 →16:1n-10, and 18:1n-9 →18:2n-9), Δ8-desaturation by acting on 20:1n-9 → 20:2n-9, 20:2n-6 → 20:3n-6, and 20:3n-3 → 20:4n-3, and Δ4-desaturation by acting on 22:4n-6 → 22:5n-6 and 22:5n-3 → 22:6n-3 [3, 10, 11]. The Δ8-desaturase activity provides an alternative pathway to LCPUFA biosynthesis, possibly available when Δ6-desaturase activity is compromised. *FADS2* Δ8-desaturates 20:2n-6 and 20:3n-3 to eicosanoid precursors as well as to precursors of 20:4n-6 and 20:5n-3 respectively [10]. This result offers an explanation as to why 20:2n-6 has been associated with *FADS* single nucleotide polymorphisms (SNPs) in genetic studies described below.

The desaturation of saturated fatty acids (SFA) and PUFA is nearly always considered separately in mammals, with the FADS acting on PUFA and the stearoyl CoA desaturase 1 (SCD1) on SFA. However, 16:0 is the only exception, being a substrate for both Scd1 (16:0→16:1n-7) and FADS2 (16:0→16:1n-10). Human skin lipids, sebum, have long been known as unique with about 25% of fatty acids as 16:1n-10 [12]. Importantly, human and mouse skin cells handle this metabolite shift with different genes: humans use FADS2 to make 16:1n-10 and mice use Scd1 to make 16:1n-7 (Figure 1.2).

Scd1 principally mediates desaturation of 18:0→18:1n-9 (Figure 1.2), however we detect no activity of FADS2 towards 18:0 in a human cell system.

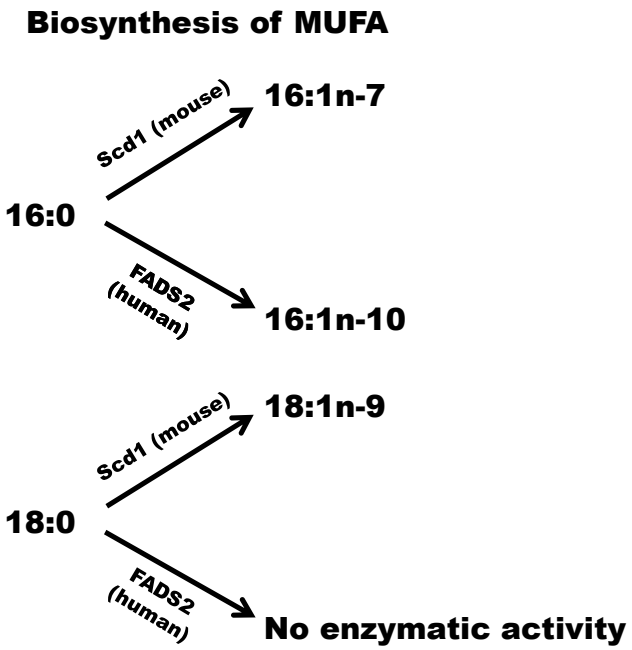

Figure 1.2 Biosynthesis of monounsaturated fatty acids (MUFA) in mouse and human skin. Human skin expresses only FADS2 while mice and all other animals' skin expresses only Scd1. Both SCD1 and FADS2 are expressed and their resulting enzymes are active in liver and other organs of both humans and mice. Mouse: Scd1 mediates 18:0 conversion to 18:1n-9 and 16:0 conversion to 16:1n-7; Human: FADS2 mediates conversion of 16:0 to 16:1n-10 but has no effect on any other saturated fatty acid (e.g. 18:0 no enzymatic activity).

A human MCF-7 cell with no detectable Δ6-desaturase (FADS2) activity stably transformed with *FADS2* mediates direct Δ4 desaturation to yield 22:6n-3 and 22:5n-6, similar to fish and many other organisms [3]. *Fads2* null mice had severe problems with fertility; once born, both female and male mice had normal viability and lifespan but were sterile [13]. The *Fads2* disruption caused an upstream deficiency in eicosanoid synthesis via reduction in 20:4n-6 substrate, unusual fatty acid biosynthesis, dermal and intestinal ulceration, reduced insulin sensitivity and perturbed cell membrane structure [14, 15]. *FADS2* is alternatively spliced to generate two isoforms (*FADS2AT1* and *FADS2AT2, "AT"=alternative transcript*) [16, 17]. We have shown that polypyrimidine tract binding protein (PTB, also known as *PTBP1* or *hnRNP I*) regulates alternative splicing of *Fads2*. Knock-down of PTB modulated the balance of omega-3 to omega-6 fatty acids by dramatically reducing (50% reduction) 20:5n-3 content [1].

1.3.2 FADS1 (Δ5-DESATURASE)

FADS1 (OMIM#606148) in humans spans 17.2 kb of genomic DNA, encodes a 444-amino acid protein with a molecular mass of 52.0 kDa and shares 61% and 52% identity with *FADS2* and *FADS3*, respectively [8]. It introduces a double bond at the Δ5 position (between carbons 5-6) by acting on at least four 20-carbon fatty acid substrates 20:3n-3, 20:4n-3, 20:2n-6 and 20:3n-6 [6]. When FADS2 is absent, FADS1 produces rare butylene-interrupted fatty

acids, for instance 5,11,14-20:3 and 5,11,14,17-20:4. These and similar PUFA are observed in cell systems [6], knockout mice, and normal domestic cats [18]. In this sense, FADS1 competes successfully with FADS2 when *FADS2* expression is negligible. Disruption of the *Fads1* gene in mouse causes massive accumulation of the 20:3n-6 substrate and 1-series-derived prostaglandins, with a concurrent decrease in the product 20:4n-6 and 2-series-derived prostaglandins [19]. *Fads1* ablated mice fail to thrive beyond 12 weeks of age; the phenotype is rescued by dietary supplementation of 20:4n-6 [19]. We recently showed *FADS1* producing several mRNA and protein isoforms. One *FADS1* isoform (*FADS1AT1*) enhances desaturation activity of *FADS2*, leading to increased production of eicosanoid precursors [7].

1.3.3 FADS3

FADS3 (OMIM#606150) is the enigmatic third member of the *FADS* gene cluster. In humans it spans 17.9 kb of genomic DNA, presumed to encode a 445-amino acid protein with a molecular mass of 51.1 kDa [8]. *Fads3* [7, 20] is translated, but no reports exist showing *FADS3* mediating front-end desaturation analogous to *FADS1* and *FADS2*. It is extensively spliced, generating at least 8 alternative transcripts that are phylogenetically conserved in several mammalian and avian species [9]. Several FADS3 isoforms have been reported using immunoblotting [20], and are phylogenetically conserved.

Some aspects of its regulation are known and provide clues to its function. Gene transcript studies show *Fads3* is highly expressed in mouse

uterus at the implantation site; in a *Fads2* null mouse, *Fads3* expression increased by 3-fold; in baboons fed 22:6n-3 and 20:4n-6 *Fads3 ATs* abundance increases while *Fads1* and *Fads2* classical transcripts decrease [9, 21]. An *in vitro* study provided evidence for *Fads3* desaturation of *trans*11-18:1 (the most abundance *trans* fatty acid in bovine milkfat) to make a conjugated isomer (*trans*11,*cis*13-18:2) by back-end desaturation at position Δ13 (between carbons 13 and 14) common in plants, though the final product structure was not positively identified [22]. We generated the first *Fads3* null mouse and found no differences in overt phenotypes (survival, fertility, growth rate) between null and wild type, but fatty acid tissue profiles support a role for *Fads3* in the synthesis of DHA during perinatal period [23]. Dosing of *trans*11-18:1 in aged wild-type mice and comparison to *Fads3* null mice provided no *in vivo* evidence for the 11,13 isomer (Zhang et al, 2015, unpublished observations).

1.4 ELONGASES

Fatty acid elongation is well known to occur in cytosol, mitochondria and predominantly microsomes. The microsomal fatty acid chain elongation system (FACES) pathway cycles through a four step process (condensation, reduction, dehydration and reduction) using fatty acids of 12 or more carbons from endogenous and exogenous sources, adding two carbons in each cycle [24]. The first, condensation step is rate limiting and is catalyzed by ELOVL family in mammals, comprised of seven members (ELOVL1-ELOVL7).

Among the seven elongases, ELOVL1, ELOVL3, ELOVL6 and ELOVL7 prefer SFA and monounsaturated fatty acids (MUFA) as substrates [24]. ELOVL2 and ELOVL5 are PUFA-specific, whereas ELOVL4 (OMIM#605512) prefer SFA and very long chain PUFA (C28-C38) [24]. A highly conserved HXXHH motif is commonly found in all 7 members [25]. Here we focus on the PUFA specific ELOVL2 and ELOVL5.

1.4.1 ELOVL5 (C18- 20 PUFA ELONGASE)

ELOVL5 (OMIM#611805), is expressed in several human tissues, with highest expression detected in Purkinje cells, lung, testis and adrenal gland. It is specific for 18 and 20 carbons PUFA [24-26]. Microsomes from *Elovl5* null mice failed to elongate 18:3n-6 to 20:3n-6 and 18:4n-3 to 20:4n-3 resulting in accumulation of 18:3n-6 and 18:4n-3 respectively, and significant lowering of their downstream products 20:4n-6 and 22:6n-3, respectively. These mice develop hepatic steatosis apparently as a consequence of decreased cellular 20:4n-6 and 22:6n-3 and upregulation of sterol regulatory element-binding protein 1c (*Srebp-1c*) and its target genes [24]. The expression of *ELOVL5* is transcriptionally regulated by SREBP-1c and a recent study in human showed existence of two novel sterol regulatory element (SRE) binding sites, one in the upstream region and one in the exon 1 of *ELOVL5* [25].

1.4.2 ELOVL2 (C20-24 PUFA ELONGASE)

ELOVL2 (OMIM#611814), is selectively expressed in human tissues, with

highest expression detected in testis and liver. It has substrate specificities for 20 and 22 carbon PUFA [24, 27-29]. Rat Elovl2 converts 22:5n-3 to 24:5n-3 [28], whereas, in chickens both Elovl2 and Elovl5 convert 22:5n-3 to 24:5n-3 demonstrating species specific differences [30]. Ablation of *Elovl2* caused complete arrest of spermatogenesis, complete absence of very long chain PUFA (carbon chain length between 24 to 30) of the n-6 family in testis and significant increase in the serum levels of 20:5n-3 and 22:5n-3, with concurrent non-significant decrease in 22:6n-3 [31]. The supplementation of 22:6n-3 for 3 months was not able to restore male fertility in these mice [31]. In the follow-up study the same group found *Elovl2* deletion caused severe reduction of 22:6n-3 and 22:5n-6 and an accumulation of 22:5n-3 and 22:4n-6 in both liver and serum. These mice had increased expression of *Srebp-1c* and its target genes (*Fasn* and *Scd1*) in liver but did not develop steatosis [32].

1.5 GENETIC VARIANTS: LCPUFA LEVELS AND HUMAN PHENOTYPES

The *FADS* and *ELOVL* are among the most prominent genes associated with human phenotypes in both candidate gene study and genome-wide association study (GWAS). It has long been known that carnivores, such as cats and higher trophic level fish, have lost the metabolic ability to synthesize long chain PUFA via loss of Fads2 desaturation activity; presumably this is due to the ubiquitous presence of 20:4n-6 and 22:6n-3 in a meat based diet. In contrast, herbivores ingest very little 20:4n-6 and 22:6n-3 and must have a robust metabolic pathway to synthesize all they need, especially at life stages

of high demand such as the brain growth spurt. The remarkable flexibility of humans to survive in environments that predominantly produce animal foods or plant foods suggests adaptive changes specifically in the *FADS* and *ELOVL* genes [33].

Converging evidence from candidate gene and GWAS available recently show large genetic variability in the level of fatty acid precursors 18:3n-3 and 18:2n-6 and their apparent conversion to physiologically important LCPUFA products, especially 20:5n-3 and 20:4n-6. These studies have shown strong associations between the minor allele carriers of single nucleotide polymorphisms (SNPs) within *FADS* gene cluster, *ELOVL2* and *ELOVL5* and fatty acid levels in serum, plasma, red cells, breast milk and adipose tissue [13, 34-36].

1.5.1 CANDIDATE (FADS GENE CLUSTER, ELOVL2 AND ELOVL5) GENE STUDIES

A *FADS* gene cluster association study showing minor allele carriers of a 11 SNP haplotype exhibited increased levels of 18:2n-6, 20:2n-6, 20:3n-6, and 18:3n-3 and decreased levels of 18:3n-6, 20:4n-6, and 20:5n-3 in serum [34]. This finding was subsequently replicated independently by others. Genetic variability was highest (28%) for 20:4n-6 [34]. Locus specific (*FADS* gene cluster) SNPs are associated with human phenotypes, for instance, inflammation and cardiovascular disorders [13, 34], levels of blood lipids (total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LCL)

and triglyceride(TG)) [37], insulin resistance in schizophrenia and bipolar patients [38], perinatal depression [13], atopic diseases [13, 34], attention/hyperactivity [13] and intelligence in children [13]. A SNP in *ELOVL5* was associated with late onset primary open-angle glaucoma [39]. Recent studies show SNPs near *FADS1* (rs174537) influencing the levels of 5-lipoxygenase products [40] and another found rs174537 associated with epigenetic alteration [41]. A minor allele of *FADS3* SNP (rs174455) was negatively associated with 22:6n-3 in erythrocyte phospholipids in the AVON longitudinal study of parents and children (ALSPAC) cohort [42]. A very distinct *FADS* haplotype was shown to be associated with enhanced ability to biosynthesize LCPUFA from PUFA precursors [43]. Major allele homozygotes of a 4 SNP haplotype showed more than 3-fold greater apparent conversion of [U-^{13}C]-18:3n-3 to [U-^{13}C]-20:5n-3 in plasma than minor allele carriers [44]. Al Saleh *et al.* [36] found minor allele carriers of 3 SNP haplotype within the *ELOVL2* gene with lower plasma 22:6n-3 than major allele carrier. An SNP within *ELOVL5* was nominally associated with decreased capacity to metabolize 20:5n-3 to 22:5n-3 [35]. Epigenetic marks within the regulatory regions of *ELOVL5* were associated with depression and suicide risk [45].

1.5.2 GENOME-WIDE ASSOCIATION STUDY

As a complement to locus specific SNPs, several GWAS identified *FADS* and *ELOVL* loci to be associated with LCPUFA levels and human phenotypes. Traditional inhabitants of the arctic region of North America are known to

subsist almost exclusively on animal foods, predominantly fish and marine mammals. GWAS show a striking adaptation of the Greenland Inuit *FADS* gene cluster attributed to high omega-3 fatty acid intake [46]. A meta-analysis of seven GWAS scans and replication with 20,623 individuals identified 30 loci including the *FADS* cluster as influencing HDL and triglyceride levels [13]. The InCHIANTI GWAS identified a minor allele of rs174537 accounted for 18.6% genetic variance in 20:4n-6 concentrations, which was confirmed in an independent sample from the GOLDN study [13] and another study found genetic variants within *FADS1* and *FADS2* to be associated with higher levels of ALA and lower levels of EPA and DPA and these associations were similar in ancestries of European, African, Chinese and Hispanic origin [47]. Lemaitre et al.[47] also showed minor alleles of SNPs in *ELOVL2* to be associated with lower 22:6n-3 levels, however, these associations were found to be less consistent in the four ancestries. Moreover, there are numerous GWAS reports that relate *FADS* and *ELOVL* SNPs to TC-HDL-LDL-TG and lipid metabolite levels [13, 48, 49], fasting glucose homeostasis [50], and resting heart rate [51]. A few studies tried to combine GWAS with metabolomics (mGWAS) to understand gene-environment impacts on homeostasis and to address missing heritability [52]. Genetic information at the *FADS1* locus (rs174547) combined with targeted metabolomics identified 36% of the observed variance in metabolite concentrations [53], in the same study SNP (rs9393903) in *ELOVL2* accounted for 9.8% variance. Similarly, Suhre et al. [54] showed “genetically

determined metabotypes" accounted for 10–60% differences in metabolite levels per allele copy in 25 loci which also includes *FADS1* and *ELOVL2*. *FADS1* SNP (rs174548) accounted for 29% of the metabolic ratio variance [13]. *ELOVL2* is associated with sleep duration [55], age and DNA methylation [56].

1.5.3 CODING GENETIC VARIANTS (FADS GENE CLUSTER, ELOVL2 AND ELOVL5)

Target captured exome sequencing showed existence of 53,081 coding SNPs (cSNPs) in the human genome [57]. cSNPs may be synonymous, resulting in no change in the resulting protein primary sequence, or non-synonymous, resulting in a substitution of one amino acid for the other at the relevant position. An October 2015 check of the NCBI dbSNP database reveals several cSNPs within the *FADS* and *ELOVL* genes. No disease causing phenotypes associated with cSNPs in the FADS gene cluster and *ELOVL2* are yet known, however, a *FADS2* promoter SNP (rs968567) enhancing *FADS2* expression has been discovered [58]. Two non-synonymous mutations within *ELOVL5* causing spinocerebellar ataxia 38 (*SCA38*) has been reported in families originating from Italy and France [26].

1.5.4 INSERTION-DELETION (INDEL) POLYMORPHISM

GWAS and candidate gene studies target SNP(s) that are tags for haplotypes which serve only as signals close to functional variants responsible for

phenotypic variation. Association strength is directly related to the physical closeness of the GWAS SNP(s) to the functional element, a basic principle underlying the Manhattan plot.

In humans, highly polymorphic small Indels (1 bp to 10,000 bp) are the second most frequent polymorphisms after SNPs and are increasingly recognized as functional contributors of genetic variation influencing multiple human phenotypes [59]. Largely due to the technical difficulties in genotyping and calling Indels from short read sequencing data, their functional effects are understood only in a few cases [59]. Recently, we discovered a 22-bp Indel polymorphic variant (rs66698963) in *FADS2* intron 1 near a SRE to be associated with desaturase expression depending on levels of SREBP-1c agonists [60]. Follow-up work showed the *FADS2* Indel strongly influencing metabolic capacity to synthesize 20:4n-6 from precursors and also showed ethnic differences in the allele frequency in general US and Indian subjects, as well as evidence for adaptive evolution (Kothapalli et al., 2015, unpublished data). Commonly reported SNP variants within intron 1 of *FADS2* (rs174575, rs174570 and rs1535) and thus the nearby Indel are associated with increased IQ scores, blood fatty acid levels and complex diseases. rs174575 and rs174570 are within 600 and 6000 bp upstream from the *FADS2* Indel, respectively [13, 34, 46]. In humans, common SNP variants are often found to follow Indels [61], suggesting that rs174575, rs174570 and/or rs1535 are tags for the functional genomic Indel that directly modulates binding at the nearby SRE. Whether or not the Indel is the functional element or it is nearby,

genotypic variation controlling basal concentrations of 20:4n-6, its immediate precursor and its products, demonstrates *in vivo* that FADS2 protein(s) is (are) a major rate limiting enzyme for LCPUFA production.

1.6 CONCLUSION

The genes mediating the endogenous synthesis of LCPUFA contribute wide variability to the efficiency of LCPUFA synthesis, likely controlled at *FADS2* but also controlled at the level of *FADS1* and the elongases depending on genotype and metabolic state. In the era of mass individual immigration and international food supplies, individuals with genotypes adapted to a food supply with high, or low, LCPUFA will find themselves exposed to diets to which they are not adapted. Precision nutrition depends on the detailed genetics controlling LCPUFA conversion efficiency, for instance, those adapted to high intakes of 20:4n-6 and 22:6n-3 via meat and fish may become deficient when consuming an otherwise heart healthy diet predominantly composed of vegetables; the risk of neurodegenerative diseases may thereby increase. Continuing mechanistic studies of genetic function are needed to address this issue.

FADS3, as a putative front-end desaturase, is reported to associate with human blood and tissue LCPUFA levels and related to risk of familial combined hyperlipidemia. Investigations of FADS3 biological functions will advance the knowledge of LCPUFA *de novo* synthesis and the regulation of LCPUFA in chronic diseases, such as cardiovascular diseases.

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Authorship Contribution

Ji Yao Zhang, Kumar S. D. Kothapalli, J. Thomas Brenna. Ji Yao Zhang drafted the article. Kumar SD Kothapalli provided conception of the work and critical revision of the article. J. Thomas Brenna provided conception of the work, critical revision of the article and final approval of the version to be published.

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CHAPTER 2

FADS3 MODULATES DOCOSAHEXAENOIC ACID (DHA) IN LIVER AND BRAIN[2]

2.1 INTRODUCTION

Highly unsaturated fatty acids (HUFA), especially docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6) are metabolically required for growth, early neural and visual development [1-3]. The perinatal brain growth spurt imposes the highest relative demand for DHA and AA for structural lipid. Rodents are altricial mammals born with immature brains that undergo the brain growth spurt in early postnatal life [4]. The human brain growth spurt is perinatal, starting at about 27 weeks and continuing past 2 years of age. Brain growth and brain size are correlated with neurogenesis [5] during which time HUFA rapidly accumulate [6].

The availability of HUFA in mammals depends on dietary intake and endogenous synthesis. In an alternating process of desaturation and elongation, HUFA can be biosynthesized from dietary PUFA precursors, linoleic acid (LA, 18:2n-6) and α-linolenic acid (ALA, 18:3n-3). Fatty acid desaturase 1 (*Fads1*) and fatty acid desaturase 2 (*Fads2*) code for key multifunctional enzymes in HUFA biosynthesis, introducing *cis* double bonds

[2] *Fads3* modulates docosahexaenoic acid (DHA) in liver and brain. Ji Yao Zhang, Xia Qin, Allison Liang, Ellen Kim, Peter Lawrence, Woo Jung Park, Kumar SD Kothapalli, J. Thomas Brenna. Submitted, April 2016.

at specific position in the carbon chain [7, 8]. *Fads1* codes for a Δ5 desaturase using 20:2n-6, 20:3n-6, 20:3n-3 and 20:4n-3 as substrates. The *Fads2* protein product catalyzes trifunctional even-numbered Δ6, Δ8 and Δ4 desaturase activities by acting on at least eleven substrates. *Fads2* Δ6-desaturase activity catalyzes the conversion of 6 competing substrates (18:2n-6 → 18:3n-6; 18:3n-3 → 18:4n-3; 24:5n-3 → 24:6n-3; 24:4n-6 → 24:5n-6; 16:0 →16:1n-10, and 18:1n-9 →18:2n-9), Δ8-desaturase activity acting on 20:1n-9 → 20:2n-9, 20:2n-6 → 20:3n-6, and 20:3n-3 → 20:4n-3, and Δ4-desaturase activity acting on 22:4n-6 → 22:5n-6 and 22:5n-3 → 22:6n-3 [9-11] .

The fatty acid desaturase 3 (*Fads3*) [12] is the third known member of *Fads* gene cluster, along with *Fads1* and *Fads2* arose evolutionarily by a gene duplication event and localizes to mouse chromosome 19 [8]. Human *FADS* homologs with similar structural organization are localized to human chromosome 11q13, a cancer hotspot locus [12, 13]. In human, *FADS3* spans 17.9 kb genomic DNA, located 6.0 kb 3' from *FADS2*, and has the same gene structure as *FADS1* and *FADS2* consisting of 12 exons and 11 introns. The amino acid sequences of *FADS3* are 52% and 62% homologous to *FADS1* and *FADS2*, respectively. The putative protein coded by *FADS3* is composed of an N-terminal cytochrome b5-like domain and three histidine motifs at the C-terminal ends, characteristic of all membrane-bound front end desaturases. *FADS3* is transcribed and extensively spliced [14] and the splice variants yield alternative transcripts (ATs) which are phylogenetically conserved in at least several mammalian and avian species [15]. Proteins have been detected that

may correspond to ATs [16]. We have shown recently using mouse embryonic fibroblast (MEF) cells and ribosome foot-printing technology the first positive-sequence-specific-proof of *Fads3* translation [17]. Despite these observations, there are no reports of *Fads3* mediated front-end desaturation.

FADS3 is reported to have some potential biological functions. Gene expression studies show that *Fads3* mRNA changes when *Fads1* and *Fads2* changes, though not in the same direction. For instance, *Fads3* expression increased 3-fold in *Fads2* knock-out (KO) mice compared to wild type (WT) [18], and when DHA and ARA were included in the diet of neonate baboons, *Fads1* and *Fads2* expression went down while *Fads3* ATs increased [19]. An earlier study showed that the expression of *Fads3* was higher in mouse uterus at the implantation site [20]. A recent *in vitro* study showed Fads3 specificity for *trans*-vaccenic acid [VA; *trans*11-18:1], catalyzing synthesis of *trans*11, *cis*13-CLA isomer (Δ13 desaturation) in the first reported case of a mammalian back-end desaturase [21]. These data, to our knowledge, have not been replicated *in vivo*. A genome-wide association study (GWAS) showed genetic variants within *FADS3* are associated with familial combined hyperlipidemia among Mexican population [22] and a 2011 AVON longitudinal study [23] found minor allele of *FADS3* SNP (rs174455) to be negatively associated with DHA in red blood cell (RBC) phospholipids.

Here we present generation of first *Fads3* KO mouse colony, with an aim to characterize its metabolic phenotype and to find clues to *in vivo* function. We focused on the early life because it is the period of rapid brain growth spurt

and where intense demand for brain accretion of the most physiologically important HUFA is at least in part mediated by liver.

2.2 MATERIALS AND METHODS

2.2.1 Generation of the Fads3 KO mouse

Fads3 KO mice were generated using a gene targeting technique [24]. The embryonic stem cell (ES, derived from JM8A1.N3) clone was purchased from KOMP Repository (ID 49707, Davis, CA). The target vector (Fig. 1A) contained a splicing acceptor (SA) followed by positive selection marker lacZ and neomycin resistance gene (neo). After the target vector was transfected into ES cells, 7.1 kb nucleotide sequence of *Fads3* located between exon 1 and exon 2 was replaced by the vector construct via homologous recombination. Long range PCR at the 5' end of the vector was used to test the accurate integration of vector gene at the targeting location. Copy number of vector inserted in transfected ES cells was determined by Taqman PCR at Murine Genetics Analysis Lab at University of California, Davis, CA.

ES cells carrying a single copy of the vector at the targeting location were microinjected into C57BL/6 blastocytes and then implanted into a pseudo pregnant recipient to produce chimeras. One male chimera was generated at the Cornell University College of Veterinary Medicine transgenic mouse facility. The male chimera was crossed with a wild type C57BL/6 female and the litter was screened by genotyping at the age of 12 to 14 days. For genotyping, DNA (from ear punch or tail snip) was extracted using a DNeasy blood & tissue kit

(Qiagen, CA) and two primer sets were designed (Figure 2.1A) to select *Fads3* KO progeny. Primer pairs used for genotyping and gene expression studies are presented in Supplemental Table 2.1.

Breeding of screened heterozygous (Het) female and male generated WT, Het, KO progeny (Figure 2.1B). Tissue expressions of Fads genes were measured to detect the efficiency of knockout technique and validate the KO model (Figure 2.1C). WT and KO pups were used for future breeding. Litters from WT × WT were regarded as the control group and litters from KO × KO were used as experimental group for this study.

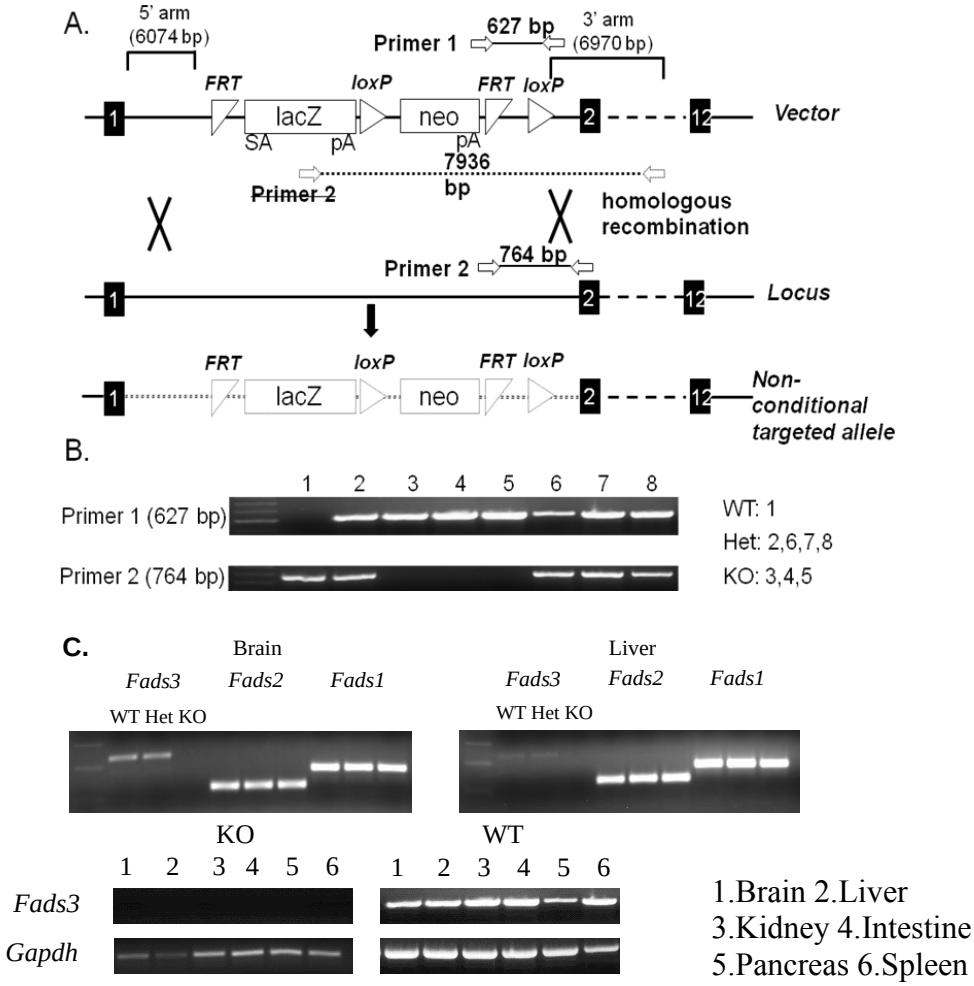

Figure 2.1 Structure of target vector, genotyping strategy and validation of knockout model.

(A) The target vector contained a splicing acceptor (SA) followed by positive selection marker lacZ and neomycin resistance gene (neo). Nucleotides of Fads3 located between exon 1 and exon 2 were replaced by the vector construct via homologous recombination. (B) Amplicons generated from primer set 1 and primer set 2. No visible product from primer 1 but visible product from primer 2 indicates that no vector exists in the mouse, indicating the mouse was wild type (WT). Mouse with visible band from primer 2 but no visible band from primer 1 was knockout mouse (KO). Heterozygotes (Het) had PCR products from both primers. (C) Tissue gene expression of *Fads* genes. KO did not express *Fads3* but expressed *Fads1* and *Fads2* in brain and liver. All six tested KO tissues did not express *Fads3*.

2.2.2 Animals

All the mice were fed *ad libitum* laboratory rodent chow (7012 Teklad LM-485, Harlan Laboratories, WI) containing 2.6%wt linoleic acid (7.6%energy) and 0.3%wt α-linolenic acid (0.9%energy) from soy oil. They were housed in a controlled environment at a 12-hour light/12-hour dark cycle. Body weights were measured three times per week. Oxymax Lab Animal Monitoring System (Columbus Instruments, OH) was used to test physical activities, specifically respiratory exchange ratio, horizontal activity (count/day), vertical activity (count/day), food intake (g/day), and water intake (ml/day). Pups (n=14/genotype/age, female: male= 1:1) on postnatal day 1, 3, 7, 13, 21, and 30 were sacrificed by CO_2 inhalation and brain and liver tissues were collected for fatty acid analysis and gene expression studies. Tissues were kept in RNA*later* stabilization solution (AM7020, Life Technologies, CA) at 4°C overnight and the next day stored at -80°C until use. Animal studies were approved by the Cornell University Institutional Animal Care and Use Committee (IACUC protocol #2011-0007).

2.2.3 Fatty acid analysis

Fatty acid methyl esters (FAME) were prepared from mouse brain and liver samples by using a modified one-step method for specific tissues [25]. Methylated fatty acids were structurally identified by covalent adduct chemical ionization tandem mass spectrometry (CACI-MS/MS, Varian Saturn 2000 Ion Trap, Agilent Technologies, Santa Clara, CA) and were quantified by gas

chromatography coupled to a flame ionization detection (GC-FID, Hewlett Packard 5890 Series II, Agilent Technologies, Santa Clara, CA) using an equal weight FAME standard mixture to verify response factors in each run with sample FAME. Triplicate injections were performed for each FAME and quantity of each fatty acid species was calculated as percentage by weight in total fatty acids (%, w/w).

2.2.4 RNA extraction and preparation of cDNA

Total RNA was extracted from mouse brain and liver tissues using RNeasy Mini Kit (Qiagen, Valencia, CA). The quality and quantity of RNA was determined by using a Nanodrop 2000 (Thermo Scientific, Waltham, MA) and one microgram of total RNA was used to prepare cDNA using the High Capacity cDNA Reverse Transcription Kit (Life Technologies, NY) according to the manufacturer's instruction. The synthesized cDNA was stored at -20°C for future studies.

2.2.5 Real-time PCR

Gene expression ontogeny of classical *Fads1* and *Fads2* transcripts were measured by real-time PCR using SYBR Green Master Mix on a LightCycler 480 instrument (Roche, Madison, WI). Mouse *Gapdh* and *β-actin* were chosen as reference genes. The real-time PCR protocol was as follows: initial denaturation: 95°C for 10 min; amplification: 45 cycles of denaturation at 95°C

for 10 s, amplification at 65°C for 20 s, extension at 72°C for 10 s; final extension: 72°C for 5 min. Melting curve and calculated primer efficiency from standard curves were used to verify the specificity of each primer pair. Based on the standard curve we selected 1:100 dilution of cDNA for testing *Fads1* and *Fads2* gene expression and each reaction was run in triplicate. Relative quantification was calculated based on the normalization of sample targeting gene quantitative cycle (Cq) values to those of geometric mean of sample reference genes using $2^{-\Delta\Delta Cq}$ method [26]. In each time point, mRNA expression of KO mice was normalized to WT (considering the *Fads1* and *Fads2* expression in WT as "1").

2.2.6 Reverse transcription-PCR (RT-PCR)

cDNA generated from mice brain and liver tissues on postnatal day 1, 7, and 30 were used to measure mRNA expression levels of nine genes related to lipid metabolism using semi-quantitative RT-PCR. Amplicons generated from *Elovl2* (elongation of long chain fatty acids 2), *Elovl5*, *Fasn* (fatty acid synthase), *Scd1* (stearoyl-CoA desaturase 1), *Srebp-1c* (sterol regulatory element-binding protein 1c), *Ppara* (peroxisome proliferator-activated receptor alpha), *Ppard*, *Pparg*, *Acaca* (acetyl-Coenzyme A carboxylase alpha) were run on 2% agarose gels stained with ethidium bromide and visualized under UV. The intensities of amplified products were quantified densitometrically by ImageJ software (National Institutes of Health, USA) and the expression levels

of *Elovl2, Elovl5*, *Fasn, Scd, Srebp* and *Acaca* transcript were normalized to expression level of *18S* control gene.

2.2.7 Investigation of Δ13 desaturase activity

Both WT and *Fads3* KO male mice aged around 15 months (n=3/genotype) were provided with 5 mg of vaccenic acid (*trans*11-18:1, Sigma-Aldrich, MO) mixed with chow diet per day for one month. Livers and hearts were collected and fatty acids were analyzed.

2.2.8 Statistical Analysis

Data was analyzed using SPSS 16.0 (IBM, Armonk, NY) software. All data in Figure are presented as mean ± 95% confidence interval. All data in tables are presented as mean ± standard deviation (SD). Student's *t*-test was used to compare the difference between WT and KO. $P<0.05$ was set as statistical significance threshold.

2.3 RESULTS

2.3.1 Fads3 KO mice showed no overt phenotype

Tissue gene expressions of *Fads3* were measured to verify the success of generation of a whole body knockout mouse model. Gel results in Figure 2.1C showed that *Fads3* KO mice did not express *Fads3* but expressed Fad2 and

Fads1 in brain and liver; the six tested tissues did not have expressions of Fads3 in KO. All the evidence listed above indicated that this knockout model was valid.

The litter size was 6 ± 2.5 and 6 ± 2.2 (n=26) in WT and KO, respectively and no significant difference was observed. The growth rate (Figure2.2 A, 2B) of KO mice was similar to WT mice. Food intake, physical activity and metabolic rate measured by Oxymax Lab Animal Monitoring System were not different between two genotypes (data not shown). The physical appearance of *Fads3* KO mice was indistinguishable from WT mice.

2.3.2 P1 Fads3 KO brain had lower DHA and AA but higher LA

The postnatal brain growth spurt was observed from birth to postnatal day 13 (P13), followed by slower growth thereafter (Figure 2.2 C), with continued DHA accretion past P30. *Fads3* KO mice had significantly lower brain DHA at P1 and P7 compared to those of WT, though the intermediate P3 was not different (Figure 2.3A). From P13 to P30 no differences in brain DHA were found. On P1, the precursor of n-6 HUFA, LA, was 0.96 ±0.13% and 0.74 ± 0.18% of total fatty acids in KO brain and WT brain, respectively ($P<0.05$, Figure 2.3B). From P3 to P21, LA was non-significantly greater in KO brains and by P30 was 0.97 ± 0.10% vs. 0.85 ± 0.09% of total fatty acids in KO and WT, respectively (Figure 2.3B). Interestingly, even though the n-6 precursor LA was higher in KO brain, its downstream product AA was lower (Figure 2.3C) compared to WT mice on P1. The complete brain fatty acid profiles were

presented in Supplemental Table 2.2.

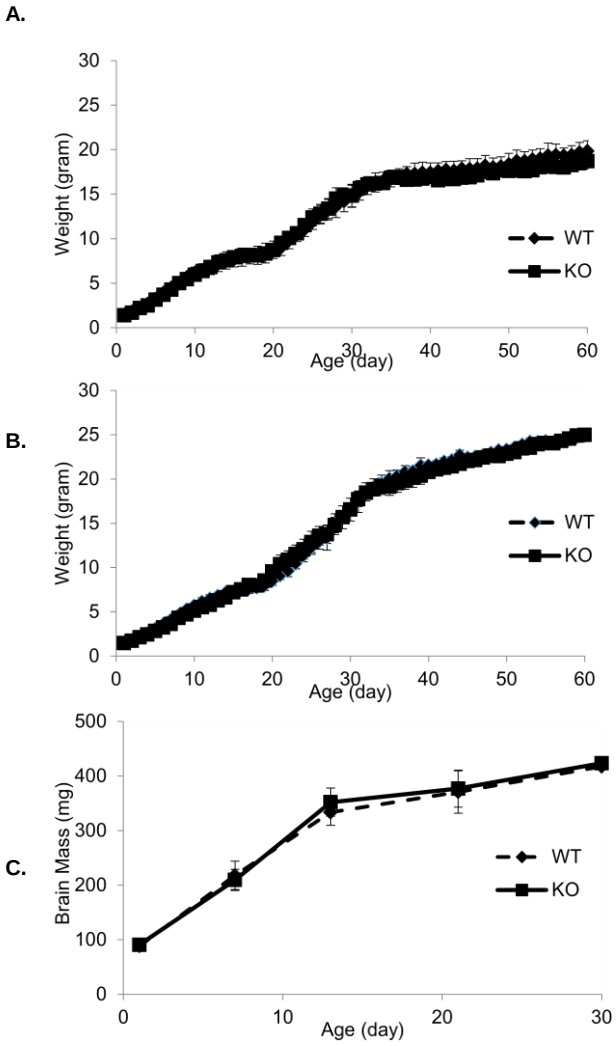

Figure 2.2 Mice growth curve and brain mass growth. (A) Female mice body weight. No differences in body weight between genotypes were observed. (B) Male mice body weight. No differences in body weight between genotypes were observed. (C) Brain growth curve. Mice brain mass was used to determine gross brain growth. From birth to P13 the rate of postnatal brain growth was higher (regarded as “spurt”) than later age point. No differences were observed between genotypes.

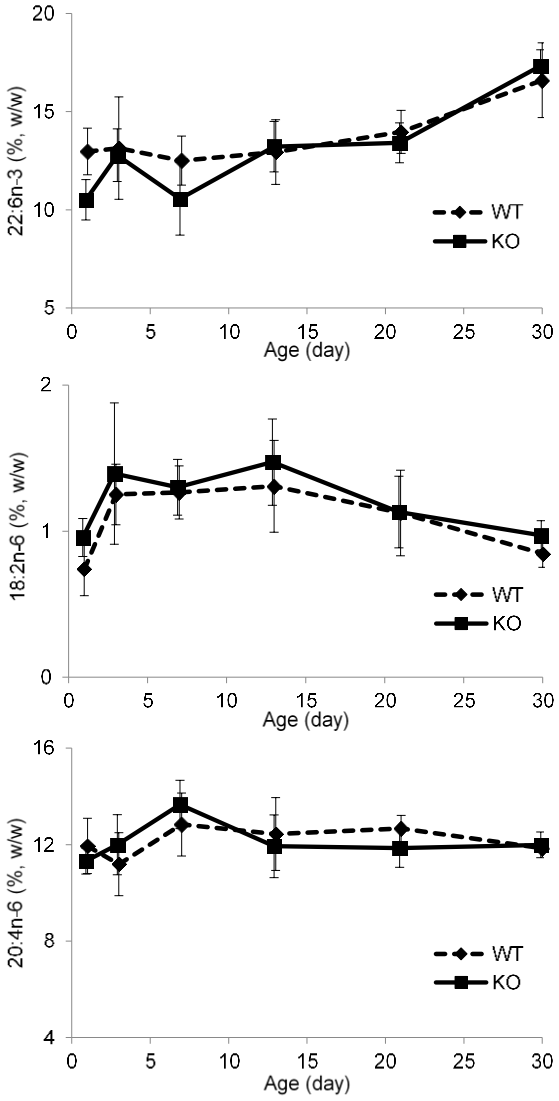

Figure 2.3 Brain PUFA ontogeny. (A) Brain DHA (22:6n-3) content. KO had significantly lower amount of brain DHA at P1 and P7 but caught up from P13. (B) Brain LA (18:2n-6) content. KO had significantly higher LA, the precursor of n-6 PUFA at P1 and P30 than WT. (C) Brain AA (20:4n-6) content. Brain AA in KO was significantly lower than WT at P1 but no differences at other ages. Values were Mean ± 95% CI (n=14) and * $P<0.05$.

2.3.3 Fads3 KO liver had higher DPA during the Brain Growth Spurt (BGS)

The precursor of n-3 HUFA, ALA, was significantly higher in KO liver than WT on P1 and P3, similar trends though not statistically significant were found from P7 to P30 (Figure 2.4A). In P1 to P13, hepatic docosapentaenoic acid (DPAn-3, 22:5n-3) levels in KO mice were found to be greater than WT, most importantly, DPA levels were 2.4 fold higher in P1 KO. However, by P30 DPAn-3 levels dropped significantly in KO (Figure 2.4B). In contrast, hepatic DHA showed no consistent pattern of differences. P3 KO mice had higher hepatic DHA (7.16 ± 1.11% vs 6.03 ± 1.62%, KO vs WT) while P21 and P30 KO mice had lower hepatic DHA compared to WT (Figure 2.4C). The proportion of DPA to DHA, calculated as DHA/(DPA+DHA) at P1, was 88% and 95% in KO and WT mice, respectively and was lower at P3 (n.s.), P7, P13, and P21. By P30 KO had risen and WT fallen, and the KO DHA proportion was significantly greater (Figure 2.5A).

Similar to brain LA, KO mice hepatic LA was found to be higher from P1 to P13, through the BGS (Figure 2.4D). Hepatic 20:4n-6 in P1 KO was significantly lower compared to WT, similar observations were seen in brain (Figure 2.4E). No changes in AA levels were observed from P3 to P21, however, at P30 AA was significantly higher in KO than WT. The complete liver fatty acid profiles were presented in Supplemental Table 2.3.

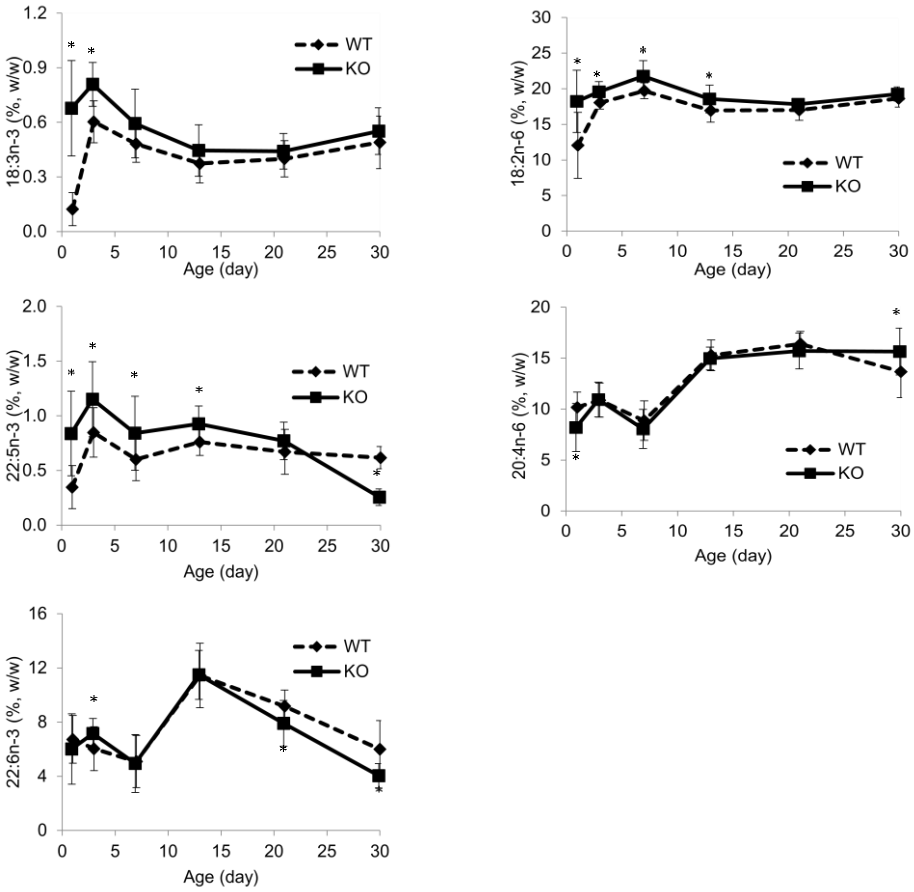

Figure 2.4 Liver PUFA ontogeny
(A) Liver ALA (18:3n-3) content. Hepatic ALA was higher at all time points but reached significance only at P1 and P3. (B) Liver DPA (22:5n-3) content. Hepatic DPA was higher from P1 to P21 (P21 not significant) but significantly lower at P30 in KO compared to WT. (C) Liver DHA (22:6n-3) content. KO liver had significantly lower amount of DHA than WT at P21 and P30. (D) Liver LA (18:2n-6) content. Hepatic LA in KO was significantly higher from P1 to P13 in contrast to WT. (E) Liver AA (20:4n-6) content. KO liver AA was significantly decreased at P1 but no differences were observed from P3 to P21, at P30 AA was significantly higher in KO. Values were Mean ± 95% CI (n=14) and * $P<0.05$.

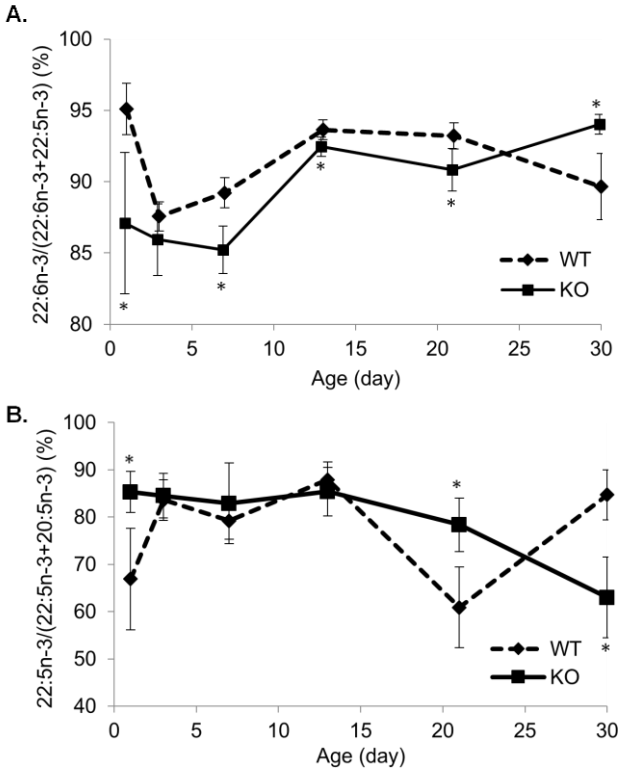

Figure 2.5 Liver Ontogeny of relative DPA to DHA.
(A) The hepatic precursor-product levels for 22:5n-3→22:6n-3 was lower during the BGS (P1, 7, 13 and 21 ($p<0.05$); P3 (n.s.)) in KO compared to WT. After weaning, the proportion was significantly increased in KO in contrast to WT (P30). (B) The hepatic precursor-product levels for 20:5n-3→22:5n-3 was greater in KO at P1 and P21 but lower at P30 compared to WT.

2.3.4 Brain and hepatic expression levels of Fads2 and Fads1 increased at weaning in KO

KO Brain *Fads2* expression levels were significantly higher on P1 and P21, whereas, they were significantly lower on P13 and P30 compared to WT (Figure 2.6A). Brain *Fads1* expression was not different between genotypes from P1-P13 and P30, the only significant increase was observed on P21, the day of weaning (Figure 2.6B). Interestingly on P21, both *Fads2* and *Fads1* expression in KO mice brains were found to be upregulated by 1.3 and 1.7 fold relative to WT, respectively (Figure 2.6A, 6B). As shown in Figure 2.6C and 6D, hepatic *Fads2* and *Fads1* expression levels were significantly downregulated on P1, P7 and P30 KO compared to WT. In contrast, on P21 hepatic *Fads2* expression was elevated by 1.4 fold in KO relative to WT.

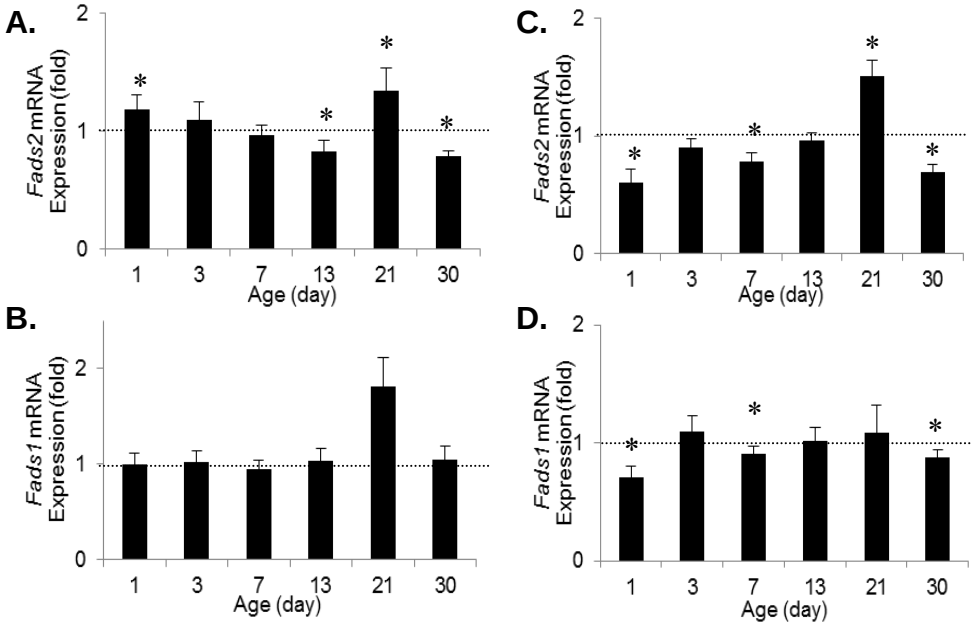

Figure 2.6 Brain and Liver Ontogeny of *Fads2* and *Fads1* gene expression levels. (A) Brain *Fads2* mRNA expression levels. KO brain *Fads2* mRNA levels were significantly decreased at P13 and P30 but increased at P1 and P21 compared WT. (B) Brain *Fads1* mRNA expression levels. Only at P21 KO brain had significantly higher expression levels of *Fads1* than WT. No differences were detected at other ages. (C) Liver *Fads2* mRNA expression levels. Except for P21, KO had lower *Fads2* levels (significant differences found at P1, 7 and 30). (D) Liver *Fads1* mRNA expression levels. *Fads1* mRNA levels in KO were significantly lower on P1, 7 and 30 compared to WT. The dash line crossing "1" represented WT and the solid bars were mRNA levels of KO mice after normalization. Values were Mean ± 95% CI (n=14) and * $P<0.05$.

2.3.5 Hepatic expression of critical genes and transcription factors related to lipid metabolism

Semi-quantitative RT-PCR was performed to test expression levels of critical genes and transcription factors related to lipid metabolism (Supplementary Figure2.1). *Srebf1*, also known as *Srebp-1c* is a key transcription factor involved in the regulation of lipid metabolism. In the absence of *Fads3*, transcript abundance of hepatic *Srebf1* was found to be higher than that of WT on P1 (not statistically significant). Elongation of very long-chain fatty acids (ELOVLs), are a family of enzymes which carry out substrate-specific elongation of fatty acids. The *Elovl2* involved in the elongation of C20–C24 PUFA and *Elovl5* involved in the elongation of C18 and C20 PUFA were found to be significantly upregulated in P1 KO mice. No obvious changes in the expression levels of *Fasn,* which catalyzes the conversion of Acetyl-CoA to palmitate [27] and *Scd,* rate limiting enzyme for the biosynthesis of monounsaturated fatty acids were observed between P1 KO and WT. *Ppard* and *Pparg* were not activated at early development in mice; *Ppara* was upregulated in KO liver on P1. *Acaca*, which catalyzes the rate limiting step in the biosynthesis of fatty acids by carboxylation of acetyl-CoA to malonyl-CoA was found to be significantly upregulated in P1 KO. We also tested brain expression levels, but no obvious differences on P1 were found in the expression levels between KO and WT brain tissue (data not shown).

2.3.6 No Δ13-desaturase activity was detected in liver or heart

One-month treatment of vaccenic acid (5 mg/day) resulted in accumulation of vaccenic acid in the livers and hearts of both WT and KO mice. *Trans*-11-18:1 was positively identified by GC-MS at 1.98 ± 0.78% and 0.97 ± 0.42% (w/w) in livers and hearts, respectively. No new peak with molecular weight corresponding to an 18:2 and that might correspond to *trans*-11,*cis*-13-18:2 was detected (data not shown). Based on the smallest peaks observed in the chromatogram, we estimated that *trans*-11, *cis*-13-18:2, if it is in the liver, was less than 1% of *trans*-11-18:1. A similar estimate led to an estimate of less than 5% for heart.

2.4 DISCUSSION

Because of similarities in gene structure and amino acid homology among the *Fads* genes, we hypothesized that *Fads3* would have a role in HUFA biosynthesis. An early study showed *Fads3* to be highly expressed at the implantation site in mice [20]. *Fads1* KO mice failed to thrive and died by 12 weeks of age [28] due to AA-deficiency, and *Fads2* KO disrupted spermatogenesis and made male *Fads2* KO mice infertile [18, 29] and we further hypothesized that *Fads3* KO mice would have impaired reproduction. However, the first *Fads3* KO mice showed no differences in fertility, litter size or longevity (data not shown) compared to WT. Moreover, *Fads3* KO mice had no overt differences in growth, metabolic rate, or overt physical appearance.

HUFA play vital roles in early development so we focused on the biochemical phenotype in early life. The HUFA-rich brain grew similarly in KO and WT mice (Figure2.2). Similarly, food intake, water intake, RER and locomotion of *Fads3* KO mice were similar to those of WT (data not shown).

Human *FADS3* was cloned in 2000 [12] but its function has been elusive. An *in vitro* report with rat *Fads3*-transfected COS-7 monkey kidney cells yielded evidence that the Fads3 protein catalyzes Δ13–desaturation, converting vaccenic acid (*trans*11-18:1, VA) to *trans*11, *cis*13-18:2, a novel conjugated linoleic acid isomer. Vaccenic acid is the major *trans* monoene in cow's milk, and its Δ13–desaturation would be the first observation of a "back-end" desaturation in mammals. We treated both WT and KO adult male mice with 5 mg of VA per day, an amount calculated to be equivalent to the amount of VA provided per day if all dietary fat was from milk fat, for 30 days. Our GC results showed accumulation of VA in liver and heart, however, we were not able to detect any desaturation product and estimate that conversion, if operating in liver would be less than 1% of the *trans*-11-18:1 and in heart would be less than 5% of the *trans*-11-18:1.

Differences in fatty acid profiles between WT and KO were detected. Fatty acid ontogeny in brain showed that HUFA precursor 18:2n-6 was higher but downstream HUFA 20:4n-6 was lower in Fads3 KO on P1; similar trend was observed in the liver. *Fads3* gene expression was upregulated by 2.5 fold in *Fads2* KO compared to WT [18]. Our study showed that brain and liver *Fads1* and *Fads2* expression were increased significantly only on P21 in KO

mice. Hepatic *Elovl2* and *Elovl5* expression levels were increased significantly in the absence of *Fads3* on P1, which may explain the significant increase of 22:5n-3 levels and indicated *Fads3* as a factor influencing elongase activity.

22:6n-3 is the most physiologically important n-3 PUFA in neural and retinal membranes, accounting for 8% of brain dry weight [30], and required for normal brain and vision development [1-3]. Mouse gross brain mass at birth is around 20% of maximum brain weight [31] and grew to 80% of maximum brain weight by P13, characteristic of altricial animals with post-natal BGS (Figure 2.2C) synchronized with rapid synaptogenesis and myelination [32, 33]. In our study, KO newborn brain 22:6n-3 was significantly lower than WT but normalized by P13 when eyes open (Supplemental Table 2), which may be the reason why we failed to observe any overt differences between two genotypes. It is plausible that low brain 22:6n-3 concentration in the P1 KO was due to residual low accumulation of brain 22:6n-3 during fetal life that was corrected in the first days of independent life. We did not check maternal 22:6n-3 levels to shed light on the cause of low neonatal brain 22:6n-3.

Brain 22:6n-3 can be obtained either from circulating preformed 22:6n-3 or from *de novo* synthesis in the brain [34]. Before P13, milk is the sole food for neonates. In the milk of chow-fed mice, 18:2n-6 is the major PUFA accounting for ~15% of total fatty acids by weight and 18:3n-3, 20:4n-6, 22:6n-3 are less than 1% of total fatty acids [35]. In liver we found 18:3n-3 and the downstream product 22:5n-3 in KO were generally higher in P1-P21 compared to age-matched WT (Supplemental Table 3). Of the sum of 22:5+22:6, the

proportion of 22:6 was greater in the WT than the KO (95% vs 88%, respectively, at P1) reflecting less net product accumulation in KO and suggesting that Fads3 played a role in 22:6n-3 synthesis or accumulation (Figure2.5A). *Fads2* expression was downregulated in P1 KO liver (Figure 2.6) where *Fads2* mediated 22:6n-3 synthesis via Δ4-desaturation (22:5n-3→22:6n-3) [11] or Δ6-desaturation (24:5n-3→24:6n-3→22:6n-3) [36] might be affected. As liver supplies 22:6n-3 to the developing brain [37], lower hepatic *Fads2* expression and reduced Fads2 mediated net conversion of 22:5n-3 to 22:6n-3 may explain lower amounts of 22:6n-3 in the KO brains. DPA is converted from EPA via elongation catalyzed by *Elovl2* or *Elovl5* [38, 39]. In P1 KO the precursor-product levels for the conversion 20:5n-3→22:5n-3 was much greater than WT (Figure 2.5B), consistent with increased hepatic expression of *Elovl2* and *Elovl5* (supplement Figure 2.1).

Our results provided evidence that *Fads3 in vivo* acted to reduce elongase expression and by implication, activity. *Fads3* is extensively spliced and it has been demonstrated that splice variants can modulate several enzymatic activities, such as substrate specificity, catalytic properties, activity regulation and also can have dominant negative effects over the catalytically active enzymes [14, 40, 41]. *Ex vivo* and *in vitro* studies show that 22:5n-3 is a potent inhibitor of platelet aggregation [42] and effective stimulator of endothelial cell migration [43], suggesting that 22:5n-3 is beneficial for cardiovascular health. Most interestingly, a recent *in vivo* study indicated that 22:5n-3 exerts neuroprotective effects on improving spatial learning task by

attenuating age-related increase in microglial activation [44].

Besides hepatic 22:6n-3 supplying brain 22:6n-3, 22:6n-3 can also be synthesized in the brain in situ [34]. The ontogeny of Δ6-desaturation enzyme activity in mouse brain is highest in late fetal life and drops continuously until weaning. In contrast, hepatic Δ6-desaturation activity is near zero in late fetal life, rises sharply to peak at 7 days about 2.5-fold greater than the highest brain activity, then plateaus at 13 days about 50% greater activity than peak brain activity [45]. We found that brain *Fads2* mRNA expression varied with development in KO, gradual decreased till P13 (significant only at P13), significant increased at P21 and then significant decreased at P30 (Figure 2.6A).

Ubiquitously present 20:4n-6 [46] is indispensable for growth and development [1], besides being precursors for eicosanoid signaling [47-49]. KO mice at P1 had significantly lower amounts of 20:4n-6 but higher amounts of precursor 18:2n-6 in both brain and liver tissues compared to age-matched controls (Supplemental Table 2, Supplemental Table 3). During development, brain 20:4n-6 can be transported from liver, the major tissue for *de novo* synthesis of HUFA in newborn pups [50]. Lower hepatic 20:4n-6 in KO than WT (8.2 ± 2.4 *vs.* 10.2 ± 1.5) and higher LA levels in KO than WT (18.2 ± 4.4 *vs.* 12.1 ± 4.6) (Supplemental Table 3) were consistent with the downregulation of *Fads1* and *Fads2* in P1 (Figure 2.6). Similarly, in KO brain, 20:4n-6 was lower than WT but 18:2n-6 was higher on P1 (Supplemental Table 3); however there was no significant changes in *Fads1* expression

levels. Adrenic acid (ADA, 22:4n-6), the elongation product of 20:4n-6, was the third most abundant PUFA in the neonatal brain in mice (Supplemental Table 2) and in humans [51]. Brain 22:4n-6 accretion was fast during the BGS consistent with its physiological role in myelination [51]. Similar to 20:4n-6, 22:4n-6 was lower in KO brains than for WT at P1 (Supplemental Table 2). However, liver 20:4n-6 was lower in P1 KO than WT, but 22:4n-6 was higher, consistent with the significant upregulation of *Elovl2* and *Elovl5* in P1 (Supplement Figure 2.1). Significantly higher amounts of 22:5n-3 and 22:4n-6, both elongation products in liver further suggested that *Fads3* influenced *Elovl2* or *Elovl5*. All the above observations indicated that *Fads3* played a role during early development as an enhancer of HUFA biosynthesis and/or regulation.

In conclusion, *Fads3* KO mice were overtly normal. The HUFA pattern pointed to a role for the liver in producing 22:6n-3 destined for the brain. Consistent with hypothesis, in *Fads3* KO neonates, hepatic *Fads1* and *Fads2* were lower and *Elovl2* and *Elovl5* were higher, reflecting compensation to produce more intermediates. It is tempting to speculate that *Fads3* may have a role in maintaining 22:6n-3 synthesis during the BGS when dietary omega-3 is limiting.

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Authorship Contribution

Ji Yao Zhang conducted experiments, collected data, analyzed and interpreted data, and drafted the article. Xia Qin helped data collection and provided critical revision of the article. Allison Liang and Ellen Kim helped data collection. Peter Lawrence provided technical support of data analysis. Woo Jung Park provided critical revision of the article. Kumar SD Kothapalli provided design of the work and critical revision of the article. J. Thomas Brenna provided conception of the work, critical revision of the article and final approval of the version to be published.

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CHAPTER 3

ALTERNATIVE SPLICING GENERATES NOVEL FADS3 TRANSCRIPT IN MICE[3]

3.1 INTRODUCTION

Polyunsaturated fatty acids (PUFA) are ubiquitous in mammalian tissue. Long chain PUFA (LCPUFA) are bioactive components of membrane phospholipids and serve as substrates for signaling molecules [1]. They can be obtained from the diet or are endogenously synthesized in the liver from dietary PUFA precursors, alpha-linolenic acid (ALA, 18:3n-3) and linoleic acid (LA, 18:2n-6) by alternating series of position-specific desaturation and carbon chain-elongation reactions [2]. The nonheme, iron-containing, oxygen-dependent fatty acid desaturases catalyze introduction of double bonds at specific positions within a fatty acid chain. *FADS1* (OMIM#606148), *FADS2* (OMIM#606149) and *FADS3* (OMIM#606150) are located as a cluster within 100 kb region on the long arm of HSA11q12-13.1 locus, while a similar organization is found on mouse chromosome 19 [3, 4]. All three fatty acid desaturase (FADS) genes have evolved by gene duplication events, share 12 exons and 11 introns, and contain a well conserved cytochrome b5 domain and three histidine repeats [4].

[3] Zhang JY, Qin X, Park HG, Kim E, Liu G, Kothapalli KS, Brenna JT. Alternative splicing generates novel Fads3 transcript in mice. Molecular Biology Report. Mol Biol Rep. 2016 May 23. [Epub ahead of print] PMID: 27216536

The *FADS1* and *FADS2* genes encode classical transcripts (CS) for the Δ5-desaturase and Δ4-/Δ6-/Δ8-desaturase, respectively [2, 5]. Direct evidence for the biochemical function of *FADS3* gene product is enigmatic. The first *Fads3* null mouse is fertile despite its high expression at the implantation site in the mouse uterus [6] and has no overt phenotype, though the biochemical phenotype suggests a role in docosahexaenoic acid (DHA, 22:6n-3) synthesis [7]. An early *in vitro* report suggests a unique role as a back end desaturase for *trans*-11 vaccenic acid [8]. Genetic studies have shown associations with familial combined hyperlipidemia in a Mexican population [9] and a minor allele of *FADS3* SNP (rs174455) was found to be negatively associated with DHA in RBC phospholipids in the Alspac cohort [10].

We established that primate *FADS3* gives rise to at least eight alternative transcripts that are highly expressed in a tissue-specific manner and are phylogenetically conserved [11]. Others have reported detection of FADS3 proteins [12]. We have shown with mouse embryonic fibroblast (MEF) cells and ribosome foot-printing technology the first positive-sequence-specific-proof of FADS3 translation [5]. Additionally, both *FADS1* and *FADS2* are also alternatively spliced, and we have established a function for a *FADS1 AT* in *FADS2* mediated desaturation [5, 13]. In this study, we report a transcript variant of *Fads3* in adult mouse liver generated by alternative splicing, evidence of its wide expression in 11 mouse tissues. We also report fatty acid profiles emphasizing the FADS target metabolites from 11 different tissues from n=4 mice. In addition, a very recent study showed that white

adipocytes and brown adipocytes accumulated different long chain polyunsaturated fatty acids during differentiation (Qin X 2006 PMID: 26802938). Here we showed the mRNA expression of *Fads3AT9* in both undifferentiated and differentiated white/brown adipocytes (n=4 for each group). Fatty acid profiles of these cells are detected for exploring the possible function of FADS3.

3.2 MATERIALS AND METHODS

3.2.1 Animals

Studies on mice were approved by Cornell University Institutional Animal Care and Use Committee (IACUC, protocol # 2011-0007). C57BL/6 mice were maintained on rodent laboratory diet (7012 Teklad LM-485, Harlan Laboratories, WI) and were fed *ad libitum*. High quality adult mouse tissues, treated with RNAlater, and maintained at -80°C, were used to isolate total RNA. Four Mice (2 males and 2 females) were on average 1 year old at the time of sacrifice.

3.2.2 Cell culture and sample preparation

The mouse white preadipocyte 3T3-L1 (gift from Dr. Ling Qi, Cornell University) and mouse SV40T-immortalized brown adipocyte cell lines (gift from Dr. Johannes Klein, University of Lübeck) were grown in DMEM media containing FBS (10% FBS for white and 20% FBS for brown adipocytes) and 1% penicillin/streptomycin (10,000 U/ml) in a humidified environment at 37°C

with 5% CO_2. At confluence white and brown adipocyte cell lines were subjected to differentiate by adding induction media. After 48 hours induction media is replaced by differentiation media and the cells were allowed to grow for 8 days to differentiate. Details of the culturing conditions are provided in Supplementary Table 3.1. Cells were collected on day 0 (i.e before adding induction media, undifferentiated) and day 8 (differentiated). Four replicates of each cell group are conducted in this study.

3.2.3 RNA isolation and cDNA synthesis

Total RNA was isolated from adult mouse tissues, and from white and brown fat cells using the E.Z.N.A. total RNA kit (Omega Bio-Tek, GA). The quality and quantity of RNA was analyzed by 260/280 nm ratios using a low volume spectrophotometer (NanoDrop, Thermo Scientific, DE). Total RNA was reverse transcribed into cDNA using the High Capacity cDNA Reverse Transcription Kit (Life Technologies, NY) according to the manufacturer's instructions.

3.2.4 RT-PCR conditions and identification of alternative transcript

We designed primers to amplify the protein coding region of mouse *Fads3* (GenBank Accession# NM_021890). *Fads3* Forward: ATGGGCGGTGTCGGGGAGCCCGGA and *Fads3* Reverse: TCATTGATGGAGGTATGCATCCAGCCA. RT-PCR was performed using these primers with adult female mouse liver cDNA as template and iProof high-fidelity DNA polymerase (BIO_RAD, CA) in a 20 µl reaction. Cycling

conditions were: initial denaturation at 98°C for 30 s followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 68°C for 30 s and extension at 72°C for 30 s, with a final extension at 72°C for 5 min. PCR products were run on 2% agarose gels containing ethidium bromide and visualized under UV. Two prominent bands were obtained when separated by electrophoresis on a 2% agarose gel. The lower band was gel purified, cloned into pGEM T-Easy vector (Promega, WI) and sequenced using T7 forward and SP6 reverse universal primers at the Cornell University life sciences core laboratories.

3.2.5 Expression of Fads3CS and AT9 in mouse tissues and adipose cells

To amplify *Fads3CS*, a forward primer was designed within the exon1 and reverse primer was designed within in the exon2 which is missing in the alternative transcript. To amplify *Fads3AT9*, the forward primer bridged the deleted parts of the exon 2. Details of the primers and PCR conditions are provided in Supplementary Table 3.2. Briefly, cDNA was prepared from 11 different mouse tissues and adipose cells (undifferentiated and differentiated white and brown cells) using the High Capacity cDNA Reverse Transcription Kit (Life Technologies, NY) to measure mRNA expression levels of *Fads3CS, Fads3AT9* and adipose cells. PCR amplification reactions were performed using EmeraldAmp GT PCR Master Mix (Clontech, CA) in a volume of 20 microliters. PCR products were resolved using 2% agarose gels and bands visualized under UV light. 18S was used as control. mRNA expression of

Fads3CS and *AT9* were quantified using ImageJ software (National Institutes of Health, USA) and the expression levels were normalized to reference gene *18S*. mRNA expression of Fads3CS was regarded as "1" and the change between *CS* and *AT9* was measured by ratio of AT9/CS.

3.2.6 Fatty acid analysis

Adult mouse tissues (11 different tissues from 4 mice) and adipocytes (4 replicates for each cell type) were used for fatty acid extraction and analysis. Fatty acid methyl esters (FAME) were prepared using modified one-step method of Garces and Mancha [14] modified for use with soft tissue. Methylated fatty acids were quantified by gas chromatography-flame ionization detection (GC-FID) using an equal weight mixture for response factor calibration and peak structures were identified by GC-covalent adduct chemical ionization tandem mass spectrometry (GC-CACI-MS/MS) [2]. Three injections were performed for each FAME and quantity of each fatty acid species was calculated as percentage by weight in total fatty acids (%, w/w).

3.2.7 Statistical analysis

Data are expressed as Mean ± SD. Gene expression levels of *Fads3CS* and *Fads3AT9* in each cell type were compared using paired *Student's* t-Test. Statistical significance of fatty acids among eleven tissues and cells was analyzed using one-way ANOVA and Tukey's post hoc test (SPSS software, version 16.0). Different alphabets shown in Table 1 represent statistical

significance (P<0.05) between groups.

3.3 RESULTS AND DISCUSSIONS

The open reading frame sequence of house mouse (*Mus musculus*) *Fads3* (GenBank Accession# NM_021890) consists of 1350 bp, encoding a protein of 449 aa and a stop codon. To clone *Fads3* ORF into expression vectors for functional studies we designed primers to amplify 1350 bp of *Fads3*, however, in addition to the expected product size we observed another prominent band using adult mouse liver cDNA (Figure 3.1, Top). We carefully gel extracted the prominent band, cloned and sequenced the product resulting in the identification of a novel splice variant of *Fads3* (*Fads3AT9*; GenBank Accession# KM975938). Sequencing analysis revealed complete absence of exon 2 (Figure 3.1, Bottom). The putative 1239 bp ORF of *Fads3AT9* identified using ORF finder <http://www.ncbi.nlm.nih.gov/gorf/gorf.html>, encodes a protein of 412 aa, resulting from an in-frame loss of 37 aa compared to classical *FADS3*. *FADS3AT9* retains all the conserved regions characteristic of front end desaturase (cytochrome b5 domain and three histidine repeats) (Supplement Figure 3.1). Rat FADS2 has two isoforms with sizes of 52 kDa and 46 kDa, respectively (Shoji Y 2003 PMID: 12834305). Similarly, besides the 51 kDa of FADS3CS, a recombinant protein at 48 kDa was detected in Cos-7 cells transfected with rat Fads3 (Pedrono 2010, PMID: 19752397). Here, based on the cDNA sequence of mouse *Fads3AT9*, we predicted its protein size of 47 kDa using the protein molecular weight calculator

<http://www.sciencegateway.org/tools/proteinmw.htm>.

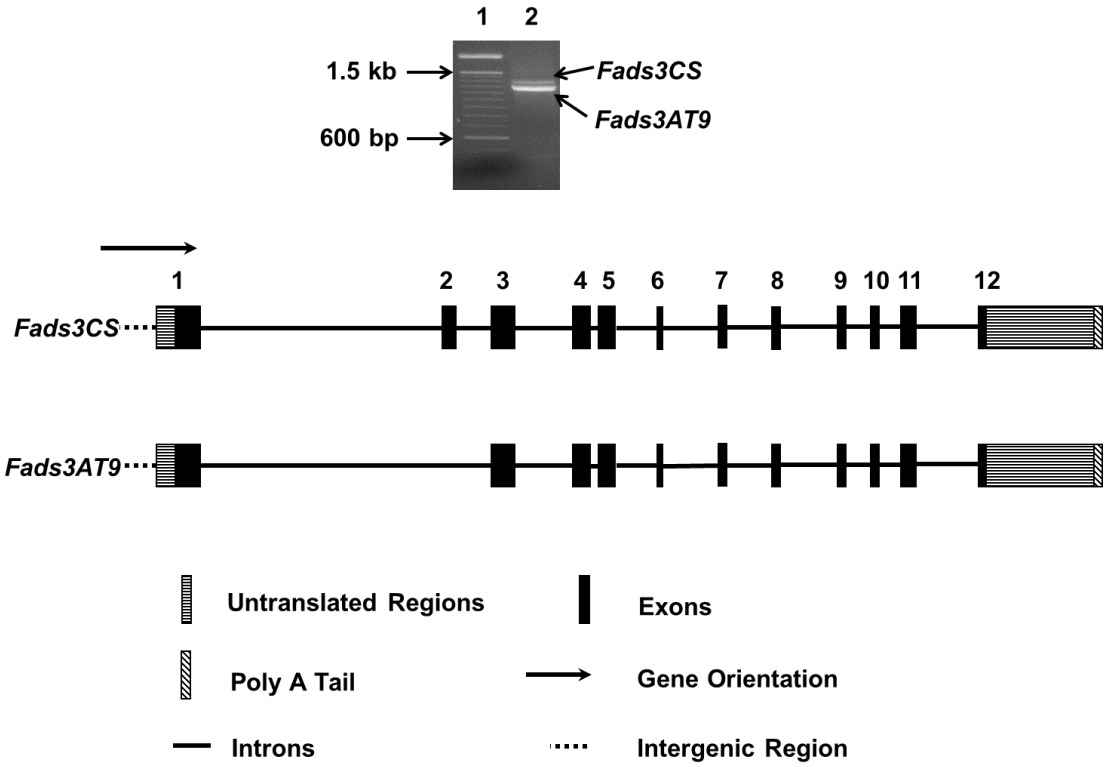

Figure 3.1 Amplicons of *Fads3CS* and *Fads3AT9* and cartoon image of *Fads3CS* and *Fads3AT9* gene structure Top: *Fads3CS* - Classical splicing, *Fads3AT9* - alternative transcript. The products were separated on 2% agarose gel and visualized under UV using ethidium bromide. Lane 1: 100 base pair molecular weight marker and Lane 2: PCR products amplified by RT-PCR. Bottom: *Fads3CS* - Classical splicing, *Fads3AT9* with missing exon 2 is shown. Numbers 1 to 12 are exons.

We studied the expression of *Fads3AT9* along with *Fads3CS* using 11 mouse tissues and adipose cells. *AT9* along with *CS* is ubiquitously expressed in all 11 tissues tested; *AT9* expression is higher than *CS* in thymus, spleen, pancreas and brown adipose tissue (Figure3.2 top, supplementary Figure.2). *CS* expression is low in pancreas while *AT9* is at much greater abundance (Figure3.2 top, supplementary Figure.2). These observations suggest that *AT9* has an important role in pancreatic LCPUFA synthesis. Both *CS* and *AT9* are expressed in the undifferentiated and differentiated white and brown cells (Figure3.2 bottom). AT9 expression is lower than CS in brown cells, but no obvious trends were observed in between undifferentiated and differentiated cells (Figure3.2 bottom, supplementary Figure.3).

We used Cell-PLoc 2.0 <http://www.csbio.sjtu.edu.cn/bioinf/euk-multi-2/> [15] to predict subcellular localization of *Fads3* and *Fads3AT9*. The protein sequences of *Fads3* and *Fads3AT9* in Fasta format were uploaded and the software predicted putative subcellular localization. Both *Fads3* and *Fads3AT9* are predicted to be localized to endoplasmic reticulum (ER) with high confidence. *Fads* proteins are known to localize to the ER though recently organelle-specific staining and Western blotting show that primate *FADS1* and *FADS2* localize to both ER and mitochondria [2, 5].

We also checked the relevant substrate-product metabolites via fatty acid profiles of the 11 tissues (Table 3.1A). Table 3.1A is arranged based on highest to lowest n-6 PUFA levels. Total n-6 PUFA levels were highest in the pancreas, followed by liver and spleen and total n-3 PUFA was highest in

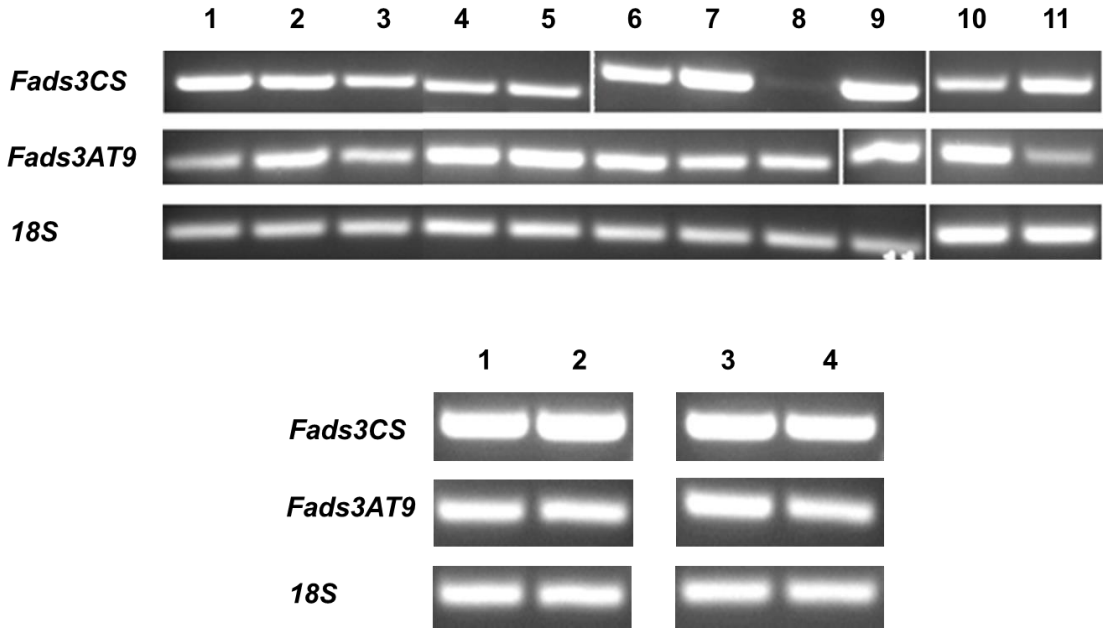

Figure 3.2 *Fads3CS* and *Fads3AT9* mRNA expression in mouse tissues and fat cells Top: *Fads3CS* and *Fads3AT9* mRNA expression in adult C57BL/6 mouse tissues (lane 1 hippocampus, lane 2 cerebellum, lane 3 liver, lane 4 thymus, lane 5 spleen, lane 6 testis, lane 7 cortex, lane 8 pancreas, lane 9 lung, lane 10 brown adipose tissue, lane 11 white adipose tissue). 18S primer is used as control. Bottom: *Fads3CS* and *Fads3AT9* mRNA expression in undifferentiated and differentiated white and brown cells (lane 1 undifferentiated white cells, lane 2 differentiated white cells, lane 3 undifferentiated brown cells, and lane 4 differentiated brown cells). 18S primer is used as control.

Table 3.1A: Fatty acid profiles of mouse tissues

Name of FA	Pancreas	Liver	Spleen	Testes	BAT	WAT	Thymus	Lung	Hippocampus	Cortex	Cerebellum
12:0	0.00±0.00	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.02	0.21±0.03	0.18±0.02	0.00 ± 0.00	0.00±0.00	0.00 ± 0.00	0.00±0.00	0.00 ± 0.00
14:0	0.32±0.10^{a}	0.34 ± 0.08^{a}	0.52 ± 0.04^{a}	0.57 ± 0.06^{ab}	1.87±0.46^{d}	1.11±0.34^{bc}	1.47 ± 0.16^{cd}	1.16±0.15^{bc}	0.14 ± 0.05^{a}	0.20±0.06^{a}	0.13 ± 0.03^{a}
15:0	0.09±0.03^{ab}	0.13 ± 0.01^{abc}	0.20 ± 0.05^{c}	0.06 ± 0.02^{ab}	0.07±0.01^{ab}	0.14±0.03^{bc}	0.10 ± 0.03^{ab}	0.21±0.06^{c}	0.05 ± 0.01^{ab}	0.06±0.03^{ab}	0.04 ± 0.01^{a}
16:0	23.90±0.83^{bc}	24.52±0.79^{c}	24.69±0.63^{c}	30.83±0.69^{d}	19.47±1.84^{a}	19.20±1.37^{a}	20.24±1.04^{a}	37.01±1.20^{e}	20.67±1.21^{ab}	25.33±2.28^{c}	22.43±0.27^{abc}
18:0	13.41±0.72^{e}	10.81±1.77^{cde}	17.31±0.29^{f}	8.45 ± 1.53^{bc}	5.82±0.83^{ab}	2.89±0.75^{a}	7.89 ± 1.02^{bcd}	11.91±0.78^{d}	22.00±0.59^{g}	21.19±0.64^{g}	21.65±0.40^{g}
20:0	0.02±0.00^{a}	0.07 ± 0.04^{ab}	0.35 ± 0.05^{cd}	0.33 ± 0.09^{cd}	0.44±0.08^{d}	0.22±0.03^{bc}	0.00 ± 0.00^{a}	0.23±0.07^{c}	0.27 ± 0.07^{c}	0.32±0.03^{cd}	0.59 ± 0.05^{e}
22:0	0.11±0.01^{bc}	0.14 ± 0.03^{c}	0.24 ± 0.06^{d}	0.03 ± 0.01^{ab}	0.06±0.00^{ab}	0.03±0.00^{ab}	0.07 ± 0.01^{bc}	0.28±0.04^{d}	0.08 ± 0.01^{abc}	0.00±0.00^{a}	0.02 ± 0.01^{ab}
23:0	0.00±0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00±0.00	0.00±0.00	0.00 ± 0.00	0.00±0.00	0.14 ± 0.02	0.09±0.04	0.10 ± 0.01
24:0	0.00±0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00±0.00	0.00±0.00	0.00 ± 0.00	0.00±0.00	0.10 ± 0.04	0.05±0.00	0.06 ± 0.01
Total SFA	**37.83±1.28^{c}**	**36.01±1.16^{c}**	**43.33±0.58^{de}**	**40.30±2.24^{cd}**	**27.95±2.89^{ab}**	**23.76±2.21^{a}**	**29.76±0.27^{b}**	**50.81±0.65^{f}**	**43.45±1.62^{de}**	**47.21±2.76^{ef}**	**45.03±0.59^{de}**
14:1	0.00±0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.00	0.11±0.03	0.12±0.03	0.12 ± 0.04	0.04±0.00	0.00 ± 0.00	0.00±0.00	0.00 ± 0.00
16:1n-9	0.08±0.01^{a}	0.38 ± 0.05^{cde}	0.29 ± 0.03^{abcd}	0.53 ± 0.04^{de}	0.54±0.13^{de}	0.51±0.03^{de}	0.34 ± 0.04^{bcde}	2.55±0.23^{f}	0.43 ± 0.04^{de}	0.17±0.02^{abc}	0.12 ± 0.01^{ab}
16:1n-7	0.89±0.24^{ab}	1.83 ± 0.51^{ab}	0.96 ± 0.20^{ab}	1.04 ± 0.43^{ab}	3.41±0.40^{cd}	5.73±0.64^{e}	4.62 ± 0.72^{d}	2.45±0.26^{bc}	0.55 ± 0.04^{a}	0.62±0.06^{a}	0.55 ± 0.06^{a}
18:1n-9+n-7	12.62±0.97^{ab}	15.67±1.05^{abc}	9.90 ± 0.45^{a}	15.45±1.42^{abc}	33.34±2.95^{d}	35.90±1.53^{d}	31.41±1.18^{cd}	11.70±0.78^{a}	18.69±1.46^{abc}	18.86±0.33^{abc}	26.11±1.65^{bcd}
20:1n-9	0.17±0.01^{a}	0.23 ± 0.04^{a}	0.27 ± 0.05^{ab}	0.34 ± 0.03^{abd}	0.74±0.11^{bc}	0.61±0.26^{ac}	0.78 ± 0.18^{bc}	0.40±0.06^{abd}	1.07 ± 0.24^{c}	0.84±0.08^{cd}	2.70 ± 0.44^{e}
20:1n-7	0.04±0.02^{a}	0.08 ± 0.04^{abd}	0.12 ± 0.03^{abd}	0.08 ± 0.04^{abd}	0.12±0.02^{abd}	0.08±0.03^{ab}	0.19 ± 0.02^{be}	0.05±0.03^{a}	0.25 ± 0.04^{e}	0.19±0.05^{de}	0.51 ± 0.10^{f}
22:1n-9	0.06±0.00^{ac}	0.07 ± 0.00^{ac}	0.08 ± 0.01^{ac}	0.00 ± 0.00^{c}	0.03±0.01^{ce}	0.02±0.00^{ce}	0.06 ± 0.02^{ac}	0.18±0.04^{b}	0.10 ± 0.04^{ae}	0.06±0.01^{ac}	0.13 ± 0.03^{ab}
24:1	0.07±0.03^{ac}	0.07 ± 0.02^{ac}	0.00 ± 0.00^{c}	0.04 ± 0.02^{ac}	0.03±0.01^{c}	0.07±0.02^{ac}	0.03 ± 0.00^{c}	0.19±0.04^{b}	0.11 ± 0.03^{ab}	0.17±0.06^{b}	0.92 ± 0.02^{d}
Total MUFA	**13.91±1.18^{a}**	**18.34±1.46^{a}**	**11.61±0.43^{a}**	**17.48±1.75^{a}**	**38.20±2.85^{d}**	**43.04±1.67^{b}**	**37.55±0.38^{d}**	**17.54±1.01^{a}**	**21.21±1.75^{a}**	**20.91±0.40^{a}**	**31.03±2.25^{d}**
18:2n-6	19.38±2.46^{ab}	21.47±2.43^{a}	9.50 ± 0.26^{c}	5.29 ±1.16^{df}	29.44±2.06^{e}	29.84±3.44^{e}	19.25±1.77^{b}	8.33±0.84^{cd}	0.83 ± 0.08^{g}	0.84±0.02^{g}	1.21 ± 0.16^{fg}
18:3n-6	0.16±0.05^{b}	0.47 ± 0.14^{c}	0.06 ± 0.01^{a}	0.10 ± 0.01^{ab}	0.06±0.01^{a}	0.09±0.02^{ab}	0.08 ± 0.02^{ab}	0.27±0.05^{d}	0.00 ± 0.00^{a}	0.00±0.00^{a}	0.00 ± 0.00^{a}
20:2n-6	0.42±0.09^{a}	0.30 ± 0.08^{a}	1.32 ± 0.24^{b}	0.10 ± 0.01^{a}	0.24±0.03^{a}	0.18±0.03^{a}	0.70 ± 0.01^{c}	0.50±0.05^{a}	0.10 ± 0.03^{a}	0.10±0.01^{a}	0.29 ± 0.02^{a}
20:3n-6	0.78±0.17^{be}	0.63 ± 0.19^{ab}	1.00 ± 0.10^{e}	1.30 ± 0.01^{c}	0.21±0.03^{d}	0.18±0.04^{d}	0.53 ± 0.00^{be}	1.03±0.09^{e}	0.32 ± 0.06^{ad}	0.29±0.02^{d}	0.40 ± 0.02^{ad}
20:4n-6	22.82±0.67^{a}	13.48±2.53^{b}	20.97±0.85^{a}	12.76±0.82^{b}	1.56±0.27^{c}	0.43±0.09^{c}	6.27 ± 1.11^{de}	11.60±0.79^{b}	12.70±0.92^{bf}	9.66±0.52^{def}	7.70 ± 0.83^{e}
22:4n-6	0.53±0.08^{ac}	0.43 ± 0.10^{ac}	3.57 ± 0.68^{b}	1.58 ± 0.03^{c}	0.31±0.05^{a}	0.33±0.08^{a}	1.47 ± 0.26^{c}	3.49±0.46^{b}	3.37 ± 0.26^{b}	2.13±0.41^{c}	1.52 ± 0.10^{c}
22:5n-6	0.42±0.10^{ad}	0.25 ± 0.08^{ad}	0.47 ± 0.09^{a}	13.68±0.19^{b}	0.06±0.02^{d}	0.07±0.03^{d}	0.42 ± 0.06^{a}	0.48±0.16^{a}	0.41 ± 0.08^{ad}	0.29±0.08^{ad}	0.07 ± 0.02^{d}
Total n-6 PUFA	**44.51±2.03^{a}**	**37.02±0.33^{b}**	**36.88±1.07^{b}**	**34.81±0.51^{bc}**	**31.87±2.02^{c}**	**31.10±3.57^{c}**	**28.70±0.36^{c}**	**25.70±0.97^{d}**	**17.73±0.94^{e}**	**13.32±0.92^{f}**	**11.18±0.87^{f}**
18:3n-3	0.25±0.02^{a}	0.91 ± 0.25^{be}	0.20 ± 0.05^{ad}	0.14 ± 0.09^{ad}	1.04±0.29^{b}	1.66±0.22^{c}	0.81 ± 0.02^{e}	0.16±0.04^{ad}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}
20:5n-3	0.49±0.07^{a}	0.28 ± 0.06^{b}	0.13 ± 0.02^{c}	0.07 ± 0.00^{de}	0.03±0.01^{e}	0.04±0.02^{e}	0.04 ± 0.01^{de}	0.11±0.02^{cd}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.04 ± 0.01^{e}
22:5n-3	0.43±0.06^{a}	0.42 ± 0.09^{a}	1.41 ± 0.24^{b}	0.26 ± 0.01^{a}	0.09±0.03^{a}	0.08±0.04^{a}	0.58 ± 0.05^{b}	1.12±0.25^{b}	0.11 ± 0.04^{a}	0.10 ± 0.02^{a}	0.19 ± 0.08^{a}
22:6n-3	2.53±0.10^{a}	6.98 ± 1.04^{b}	6.29 ± 0.03^{b}	6.41 ± 0.10^{b}	0.69±0.07^{c}	0.22±0.03^{c}	2.36 ± 0.47^{ad}	4.49±0.66^{d}	17.38±0.39^{e}	18.37±2.25^{e}	12.41±1.07^{f}
Total n-3 PUFA	**3.71±0.07^{a}**	**8.58 ± 0.94^{b}**	**8.03 ± 0.28^{b}**	**6.87 ± 0.01^{bc}**	**1.85±0.37^{e}**	**2.00±0.29^{e}**	**3.80 ± 0.52^{ac}**	**5.88±0.48^{c}**	**17.49±0.36^{f}**	**18.46±2.26^{f}**	**12.64±1.12^{g}**
20:2n-9	0.02±0.00	0.04 ± 0.02	0.07 ± 0.00	0.05 ± 0.01	0.02±0.01	0.04±0.01	0.01 ± 0.00	0.00±0.00	0.09 ± 0.04	0.08 ± 0.01	0.10 ± 0.02
20:3n-9	0.07±0.02	0.06 ± 0.03	0.00 ± 0.00	0.15 ± 0.04	0.04±0.02	0.04±0.01	0.07 ± 0.00	0.09±0.02	0.09 ± 0.02	0.04 ± 0.01	0.07 ± 0.01
Total n-9 PUFA	**0.09±0.03^{b}**	**0.10 ± 0.03^{ab}**	**0.07 ± 0.00^{a}**	**0.19 ± 0.05^{c}**	**0.07±0.03^{ab}**	**0.06±0.01^{ab}**	**0.08 ± 0.00^{ab}**	**0.09±0.02^{bc}**	**0.18 ± 0.05^{bc}**	**0.12 ± 0.03^{ab}**	**0.18 ± 0.02^{b}**

Different alphabet represents statistical significance.

Table 3.1B: Fatty acid profiles of adipocytes

Name of FA	WAT day 0	WAT Day 8	BAT day 0	BAT day 8
14:0	0.87±0.08^{a}	2.92±0.55^{b}	2.00±0.54^{ab}	3.55±1.37^{b}
15:0	1.13±0.09^{a}	6.25±0.42^{b}	0.99±0.25^{a}	1.27±0.13^{a}
16:0	18.26±0.27^{a}	27.99±1.75^{b}	20.66±1.14^{a}	27.08±2.30^{b}
18:0	19.75±0.73^{a}	5.88±0.66^{b}	14.75±0.82^{c}	11.75±1.38^{d}
20:0	0.39±0.07^{a}	0.16±0.06^{a}	1.20±0.61^{b}	0.14±0.04^{a}
22:0	2.38±0.54^{a}	0.12±0.04^{b}	0.70±0.25^{b}	0.14±0.12^{b}
24:0	0.00±0.00^{a}	0.21±0.02^{b}	0.00±0.00^{a}	0.27±0.12^{b}
Total SFA	**42.78±1.09^{ab}**	**43.52±2.07^{ab}**	**40.30±1.45^{a}**	**44.21±0.81^{b}**
14:1	0.00±0.00^{a}	0.74±0.08^{b}	0.00±0.00^{a}	0.64±0.21^{b}
16:1n-9	1.34±0.04^{a}	31.58±1.30^{b}	2.84±0.38^{a}	25.73±1.39^{c}
16:1n-7	1.48±0.03^{a}	0.00±0.00^{b}	2.83±0.32^{c}	0.00±0.00^{b}
18:1n-9	13.3±1.61^{a}	11.82±0.36^{a}	17.66±3.25^{b}	13.10±0.72^{ab}
18:1n-7	3.03±0.08^{a}	2.99±0.32^{a}	6.66±0.95^{b}	4.34±0.97^{a}
20:1n-9	0.79±0.14^{a}	0.48±0.20^{a}	0.55±0.06^{a}	0.12±0.02^{b}
20:1n-7	0.00±0.00^{a}	0.10±0.05^{a}	0.00±0.00^{a}	0.25±0.10^{b}
22:1n-9	0.69±0.25^{a}	0.11±0.02^{b}	0.26±0.24^{ab}	0.02±0.02^{b}
24:1	1.40±0.12^{ab}	0.58±0.22^{bc}	1.94±0.75^{a}	0.36±0.14^{c}
Total MUFA	**22.03±1.60^{a}**	**48.41±0.58^{b}**	**32.74±3.6^{c}**	**44.57±0.18^{b}**
18:2n-6	2.31±0.42^{a}	0.70±0.11^{b}	1.13±0.13^{b}	0.59±0.16^{b}
18:3n-6	0.00±0.00^{a}	0.15±0.00^{b}	0.00±0.00^{a}	0.09±0.02^{c}
20:3n-6	1.35±0.13^{a}	0.27±0.03^{b}	0.65±0.16^{c}	0.39±0.10^{bc}
20:4n-6	14.61±2.11^{a}	3.28±0.51^{b}	11.42±2.09^{a}	5.78±0.62^{b}
22:4n-6	1.31±0.11^{a}	0.55±0.10^{b}	3.02±0.21^{c}	0.63±0.09^{b}
Total n-6 PUFA	**19.02±2.07^{a}**	**4.94±0.46^{b}**	**16.21±2.16^{a}**	**7.48±0.30^{b}**
20:5n-3	5.00±0.46^{a}	0.82±0.19^{b}	1.40±0.37^{b}	0.69±0.04^{b}
22:3	1.19±0.25^{a}	0.10±0.03^{b}	0.91±0.25^{a}	0.13±0.03^{b}
22:5n-3	2.27±0.31^{ac}	0.55±0.07^{b}	3.25±1.25^{a}	0.91±0.17^{bc}
22:6n-3	3.88±0.52^{a}	1.01±0.12^{b}	3.92±1.47^{a}	1.67±0.25^{b}
Total n-3 PUFA	**12.35±0.98^{a}**	**2.48±0.26^{b}**	**9.48±2.81^{a}**	**3.40±0.17^{b}**

cortex and hippocampus, followed by cerebellum. Among these tissues, docosahexaenoic acid (22:6n-3) was highest in the cortex and hippocampus. We found that the twenty carbon eicosanoid precursor arachidonic acid (20:4n-6), the immediate product of the *FADS1* Δ5-desaturase, was highest in pancreas and spleen (Table 3.1A). Earlier, it has been shown that 20:4n-6 is significantly higher in the pancreatic phospholipid fraction compared to liver and adipose [16]. AT9 expression was high but *Fads3CS* low in pancreas, suggesting that AT9 may be playing a key role in LCPUFA biosynthesis in this tissue via direct catalysis by an AT9 protein, or some modification of the Fads1CS catalytic function. Alternative splicing regulates enzymatic activities of the proteins, their interactions with ligands and substrate specificity [17]. We previously reported that a *FADS1* splice variant (*FADS1AT1*) potentiates *FADS2* mediated production of 18:3n-6 [5]. Different from tissue, brown fat cells has lower expression of *Fads3AT9* than *Fads3CS*. The fatty acid profiles of tissues and cells are incomparable due to the differences of PUFA precursors in chow and in culture medium. The fatty acid profiles of fat cells are showed in Table 3.1B and no big difference is observed between day 0 white and brown fat cells or day 8 fat cells. Widespread expression patterns and fatty acid profiles in mouse tissues suggest that *Fads3AT9* may play an important role in the regulation and/or biosynthesis of LCPUFA from precursors.

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Authorship Contribution

Ji Yao Zhang, Xia Qin, Hui Gyu Park, Ellen Kim, Guowen Liu, Kumar S. D. Kothapalli, J. Thomas Brenna. Ji Yao Zhang conducted experiments, collected data, analyzed and interpreted data, and drafted the article. Xia Qin conducted experiments, collected data, analyzed and interpreted data and helped revise the article. Hui Gyu Park provided technical support and critical revision. Ellen Kim helped data collection. Guowen Liu provided critical revision of the article. Kumar SD Kothapalli provided design of the work and critical revision of the article. J. Thomas Brenna provided conception of the work, critical revision of the article and final approval of the version to be published.

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CHAPTER 4

HIGH OLEIC SUNFLOWER OIL DIET ENHANCES N-3 AND REDUCES N-6 IN BRAIN AND HEART

4.1 INTRODUCTION

Epidemiological, clinical and pre-clinical studies show that environmental factors, particularly diets contribute to human health outcome profoundly. It is known that unsaturated fatty acid intake is negatively related to incidence of chronic diseases. However, due to the high availability of n-6 polyunsaturated fatty acid (PUFA) in western diet, the unbalanced ratio of n-6 to n-3 PUFA may be a new detrimental factor for cardiovascular diseases (CVD) development. How to shift the undesired ratio of PUFA to an optimal ratio arouses public attention.

In recent years, the Mediterranean diet has grasped a great amount of public attention due to studies reported that people following the Mediterranean dietary pattern have an inverse association with coronary heart disease [3], body weights [1] , diabetes [2], aging [4] and cancer [5]. A typical Mediterranean diet contains whole grains, legumes, vegetables, fruits, nuts, moderate red wine, limited red meat, sufficient fish and poultry and abundant amount of olive oil. Different from other healthy diets, using olive oil as the predominant fat source is one of the biggest traits of Mediterranean diet.

With the increasing interests of the Mediterranean diet, the contributions of olive oil intake are reported in animal models and clinical

studies. Randomized clinical trials show that virgin olive oil (VOO) possesses cardio protective functions via improving lipoprotein profiles [6, 7] and endothelial function [8] and reducing inflammation [8]. Patients treated with extra VOO reduce glycemic response to a high-glycemic index meal [9]. A recent cell culture study shows that olive oil derived phenolic compound, tyrosol, can inhibit endoplasmic reticulum stress-induced ß-cell apoptosis [10]. High adherence to Mediterranean diet with VOO is inversely associated with cognitive decline [11] via reducing aging-related DNA damage [12] and mitochondrial oxidation [13]. High olive oil intake contributes to a 38%, and 30% reduction in risk of breast cancer and digestive system cancers, respectively [14, 15]. Preclinical models suggest that VOO can prevent against cellular oxidative stress [16] and regulate progression of carcinogenesis [17].

Though the little amount of bioactive phenols derived from olive oil contributes to the antioxidant and anti-inflammatory property of this edible oil, we should appreciate that olive oil is rich in monounsaturated fatty acid (MUFA), particularly containing abundant of oleic acid (18:1n-9, around 75% of total fatty acid by weight). Oleic acid is reported to ameliorate lipid/lipoprotein profiles by reduction of serum total cholesterol, LCL-C, LDL particle size and ratio of triglyceride/HDL-C to change CVD risk status [18, 19]. Oleic acid also has impacts on structure of cell membrane, regulation of gene expressions related to cell apoptosis and immune system [17, 20].

Positive effects of olive oil- or avocado-derived oleic acid on health outcome promote the breeding and production of high oleic crops and/or their

oils on current market. The high oleic sunflower oil contains around 85% of oleic acid and little amount of long chain PUFA precursors which are normally provided sufficiently from soybean oil in rodent laboratory feed. Previously we determined the effects of *Fads3* ablation on tissue fatty acid profiles and significant differences between genotypes were observed only at perinatal age. It is possible that under severe condition, with limited amount of linoleic acid and alpha-linolenic acid for making long chain PUFA, the function of *Fads3* in long chain PUFA biosynthesis will be detected. The objective of this project is to examine the impact of high oleic sunflower oil on fatty acid metabolisms in the absence of *Fads3* from early to late stage of life.

4.2 MATERIALS AND METHODS

4.2.1 Animals

Whole body *Fads3* knockout mice (KO) were generated at the College of Veterinary Medicine transgenic mouse facility at Cornell University. The male chimera was bred with C57BL/6 female and the offspring was genotyped (details are elaborated in Chapter 2). They were maintained at 20 °C, a 12-to-12 hour light-dark cycle. All cares and procedures related to mice used in this study were approved by the Cornell Institutional Animal Care and Use Committee and by the American Association for Laboratory Animal Care.

Mice at age of two months (n=6/genotype) were started to feed with customized high oleic (HO) sunflower oil diet *ad libitum*. HO-treated mice started breeding after 1-month environmental adaptation. Food intake and

body weight were measured every two days. Litters from both wild type and KO mice were euthanized on postnatal day 1, 7, 13, 21, 30, 60 and 365 using CO_2 gas at the flow of 1.4 L/min/cage. Tissues (e.g. brain, liver, heart brown adipocytes and white adipocytes (epididymal/parameterial and perirenal fat) were collected and weighted during dissections. A piece of tissue (1 mm thick, 5 × 5 mm^2) was taken for histological diagnosis. Samples were fixed in 70 % ethonal and then were embedded, sliced and stained with haematoxylin/eosin at the Section of Anatomic Pathology, Cornell University. The rest tissues were stored at -80 °C for future fatty acid and gene expression analysis. Age-matched mice fed with normal laboratory rodent chow (7012 Teklad LM-485, Harlan Laboratories, WI) *ad libitum* were regarded as control group.

4.2.2 Diets

In the customized oil, we only substituted soybean oils with formulated high oleic sunflower oils and kept other nutrients unchanged in AIN-93G. The percentage of fat in diet is around 17% of energy which is consistent with the amount of fat in the laboratory rodent chow. High oleic sunflower oils were generously provided by Oilseeds International Ltd, San Francisco, CA. Due to the low amount of n-6 and n-3 PUFA in HO sunflower oil, we blended this HO oil with flaxseed oil (Barlean's organic oils, Ferndale, WA) to avoid essential fatty acid deficiency. The formulated oil was sent to Harlan Laboratories (Indianapolis, IN) to produce desired pellet customized HO diet. Table 4.1A presented the fatty acid profiles of original oils and formulated oil and Table

4.1B showed the brief nutrition information of two diets.

Table 4.1A Fatty acid profiles of experimental oils

Fatty Acid Composition (%, w/w of total FA)	High oleic sunflower oil	Flaxseed oil	Designed HO sunflower oil
Total SFA	9.57	10.02	9.84
Total MUFA	86.02	20.93	80.28
Total n-6 PUFA	4.17	14.22	5.16
Total n-3 PUFA	0.12	54.74	4.57

Table 4.1B Brief nutrition information of diets

% of energy	Rodent Feed (Control)	High Oleic Sunflower Oil Diet
Protein	25	19
Carbohydrate	58	64
Fat	17	17
Saturated Fat	3.3	2.2
Monounsaturated Fat	4.0	12.9
n-6 Polyunsaturated Fat	8.4	1.1
n-3 Polyunsaturated Fat	1.3	0.8
Kcal/g	3.1	3.8

4.2.3 Fatty acid analysis

Fatty acid methyl esters (FAME) from different tissues were extracted using modified one-step method [21]. FAME were qualified by chemical ionization tandem mass spectrometry (CI-MS, Varian Saturan 2000, CA) and was quantified by gas chromatography coupled to a flame ionization detection (GC-FID, Agilent Technologies Hewlett Packard 5890 Series II, CA) using an equal weight FAME standard mixture to verify response factors in each run with sample FAME. Triplicate injections were performed for each FAME and quantity of each fatty acid specie was calculated as percentage by weight in total fatty acids (%, w/w).

4.2.4 Quantification of gene expression

mRNA extraction and cDNA synthesis

Total mRNA was purified from mice hearts (postnatal day 1,7,13, 21, and 30) using the E.Z.N.A. total RNA kit (Omega Bio-Tek, GA). The quality and quantity of RNA were analyzed using NanoDrop (Thermo Scientific, DE). cDNA was reversely transcribed from 1 ug of total RNA using the High Capacity cDNA Reverse Transcription Kit (Life Technologies, NY) according to the manufacturer's instructions. The synthesized cDNA was stored at -20 °C for future gene expression level measurements.

Real-time PCR

Expression levels of *Fads1* and *Fads2* were detected via Real-time PCR using SYBR Green Master Mix on a LightCycler 480 instrument (Roche, Madison, WI). *Fads1* Forward: 5'-TACCTGCTTCACATCCTGCT-3'; *Fads1* Reverse: 5'-GTCGAGGTGCCAAAGACTGA-3'; Fads2 Forward: 5'-AGGCCCAAGCTGGATGGCTGC-3'; Fads2 Reverse: 5'-AGTTGGCTGAGGCACCCTTTA-3'. The real-time PCR protocol was as follows: initial denaturation: 95°C for 10 min; amplification: 45 cycles of denaturation at 95°C for 10 s, amplification at 65°C for 20 s, extension at 72°C for 10 s; final extension: 72°C for 5 min. Melting curve and calculated primer efficiency from standard curves were used to verify the specificity of each primer pair. Based on the standard curve we selected 1:10 dilution of cDNA for testing *Fads1* and *Fads2* gene expression and each reaction was run in

triplicate. The Cq values were normalized according to the geometric means of reference genes *Gapdh* and *β-actin*. Gene expression level was calculated using $2^{-\Delta\Delta Cq}$ method [22]. Gene expression of HO-fed mice was normalized to chow-fed mice (considering the *Fads1* and *Fads2* expression in chow diet as "1") at each time point.

4.2.5 Statistical analysis

Data was presented as Mean ± Standard Deviation (SD). Independent Student's *t*-Test (SPSS software, version 16.0) was used to determine statistical differences of fatty acid profiles and gene expressions between two diets. $P<0.05$ was set as a statistical significance threshold.

4.3 RESULTS

4.3.1 Food intake and body weight

No difference in food intake was observed between diets in both genotypes (Supplementary Figure 4.1). Ablation of *Fads3* did not affect body weight either. Interestingly, during the early development (postnatal day 1 to day 30), body weights of HO-fed mice were similar to control. However, adult HO-fed mice over 3 month-old were around 10 grams heavier than age-matched Chow-fed mice (Figure 4.1).

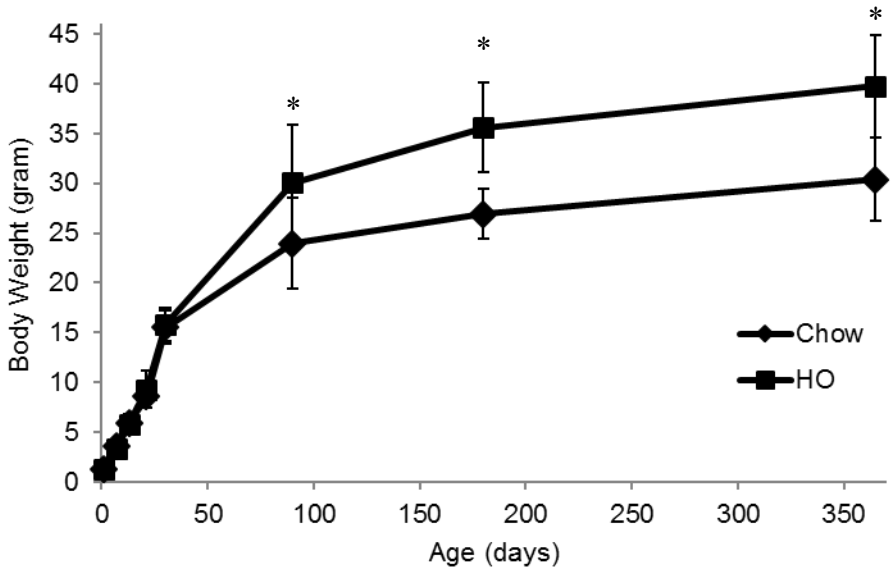

Figure 4.1 Body weights of mice fed with different diets. Mice weights were measured on postnatal day 1, 7, 13, 21, 30, 90, 180, 365 (n=12). Student's *t*-Test is used to analyze differences between groups and * $P<0.05$. During the first month after birth, HO diet did not show any impact on body weight compared to age-matched Chow-fed mice. HO-fed mice started to be heavier than Chow-fed mice at the age of 3 months.

4.3.2 Tissue weights

The weights of liver, white adipose tissue (WAT-epididymal/parameterial and WAT-perirenal), brown adipose tissue (BAT), thymus, brain, heart, kidney, pancreas, and lung were normalized to the body weight in order to minimize the biological difference in body size. Among all the ages we tested (from postnatal day 1 to 1 year), *Fads3* KO tissues were not significantly different from WT when they were fed by the same diet. However, HO diet contributed prominently to the weight gain of tested tissues. In one-year old WT mice, liver, adipose tissues, and thymus were significantly increased in HO-fed mice compared to Chow-fed mice. Particularly, both white fat depots (epididymis/ parametrium and perirenal) and brown fat depot (inter-scapular) from HO-fed mice are twice as heavy as those from Chow-fed mice (Figure4.2). Different from liver and fat pads, the masses of brain, heart, kidney, pancreas and lung were significantly lower in HO-fed mice (Figure 4.2). Similar findings are also observed in 1 year-old KO mice (supplementary Figure 4.2).

In three month old mice, KO mice tissue weights were not significantly different from WT mice when they were fed by chow diet; on HO diet, only perirenal white adipose depots were heavier in KO mice compared to WT mice (data not shown). Only brain and lung weights were significantly lower in HO-fed mice compared to chow-fed mice at the age of 3 months (Figure 4.3) though the body weight of HO-fed mouse was significantly higher than chow-fed mouse (Figure 4.1). Similar trend was detected in three-month old KO mice that epididymis white depot was increased while brain and lung were

decreased in HO diet with the comparison of chow-diet fed KO mice (supplementary Figure 4.3).

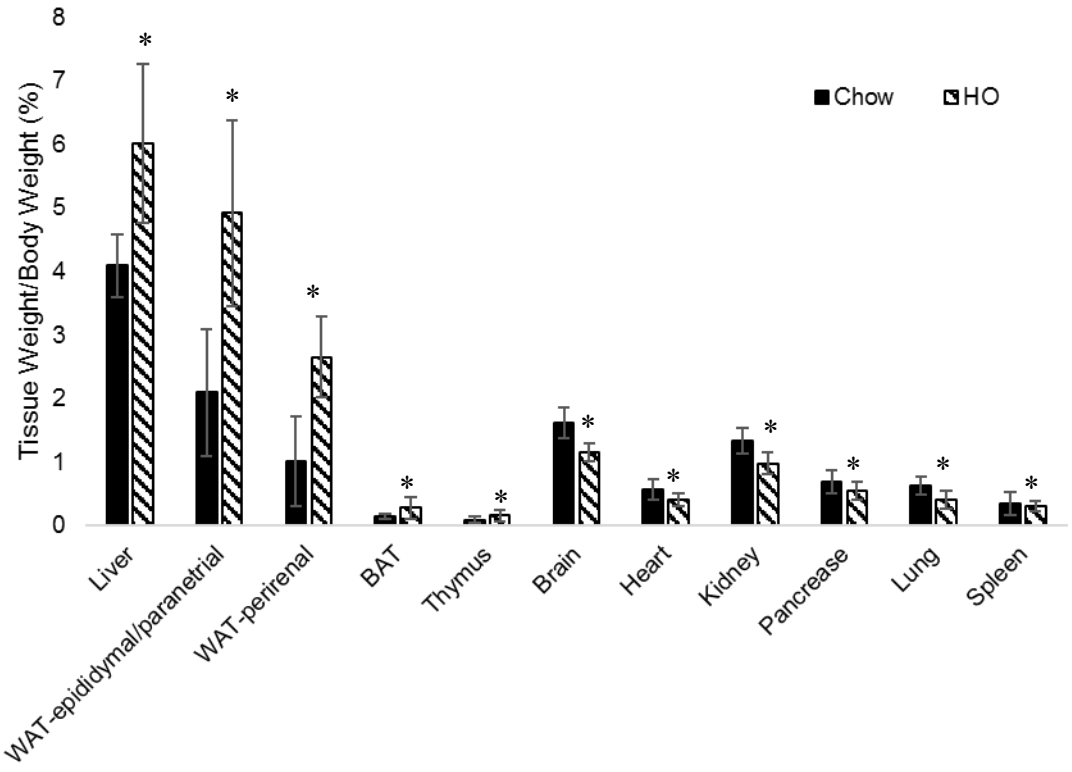

Figure 4.2 Normalized tissue weights from Chow- and HO-fed WT mice at the age of one year (n=6). Tissue mass was normalized to total body weight to minimize the biological difference in body size. Solid bar represented chow-fed WT mice and the patched bar represents HO-fed WT mice. The differences between two diets were analyzed using Student's *t*-Test (* *P*<0.05). The weights of liver, adipose tissues and thymus were increased while brain, heart, kidney, pancreas, lung and spleen were decreased with the treatment of HO diet compared to chow diet.

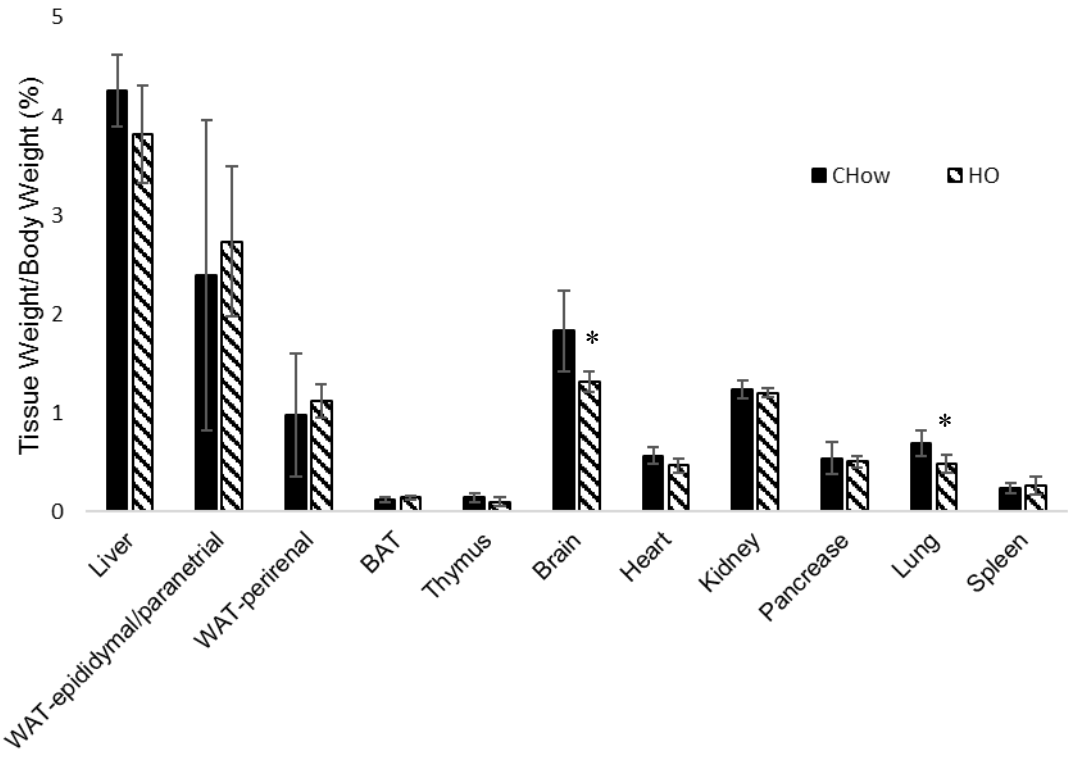

Figure 4.3 Normalized tissue weights from Chow- and HO-fed WT mice at the age of three months (n=6). Tissue mass was normalized to total body weight to minimize the biological difference in body size. Solid bar represented chow-fed WT mice and the patched bar represents HO-fed WT mice. The differences between two diets were analyzed using Student's *t*-Test (* $P<0.05$). Only brain and lung weights were significantly lower in HO-fed mice compared to Chow-fed mice.

4.3.3 Fatty acid profiles of tissues

Mice fed by either Chow or High Oleic sunflower oil more than one month before breeding. Litters from same dam were sacrificed at postnatal day 1, 7, 13, 21, 30, 90, 365 and tissues (liver, heart and brain) were collected for fatty acid analysis.

4.3.3.1 Monounsaturated fatty acids (MUFA)

The ablation of *Fads3* changed the levels of MUFAs in tissues during the early developmental periods and the effects diminished with growth. KO livers contained higher amount of 18:1n-9 at P1 but lower amount at P21 and P30 compared to WT. No significant differences of MUFA profiles were found between adult or aged KO and WT (Table 4.2). At P1 and P13, KO hearts had higher levels of 18:1n-9 and 20:1n-9 but no differences in MUFA at other time points in contrast to WT (Table 4.3). KO brain MUFA profiles were quite similar to that of WT (Table 4.4).

In this study the effects of genotype were not as prominent as dietary intervention. After mice were fed by HO diet, WT mice livers contained significantly higher amounts of 18:1n-9 compared to Chow-fed mice (Figure 4.4A). The levels of 18:1n-9 were quite stable across ages in chow-fed mice but the levels were increasing (P1: 33.74 ± 2.88% *v.s.* P365: 58.11 ± 5.74%) in HO-fed livers (Supplementary Table 4.1). In chow-fed mice heart, the content of 18:1n-9 decreased with aging which did not change much in HO-fed mice (Supplementary Table 4.2). Similar to livers, hearts collected

Table 4.2 Liver fatty acid profiles of mice fed by high oleic sunflower oil diet.

	P1		P7		P13		P21	
	WT	KO	WT	KO	WT	KO	WT	KO
'12:0	0.15±0.05	0.33±0.08*	1.63±0.40	1.99±0.51	2.05±0.44	1.18±0.50*	1.48±0.17	1.42±0.40
'14:0	0.82±0.27	1.03±0.32	4.59±1.21	4.67±1.04	3.76±0.54	2.98±0.86	1.56±0.44	1.37±0.61
'15:0	0.23±0.05	0.12±0.03*	0.08±0.01	0.07±0.01	0.11±0.02	0.13±0.03	0.13±0.02	0.14±0.02
'16:0	22.20±1.73	18.43±2.84*	23.99±1.65	22.55±2.00	23.48±1.67	25.37±1.54	22.86±1.65	22.63±1.64
'18:0	15.88±2.43	9.92±1.85*	5.11±0.75	5.05±0.61	10.77±1.29	12.47±1.65	9.36±0.85	9.68±1.53
'20:0	0.17±0.04	0.13±0.05	0.05±0.04	0.07±0.04	0.12±0.02	0.07±0.04*	0.11±0.03	0.09±0.04
'22:0	0.00+0.00	0.00±0.00	0.03±0.01	0.02±0.01	0.10±0.03	0.11±0.03	0.23±0.03	0.20±0.06
Total SFA	**39.79±3.90**	**30.16±4.49***	**35.91±2.84**	**34.81±3.42**	**40.78±1.83**	**42.57±3.34**	**36.16±1.95**	**36.01±2.53**
'16:1n-9	1.13±0.27	0.94±0.11	0.79±0.08	0.76±0.09	0.37±0.08	0.35±0.05	0.91±0.14	0.92±0.21
'16:1n-7	3.46±0.57	2.84±0.48	3.37±0.69	3.64±0.68	1.28±0.21	1.25±0.26	2.90±0.94	2.52±0.62
'18:1n-9	33.74±2.88	44.74±3.42*	40.50±2.28	41.67±2.76	23.09±1.77	24.20±1.27	35.88±1.91	31.61±1.10*
'18:1n-7	4.31±0.35	2.95±0.76*	2.08±0.45	2.14±0.17	1.87±0.13	2.29±0.27*	3.53±0.63	3.65±0.80
'20:1n-9	0.29±0.05	0.36±0.10	0.58±0.07	0.55±0.03	0.58±0.09	0.68±0.05*	0.84±0.13	0.90±0.18
'20:1n-7	0.08±0.01	0.07±0.01	0.06±0.03	0.12±0.04*	0.44±0.00	0.04±0.01	0.10±0.03	0.09±0.02
'22:1	0.06±0.00	0.03±0.02	0.03±0.03	0.04±.0.00	0.07±0.02	0.10±0.03	0.09±0.02	0.10±0.02
'24:1	0.00±0.00	0.01±0.01	0.26±0.06	0.24±0.03	0.40±0.05	0.72±0.16*	0.46±0.08	0.42±0.12
Total MUFA	**42.99±3.80**	**51.93±2.55***	**47.68±2.94**	**49.16±2.92**	**27.66±2.18**	**29.61±1.35**	**44.70±1.49**	**40.19±2.20***
'18:2n-6	3.42±0.88	3.48±0.85	3.30±0.36	3.41±0.40	5.87±0.52	5.67±0.47	5.25±0.68	6.03±0.91
'18:3n-6	0.21±0.04	0.20±0.04	0.10±0.04	0.08±0.05	0.11±0.04	0.12±0.03	0.12±0.03	0.21±0.07*
'20:2n-6	0.05±0.03	0.06±0.02	0.11±0.03	0.12±0.04	0.34±0.06	0.23±0.06*	0.16±0.03	0.16±0.06
'20:3n-6	0.38±0.06	0.29±0.03*	0.44±0.06	0.44±0.06	0.79±0.06	0.94±0.12*	0.64±0.07	0.87±0.11*
'20:4n-6	5.61±1.85	4.58±1.06	2.81±0.78	2.79±0.66	7.27±0.92	6.64±1.01	4.45±0.63	5.88±0.99*
'22:4n-6	0.25±0.10	0.32±0.11	0.24±0.08	0.24±0.09	0.22±0.05	0.21±0.06	0.22±0.08	0.14±0.07
'22:5n-6	0.13±0.04	0.11±0.04	0.05±0.01	0.06±0.01	0.11±0.04	0.09±0.01	0.10±0.03	0.11±0.04
Total n6 PUFA	**10.04±2.88**	**9.03±1.50**	**7.05±1.13**	**7.14±1.07**	**14.71±1.43**	**13.90±1.27**	**10.92±1.29**	**13.40±1.70***
'18:3n-3	0.20±0.05	0.26±0.08	0.57±0.10	0.57±0.10	0.55±0.08	0.39±0.03*	0.49±0.09	0.51±0.17
'20:5n-3	0.59±0.11	0.82±0.19*	0.91±0.17	0.84±0.09	1.55±0.42	1.55±0.44	1.24±0.34	1.68±0.61
'22:5n-3	0.14±0.04	0.34±0.10*	0.60±0.16	0.55±0.03	0.99±0.16	0.85±0.14	0.36±0.04	0.49±0.11*
'22:6n-3	4.64±1.33	6.08±2.69	5.99±1.44	5.66±1.02	12.49±1.62	9.79±1.86*	4.85±0.96	6.07±1.37
Total n3 PUFA	**5.57±1.51**	**7.50±2.87**	**8.08±1.71**	**7.62±0.95**	**15.57±1.92**	**12.58±2.34***	**6.94±1.32**	**8.76±2.08**
'20:2n-9	0.18±0.07	0.13±0.03	0.19±0.02	0.20±0.02	0.22±0.08	0.16±0.08	0.19±0.06	0.22±0.08
'20:3n-9	1.35±0.33	1.14±0.23	0.89±0.19	0.84±0.11	0.85±0.11	0.96±0.07	0.97±0.28	1.28±0.28
'22:3	0.14±0.05	0.13±0.03	0.09±0.02	0.09±0.01	0.10±0.02	0.12±0.03	0.10±0.03	0.17±0.07*
Total n9 PUFA	**1.67±0.35**	**1.40±0.25**	**1.18±0.22**	**1.13±0.12**	**1.17±0.10**	**1.24±0.06**	**1.26±0.34**	**1.67±0.35**

Values are Mean ± SD. * P<0.05.

Table 4.2 Liver fatty acid profiles of mice fed by high oleic sunflower oil diet (cont.).

	P30		P90		P365	
	WT	KO	WT	KO	WT	KO
'12:0	0.91±0.18	1.16±0.31	0.26±0.19	0.34±0.32	0.04±0.01	0.03±0.01
'14:0	0.51±0.13	0.68±0.14	0.67±0.04	0.55±0.15	0.45±0.04	0.48±0.04
'15:0	0.10±0.03	0.12±0.02	0.10±0.00	0.12±0.03	0.04±0.02	0.04±0.01
'16:0	20.31±1.96	20.24±1.98	2.34±0.37	2.52±0.67	0.06±0.02	0.06±0.01
'18:0	6.39±0.69	7.30±1.17	3.89±0.65	4.00±0.95	3.06±1.68	2.92±2.02
'20:0	0.06±0.03	0.07±0.04	0.33±0.10	0.20±0.06	0.08±0.06	0.07±0.06
'22:0	0.12±0.04	0.14±0.04	0.26±0.10	0.19±0.00	0.02±0.01	0.02±0.00
Total SFA	**28.60±1.72**	**29.96±2.03**	**27.58±0.87**	**26.13±2.27**	**24.53±3.46**	**23.70±0.95**
'16:1n-9	1.39±0.45	1.34±0.46	1.92±0.22	1.91±0.41	2.77±0.53	2.61±1.02
'16:1n-7	2.35±0.44	2.45±0.79	4.17±1.41	4.13±0.84	3.91±0.47	4.13±0.41
'18:1n-9	47.50±2.06	42.53±3.27*	49.91±0.81	49.14±1.26	58.11±5.74	59.65±1.31
'18:1n-7	2.60±0.74	2.40±0.69	2.66±0.30	2.38±0.54	0.00±0.00	0.00±0.00
'20:1n-9	0.63±0.14	0.90±0.26*	0.99±0.17	0.82±0.29	1.15±0.26	1.15±0.46
'20:1n-7	0.06±0.01	0.08±0.05	0.34±0.13	0.22±0.17	0.18±0.16	0.18±0.17
'22:1	0.04±0.02	0.06±0.02	0.07±0.02	0.08±0.01	0.02±0.02	0.02±0.02
'24:1	0.15±0.06	0.18±0.07	0.12±0.01	0.15±0.05	0.07±0.01	0.07±0.03
Total MUFA	**54.27±2.74**	**49.94±4.65**	**60.18±1.57**	**58.82±2.58**	**66.20±4.97**	**67.82±1.48**
'18:2n-6	4.03±0.63	4.85±0.71	4.56±0.46	5.87±1.63	2.84±0.37	2.55±0.59
'18:3n-6	0.16±0.07	0.13±0.03	0.13±0.07	0.34±0.05	0.08±0.04	0.08±0.05
'20:2n-6	0.04±0.01	0.07±0.03*	0.20±0.01	0.06±0.04*	0.02±0.01	0.02±0.01
'20:3n-6	0.56±0.06	0.68±0.10*	0.43±0.11	0.49±0.02	0.39±.15	0.30±0.06
'20:4n-6	3.76±0.36	4.42±1.31	2.85±0.42	3.36±0.63	2.34±0.55	1.97±0.49
'22:4n-6	0.10±0.04	0.07±0.04	0.14±0.02	0.17±0.02	0.23±0.03	0.15±0.04*
'22:5n-6	0.06±0.02	0.06±0.02	0.10±0.04	0.10±0.04	0.03±0.00	0.01±0.00
Total n6 PUFA	**8.71±1.00**	**10.28±1.97**	**8.30±0.77**	**10.27±2.44**	**5.92±1.06**	**5.08±1.13**
'18:3n-3	0.51±0.08	0.52±0.09	0.67±0.12	0.66±0.12	0.23±0.10	0.22±0.12
'20:5n-3	1.15±0.29	1.43±0.51	0.86±0.09	0.76±0.02	0.40±0.12	0.36±0.14
'22:5n-3	0.31±0.12	0.21±0.06	0.32±0.07	0.41±0.13	0.10±0.02	0.11±0.02
'22:6n-3	5.89±1.39	6.46±2.24	4.00±0.42	4.82±0.97	1.92±0.56	2.10±0.70
Total n3 PUFA	**7.86±1.71**	**8.63±1.96**	**5.85±0.57**	**6.66±1.20**	**2.66±0.76**	**2.79±0.95**
'20:2n-9	0.25±0.13	0.21±0.0.9	0.20±0.06	0.13±0.06	0.20±0.06	0.19±0.07
'20:3n-9	0.68±0.14	0.91±0.18*	0.38±0.09	0.43±0.08	0.50±0.25	0.42±0.06
'22:3	0.08±0.05	0.12±0.05	0.04±0.01	0.04±0.01	0.04±0.03	0.05±0.02
Total n9 PUFA	**1.01±0.22**	**1.23±0.22**	**0.62±0.15**	**0.60±0.10**	**0.74±0.34**	**0.65±0.14**

Values are Mean ± SD. * P<0.05.

Table 4.3 Heart fatty acid profiles of mice fed by high oleic sunflower oil diet.

	P1		P7		P13		P21	
	WT	KO	WT	KO	WT	KO	WT	KO
14:0	0.86±0.29	0.74±0.14	2.04±0.93	2.35±0.52	2.30±0.73	2.24±0.42	1.03±0.31	0.72±0.14
15:0	0.34±0.08	0.25±0.06	0.14±0.08	0.07±0.02	0.07±0.03	0.07±0.02	0.07±0.02	0.08±0.04
16:0	20.55±0.95	20.34±2.60	19.83±1.07	20.79±1.21	17.80±1.73	18.07±1.22	13.62±1.51	14.26±1.51
18:0	15.21±1.88	13.22±0.85*	14.77±1.68	14.36±1.17	15.99±1.01	15.39±0.90	17.51±0.93	17.88±1.63
20:0	0.21±0.04	0.27±0.06	0.34±0.08	0.41±0.12	0.37±0.15	0.62±0.19*	0.32±0.08	0.35±0.08
22:0	0.27±0.08	0.17±0.05*	0.20±0.07	0.23±0.07	0.25±0.03	0.28±0.08	0.31±0.06	0.34±0.11
Total SFA	37.44±1.20	34.99±2.66	37.32±2.10	38.21±2.00	36.79±1.36	36.66±1.78	32.86±1.54	33.63±2.74
16:1n-9	0.47±0.14	0.37±0.10	0.24±0.08	0.30±0.09	0.12±0.05	0.26±0.14	0.22±0.08	0.26±0.04
16:1n-7	2.27±0.41	1.79±0.30*	0.85±0.13	1.14±0.33	0.45±0.10	0.76±0.25*	0.41±0.08	0.48±0.06
17:1	0.70±0.11	0.65±0.12	0.73±0.13	0.60±0.17	0.92±0.19	0.83±0.18	1.01±0.32	0.78±0.19
18:1n-9	21.76±2.28	27.57±2.42*	19.14±3.07	21.88±2.43	14.84±1.00	19.39±2.47*	19.29±2.69	18.98±1.85
18:1n-7	3.69±0.56	3.50±0.61	3.32±0.28	3.40±0.45	2.92±0.20	3.31±0.38	3.42±0.32	3.55±0.38
20:1n-9	0.44±0.02	0.70±0.15*	1.19±0.38	1.14±0.23	0.75±0.20	0.92±0.18	0.84±0.15	0.94±0.13
20:1n-7	0.22±0.06	0.23±0.08	0.26±0.14	0.17±0.05	0.21±0.09	0.34±0.10*	0.07±0.03	0.09±0.03
22:1n-9	0.28±0.06	0.26±0.07	0.29±0.07	0.29±0.04	0.21±0.06	0.28±0.09	0.13±0.02	0.16±0.04
Total MUFA	29.75±2.22	35.06±2.62*	26.02±3.67	28.92±2.69	20.43±0.99	26.09±2.15*	25.39±2.81	25.24±1.82
18:2n-6	4.70±0.37	4.34±0.41	4.56±1.15	5.11±1.42	5.37±0.47	5.13±0.69	6.66±0.91	6.76±0.88
20:2n-6	0.66±0.39	0.60±0.28	0.30±0.05	0.42±0.10*	0.33±0.03	0.55±0.12*	0.32±0.5	0.33±0.07
20:3n-6	0.88±0.10	0.87±0.22	1.69±0.30	1.59±0.45	1.93±0.22	1.76±.10	1.89±0.23	2.08±0.29
20:4n-6	11.28±1.58	10.22±1.71	10.25±2.07	9.33±1.32	11.09±1.07	9.13±0.88*	9.27±1.26	9.74±0.65
22:4n-6	1.06±0.02	1.10±0.19	0.85±0.17	0.69±0.08	0.74±0.17	0.64±0.15	0.50±0.11	0.49±0.13
22:5n-6	0.43±0.17	0.32±0.13	0.39±0.08	0.26±0.09*	0.31±0.10	0.24±0.09	0.34±0.14	0.25±0.09
Total n-6 PUFA	19.01±1.23	17.45±1.71	18.03±1.93	17.41±0.78	19.78±1.21	17.46±0.81*	18.98±1.91	19.65±0.97
18:3n-3	0.33±0.05	0.28±0.06	0.13±0.02	0.20±0.08	0.26±0.10	0.37±0.09	0.31±0.13	0.29±0.07
20:5n-3	0.76±0.06	1.08±0.22*	1.56±0.31	1.19±0.36	0.95±0.20	1.01±0.20	0.48±0.05	0.57±0.09
22:5n-3	1.30±0.18	1.65±0.32*	3.75±0.46	3.22±0.79	3.98±0.71	3.48±0.48	2.97±0.52	3.20±0.93
22:6n-3	9.53±1.83	7.76±1.37	11.30±1.97	9.22±1.57	16.49±1.22	13.36±1.40*	17.91±2.01	16.16±2.66
Total n-3 PUFA	11.91±1.98	10.76±1.72	16.75±2.22	13.82±2.25	21.69±1.21	18.22±1.56*	21.67±2.43	20.22±3.56
20:2n-9	0.21±0.02	0.16±0.02*	0.22±0.04	0.22±0.06	0.16±0.03	0.17±0.05	0.16±0.07	0.23±0.06
20:3n-9	1.79±0.67	1.63±0.33	1.62±0.38	1.32±0.46	1.03±0.14	1.23±0.13*	0.88±0.09	0.99±0.26
Total n-9 PUFA	1.93±0.73	1.79±0.33	1.80±0.44	1.54±0.47	1.18±0.15	1.40±0.16*	1.03±0.10	1.22±0.27

Values are Mean ± SD. * P<0.05.

Table 4.3 Heart fatty acid profiles of mice fed by high oleic sunflower oil diet (cont.).

	P30		P90		P365	
	WT	KO	WT	KO	WT	KO
14:0	0.61±0.09	0.89±0.17*	0.11±0.01	0.16±0.03*	0.25±0.08	0.14±0.02*
15:0	0.19±0.08	0.11±0.03	0.29±0.03	0.18±0.09	0.09±0.04	0.17±0.10
16:0	12.73±0.52	13.07±0.71	12.18±0.61	12.12±0.97	13.59±0.78	14.62±0.77
18:0	15.49±1.14	15.75±1.52	14.28±0.93	13.11±0.65	16.19±0.14	17.15±0.71
20:0	0.29±0.12	0.22±0.05	0.34±0.02	0.51±0.24	0.34±0.07	0.41±0.03
22:0	0.50±0.10	0.37±0.07*	0.02±0.00	0.30±0.14	0.36±0.07	0.27±0.03
Total SFA	29.71±1.42	30.41±2.19	27.20±0.52	26.36±1.40	30.82±1.00	32.76±0.95*
16:1n-9	0.46±0.15	0.45±0.18	0.26±0.04	0.50±0.07*	0.15±0.06	0.16±0.05
16:1n-7	0.61±0.09	0.58±0.11	0.80±0.12	0.94±0.17	0.43±0.12	0.44±0.05
17:1	0.98±0.18	0.85±0.13	0.78±0.09	0.65±0.05	0.60±0.06	0.63±0.09
18:1n-9	21.28±1.70	22.47±3.41	19.23±1.31	24.82±3.79	7.00±1.18	7.79±1.35
18:1n-7	3.23±0.32	3.41±0.18	1.81±0.16	1.89±0.71	1.82±0.07	2.02±0.16
20:1n-9	0.60±0.06	0.75±0.19	0.55±0.01	0.66±0.09	0.40±0.06	0.39±0.11
20:1n-7	0.18±0.08	0.18±0.10	0.09±0.01	0.13±0.05	0.12±0.04	0.08±0.02
22:1n-9	0.12±0.03	0.14±0.04	0.00±0.00	0.02±0.01	0.06±0.01	0.07±0.01
Total MUFA	27.37±1.70	28.74±3.59	23.51±1.40	29.61±3.07*	10.58±1.30	11.59±1.55
18:2n-6	9.58±1.44	9.43±0.86	6.43±0.84	6.53±0.86	20.42±2.17	23.22±2.41
20:2n-6	0.22±0.08	0.55±0.33	0.05±0.01	0.06±0.00	0.55±0.12	0.39±0.06
20:3n-6	1.61±0.23	1.55±0.09	1.15±0.11	1.13±0.14	0.65±0.12	0.70±0.16
20:4n-6	7.81±0.65	7.66±0.35	6.59±0.63	5.68±0.59	8.83±0.49	9.10±0.80
22:4n-6	0.26±0.11	0.21±0.05	0.07±0.04	0.12±0.08	0.68±0.19	0.51±0.08
22:5n-6	0.27±0.05	0.27±0.06	0.42±0.02	0.32±0.08	1.58±0.35	1.04±0.10*
Total n-6 PUFA	19.72±1.88	19.68±1.00	14.71±1.43	13.81±1.00	32.71±2.67	34.96±2.15
18:3n-3	0.45±0.11	0.52±0.17	0.24±0.02	0.24±0.05	0.30±0.02	0.25±0.03*
20:5n-3	0.62±0.08	0.74±0.21	0.34±0.04	0.47±0.24	0.19±0.10	0.07±0.03
22:5n-3	1.81±0.23	1.67±0.31	1.99±0.18	1.79±0.22	1.36±0.66	1.28±0.37
22:6n-3	19.00±1.66	17.05±1.68	31.15±0.22	27.04±1.33*	23.91±2.78	19.01±3.25
Total n-3 PUFA	21.89±1.69	19.98±1.71	33.73±0.40	29.53±1.43*	25.76±2.68	20.61±3.19*
20:2n-9	0.20±0.06	0.16±0.08	0.13±0.02	0.21±0.08	0.09±0.07	0.03±0.01
20:3n-9	1.11±0.36	1.02±0.12	0.67±0.08	0.45±0.10*	0.04±0.02	0.04±0.01
Total n-9 PUFA	1.32±0.39	1.18±0.18	0.80±0.07	0.66±0.13	0.13±0.06	0.06±0.02

Values are Mean ± SD. * P<0.05.

Table 4.4 Brain fatty acid profiles of mice fed by high oleic sunflower oil diet.

	P1		P7		P13		P21	
	WT	KO	WT	KO	WT	KO	WT	KO
14:0	2.57±0.36	2.51±0.43	3.08±0.45	3.19±0.2	1.68±0.34	1.75±0.32	0.74±0.19	0.67±0.08
15:0	0.4±0.04	0.4±0.08	0.56±0.13	0.38±0.04	0.71±0.17	0.66±0.11	0.94±0.24	0.67±0.16
16:0	26.79±0.69	26.5±1.17	28.3±1.78	28.66±2.46*	26.58±0.71	27.17±0.95	25.07±1.93	24.97±1.49
18:0	15.84±0.96	15.77±0.64	16.21±0.59	16.34±0.87	18.65±0.91	17.92±0.71	21.14±1.20	20.73±0.79
20:0	0.28±0.18	0.2±0.03	0.13±0.02	0.14±0.03	0.19±0.04	0.22±0.04	0.33±0.03	0.36±0.08
22:0	0.00±0.00	0.00±0.00	0.07±0.01	0.06±0.02	0.12±0.03	0.08±0.02*	0.22±0.03	0.22±0.07
Total SFA	**45.88±2.08**	**45.37±1.84**	**48.34±2.31**	**48.78±3.05**	**47.93±0.63**	**47.8±1.00**	**48.44±3.17**	**47.62±1.02**
16:1n-9	3.49±0.28	3.52±0.29	2.81±0.17	2.76±0.16	1.66±0.3	1.44±0.21	0.61±0.10	0.57±0.09
16:1n-7	2.73±0.09	2.85±0.24	2.57±0.18	2.65±0.12	1.37±0.24	1.32±0.18	0.78±0.11	0.77±0.09
18:1n-9	15.91±0.55	15.92±0.7	12.61±0.57	13.31±0.85	12.24±0.25	12.39±0.86	13.58±0.77	13.34±0.44
'18:1n-7	3.87±0.16	3.85±0.13	2.92±0.32	2.96±0.15	2.83±0.07	2.82±0.15	3.08±0.12	3.09±0.08
20:1n-9	0.4±0.03	0.43±0.03	0.36±0.05	0.34±0.06	0.37±0.07	0.40±0.08	0.54±0.05	0.59±0.07
'20:1n-7	0.04±0.02	0.07±0.01	0.07±0.02	0.05±0.01	0.06±0.01	0.08±0.03	0.14±0.04	0.17±0.03
22:1n-9	0.06±0.02	0.06±0.02*	0.07±0.01	0.04±0.03	0.04±0.00	0.07±0.03	0.06±0.02	0.08±0.01*
24:1n-9	0.00±0.00	0.00±0.00	0.11±0.02	0.09±0.02	0.10±0.02	0.16±0.06	0.27±0.05	0.37±0.08*
Total MUFA	**26.47±0.59**	**26.67±1.12**	**21.51±0.75**	**22.18±0.88**	**18.61±0.47**	**18.60±1.03**	**19.01±1.05**	**187.9±0.67**
18:3n-6	0.22±0.01	0.37±0.07	0.73±0.14	0.83±0.12	0.80±0.18	0.95±0.29	0.67±0.20	0.71±0.25
20:2n-6	0.16±0.03	0.19±0.04*	0.11±0.04	0.14±0.06	0.18±0.06	0.18±0.05	0.16±0.07	0.21±0.10
20:3n-6	0.22±0.04	0.28±0.04	0.72±0.07	0.74±0.05	0.83±0.07	0.90±0.05	0.67±0.11	0.80±0.12
20:4n-6	8.77±0.9	9.04±0.86*	9.35±0.79	9.3±1.15	11.13±0.18	10.71±0.41*	10.27±1.10	10.76±0.49
22:4n-6	1.38±0.35	1.56±0.31	1.31±0.14	1.07±0.32	1.45±0.19	1.33±0.27	1.51±0.25	1.60±0.12
22:5n-6	0.52±0.06	0.47±0.08	0.35±0.07	0.36±0.13	0.35±0.04	0.39±0.09	0.31±0.06	0.36±0.07
Total n-6 PUFA	**11.28±1.3**	**11.91±1.25**	**12.56±0.89**	**12.44±1.52**	**14.74±0.36**	**14.47±0.66**	**13.57±1.60**	**14.43±0.53**
'20:5n-3	0.19±0.05	0.25±0.06	0.4±0.08	0.33±0.06	0.39±0.06	0.44±0.12	0.22±0.09	0.27±0.13
22:5n-3	0.35±0.07	0.4±0.09	0.77±0.05	0.67±0.08	0.69±0.05	0.71±0.15	0.43±0.08	0.52±0.08
22:6n-3	14.01±1.16	13.44±1.92	14.62±1.89	13.96±2.32*	16.11±1.11	16.43±1.16	17.40±2.87	17.6±1.20
Total n-3 PUFA	**14.55±1.23**	**14.05±2.07**	**15.8±1.94**	**14.96±2.32**	**17.19±1.14**	**17.58±1.19**	**18.05±2.95**	**18.39±1.21**
'20:2n-9	0.14±0.04	0.13±0.03	0.21±0.03	0.19±0.08	0.19±0.04	0.23±0.06	0.15±0.04	0.17±0.05
20:3n-9	1.16±0.07	1.24±0.3	1.13±0.13	0.98±0.17	0.89±0.07	0.89±0.13	0.48±0.08	0.52±0.09
22:3	0.6±0.07	0.56±0.08	0.38±0.08	0.31±0.06	0.30±0.02	0.30±0.07	0.19±0.03	0.20±0.02
Total n-9 PUFA	**1.91±0.1**	**1.92±0.37**	**1.72±0.2**	**1.48±0.26**	**1.33±0.12**	**1.36±0.18**	**0.78±0.12**	**0.83±0.16**

Values are Mean ± SD. * P<0.05.

Table 4.4 Brain fatty acid profiles of mice fed by high oleic sunflower oil diet (cont.).

	P30		P90		P365	
	WT	KO	WT	KO	WT	KO
14:0	0.38±0.11	0.32±0.09	0.16±0.01	0.15±0.01	0.16±0.01	0.18±0.06
15:0	0.51±0.10	0.56±0.11	0.55±0.03	0.47±0.03*	0.55±0.04	0.45±0.02*
16:0	22.87±0.42	22.19±0.58*	21.90±0.65	21.19±0.45	21.6±0.37	21.13±0.29
18:0	22.17±0.45	22.17±0.29	21.91±0.07	21.44±0.30*	21.87±0.31	21.81±0.33
20:0	0.38±0.03	0.38±0.10	0.21±0.05	0.20±0.01	0.22±0.01	0.23±0.05
22:0	0.30±0.06	0.37±0.07	0.14±0.08	0.17±0.04	0.15±0.06	0.97±0.66
Total SFA	**46.61±0.74**	**45.99±0.44**	**45.04±0.48**	**43.81±0.45***	**44.72±0.35**	**44.96±1.56**
16:1n-9	0.30±0.01	0.30±0.02	0.16±0.01	0.15±0.01	0.17±0.01	0.18±0.02
16:1n-7	0.61±0.04	0.59±0.05	0.64±0.04	0.66±0.02	0.62±0.02	0.65±0.04
18:1n-9	14.63±0.51	15.17±0.80	15.70±0.97	16.09±0.52	16.2±0.55	16.87±1.07
'18:1n-7	3.30±0.10	3.40±0.11	3.60±0.07	3.72±0.10	3.71±0.18	3.87±0.13
20:1n-9	0.75±0.07	0.86±0.14	0.87±0.25	0.94±0.16	1.04±0.11	1.12±0.33
'20:1n-7	0.33±0.13	0.31±0.12	0.22±0.03	0.25±0.02	0.23±0.02	0.35±0.13
22:1n-9	0.08±0.02	0.12±0.04*	0.03±0.02	0.04±0.02	0.02±0.02	0.05±0.02
24:1n-9	0.00±0.00	0.00±0.00	0.22±0.04	0.12±0.09	0.16±0.06	0.22±0.03
Total MUFA	**19.99±0.77**	**20.75±1.04**	**21.44±1.22**	**21.96±0.72**	**22.16±0.81**	**23.31±1.53**
18:3n-6	0.53±0.12	0.49±0.08	0.35±0.01	0.41±0.06	0.33±0.03	0.36±0.07
20:2n-6	0.09±0.03	0.09±0.02	0.04±0.01	0.05±0.01	0.06±0.03	0.04±0.04
20:3n-6	0.64±0.05	0.70±0.04	0.48±0.02	0.52±0.03	0.4±0.04	0.43±0.04
20:4n-6	10.44±0.37	10.28±0.49	10.65±0.12	10.49±0.30	10.94±0.28	9.64±1.82
22:4n-6	1.50±0.20	1.60±0.23	2.17±0.07	2.23±0.04	2.45±0.17	2.36±0.09
22:5n-6	0.27±0.05	0.25±0.03	0.30±0.02	0.32±0.03	0.24±0.03	0.25±0.03
Total n-6 PUFA	**13.48±0.48**	**13.41±0.70**	**13.99±0.16**	**14.02±0.23**	**14.43±0.49**	**13.09±1.64**
'20:5n-3	0.18±0.06	0.17±0.07	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
22:5n-3	0.36±0.03	0.36±0.04	0.26±0.03	0.30±0.05	0.19±0.01	0.24±0.04
22:6n-3	18.59±1.02	18.65±1.02	18.83±0.79	19.51±0.74	17.99±0.72	17.83±0.95
Total n-3 PUFA	**19.13±1.01**	**19.17±0.96**	**19.09±0.82**	**19.81±0.76**	**18.19±0.73**	**18.07±0.97**
'20:2n-9	0.17±0.02	0.13±0.04*	0.12±0.02	0.11±0.01	0.13±0.01	0.16±0.03
20:3n-9	0.49±0.09	0.41±0.05	0.34±0.03	0.33±0.06	0.37±0.03	0.40±0.05
22:3	0.00±0.00	0.00±0.00	0.15±0.03	0.15±0.02	0.19±0.00	0.20±0.03
Total n-9 PUFA	**0.66±0.08**	**0.54±0.07***	**0.61±0.05**	**0.59±0.08**	**0.69±0.03**	**0.76±0.04***

Values are Mean ± SD. * P<0.05.

from HO-fed mice also have higher amount of 18:1n-9 from neonates to ages compared to chow-fed (Figure4.4B). Interestingly, chow-fed mice tended to accumulate 18:1n-9 with growth (P1:14.45 ± 0.99% *vs.* P365: 21.86 ± 1.73%) but HO-fed mice maintained the concentration of 18:1n-9 (Supplementary Table 4.3). HO-fed brain 18:1n-9 was significantly higher at P1 and P30 but was significantly lower at P90 and P365 compared to Chow-fed mice (Figure 4.4C). Similar trends of MUFA profiles in tissues were observed in KO mice fed by different diets (Supplementary Table 4.4-4.6).

4.3.3.2 Saturated fatty acids (SFA)

Only at P1, KO mice livers contained significantly lower amount of 16:0 (WT *vs.* HO: 22.20 ± 1.73% *vs.* 18.43 ± 2.84%) and 18:0 (WT *vs.* HO: 15.88 ± 2.43% *vs.* 9.92 ± 1.85%) compared to WT and in the rest of life no differences in saturated fatty acids were found between WT and KO livers (Table 4.2). KO HO-fed mice heart have lower amount of 18:0 and 22:0 at P1 while they have higher amount of total SFA at the age of one year compared to WT (Table 4.3). Though statistical significances between WT and KO were found in HO-fed mice brains, the biological differences were not clear (Table 4.4). Hepatic 16:0 in HO-fed WT mice was significantly lower than Chow-fed mice from P1 to P365 (except for P21 and P30). Similarly, hepatic 18:0 was significantly decreased by HO diet from P7 to P365 (Figure 4.5A, 4.5B). Especially adult and old HO-fed mice livers had only half amount of 18:0 in age matched Chow-fed mice (Supplementary Table 4.1). Heart 16:0 was not

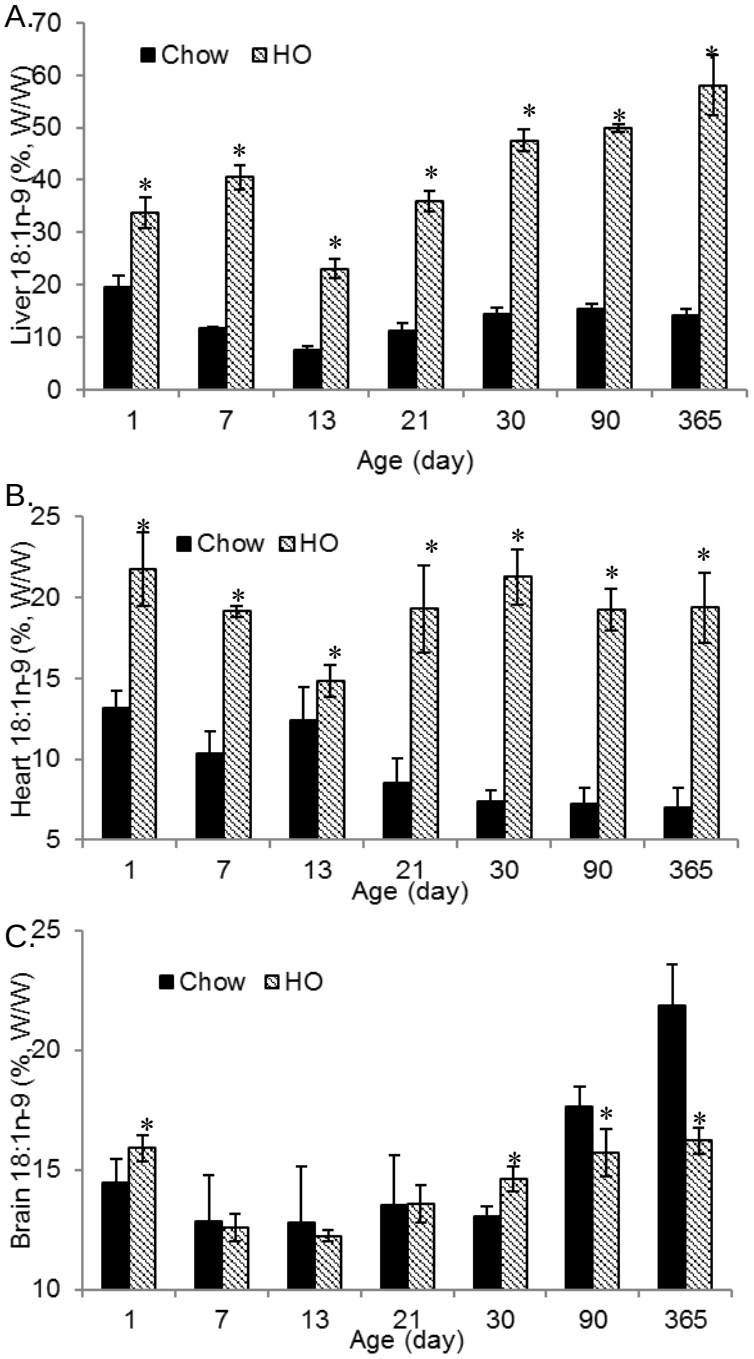

Figure 4.4 Ontogenies of oleic acid from mice tissues. A) WT Liver 18:1n-9 from neonates to ages (n=6). Oleic acids were more abundant in HO-fed mice livers compared to Chow livers and livers accumulated oleic acids with age. B) Heart 18:1n-9 from neonates to ages (n=6). With growth, the amount of 18:1n-9 gradually decreased in Chow but the amount was stable in HO heart. C) Brain 18:1n-9 from neonates to ages (n=6). At postnatal day 1 and 30, HO had higher amount of 18:1n-9 but with growth they had lower amount.

influenced significantly by dietary intervention from youth to aging except for the lower amount found in HO-fed heart at P7 (Figure 4.5C). Heart 18:0 was significantly decreased in HO-fed mice at P1, P7, P21 and P30 compared to Chow-fed mice (Figure4.5D). In both Chow and HO group, the amounts of heart 16:0 in old mice were half of that in neonates (P1 and P7) shown in Supplementary Table 4.2. Brain 16:0 of HO-fed neonates was significantly higher while the adult and old HO-fed mice had lower amounts of brain 16:0 compared to Chow-fed group (Figure 4.5E). As shown in supplementary Table 4.3, mouse brain tended to accumulated 18:0 in both diet (e.g. Chow P1 *vs.* P365: 15.41±0.31 *vs.* 21.65±0.40%). Brain 18:0 of HO-fed mice was significantly lower at P7, P13 and P90 compared to Chow-fed mice (Figure 4.5F). Similar trends of SFA profiles in tissues were observed in KO mice fed by different diets (Supplementary Table 4.4-4.6).

4.3.3.3 n-6 polyunsaturated fatty acids

The hepatic n-6 LCPUFA precursor 18:2n-6 was not significantly different between WT and KO in HO-fed group through young to aging mice. The difference of hepatic 20:3n-6 level between WT and KO varied through age, e.g. KO was lower at P1 but higher at P13-P30 compared to WT (Table 4.2). Only at P21, the hepatic 20:4n-6 in KO was significantly higher than WT. Heart 20:4n-6 was lower in KO compared to WT at P13 (WT vs KO: 11.09±1.07% *vs.* 9.13±0.88%). No differences of heart n-6 PUFA profiles were found between WT and KO at other tested time point (Table 4.3). Brain 20:4n-6

levels were higher in KO at P1 but lower in KO at P13 compared to WT (Table 4.4).

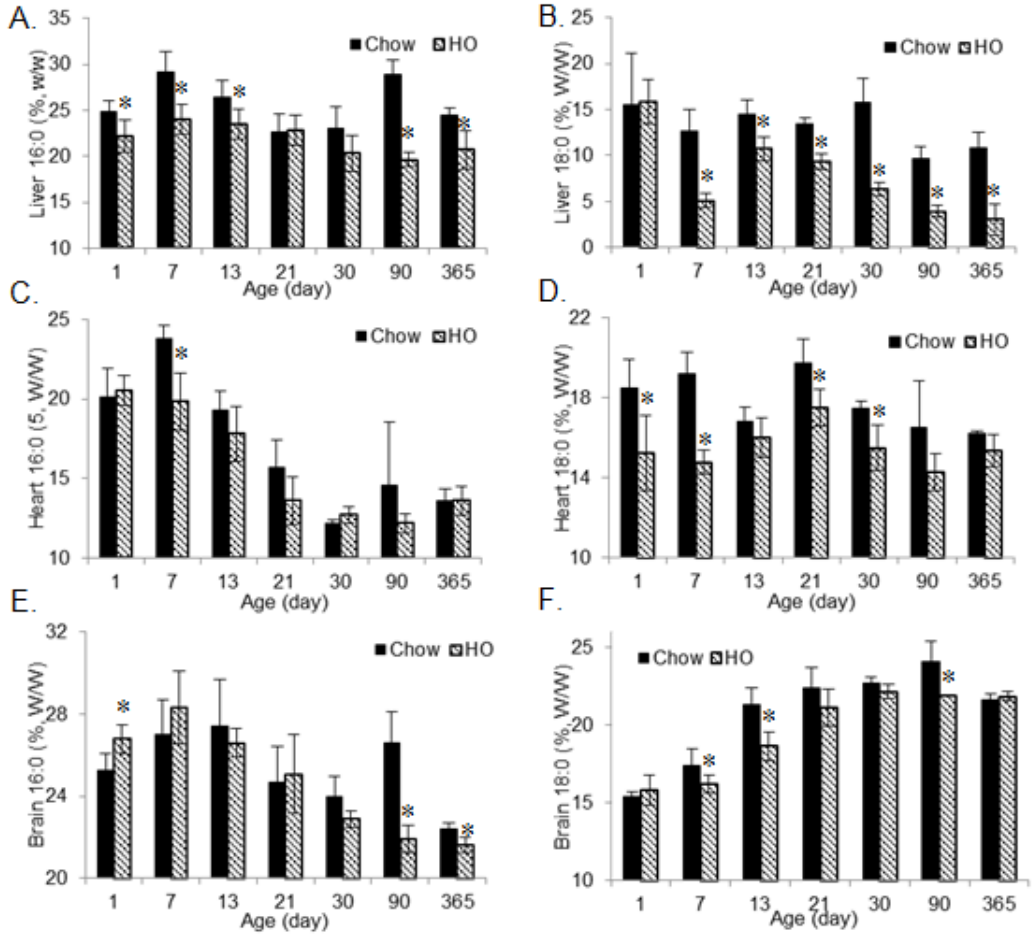

Figure 4.5 Ontogenies of important saturated fatty acids from mice tissues. Solid bar represented chow-fed WT mice and the patched bar represented HO-fed WT mice. A) WT Liver 16:0 from neonates to ages (n=6). Hepatic 16:0 was significantly lower in HO-fed mice livers at postnatal day 1, 7, 13, 90 and 365 compared to Chow. B) Liver 18:0 from neonates to ages (n=6). Hepatic 18:0 was significantly lower in HO-fed mice through postnatal day 7 to 365. C) Heart 16:0 from neonates to ages (n=6). No difference of heart 16:0 was observed across age expect for the lower amount found in HO-fed heart at P7. D) Heart 18:0 from neonates to ages (n=6). Heart 18:0 was significantly decreased in HO-fed mice at P1, P7, P21 and P30 compared to Chow-fed mice. E) Brain 16:0 from neonates to ages (n=6). Brain 16:0 was higher in HO-fed WT at P1 but lower at P90 and P365 compared to control group. F) Brain 18:0 from neonates to ages (n=6). Brain 18:0 of HO-fed mice was significantly lower at P7, P13 and P90 compared to control group.

Figure 4.6 A-D showed that when mice were fed by HO diet, the concentrations of 18:2n-6 and 20:4n-6 in livers and hearts were significantly lower with growth compared to age matched Chow-fed mice (except for P13 Heart 20:4n-6). The amounts of hepatic 18:2n-6 and 20:4n-6 and heart 18:2n-6 in Chow diet were two-fold of those detected from HO-fed livers (Supplementary Table 4.1, Supplementary Table 4.2). Different from other heart n-6 PUFAs, heart 20:4n-6 had a tendency to decrease with growth and the level of heart 20:4n-6 at the age of one year was as half as that at the age of postnatal day 1 (Supplementary Table 4.2). The concentrations of brain 18:2n-6 in HO-fed mice were significantly decreased in contrast to Chow-fed mice (Figure 4.6E). The quantity of brain 20:4n-6 decreased significantly compared to control (Supplementary Table 4.3). The differences of brain 20:4n-6 in HO-fed mice were lower from P1 to P30 (excluding P13) but higher at 3 month and 1 year after birth compared to Chow-fed mice (Figure 4.6F). Similar trends of n-6 PUFA profiles in tissues were observed in KO mice fed by different diets (Supplementary Table 4.4-4.6).

4.3.3.4 n-3 polyunsaturated fatty acids

KO livers contained higher amount of 20:5n-3 and 22:5n-3 at P1 and lower amount of 22:6n-3 and total n-3 PUFA at P13 compared to WT livers with HO diet intervention (Table 4.2). Similar to livers, KO hearts had higher levels of 20:5n-3 and 22:5n-3 at P1 and lower amount of 22:6n-3 and total n-3

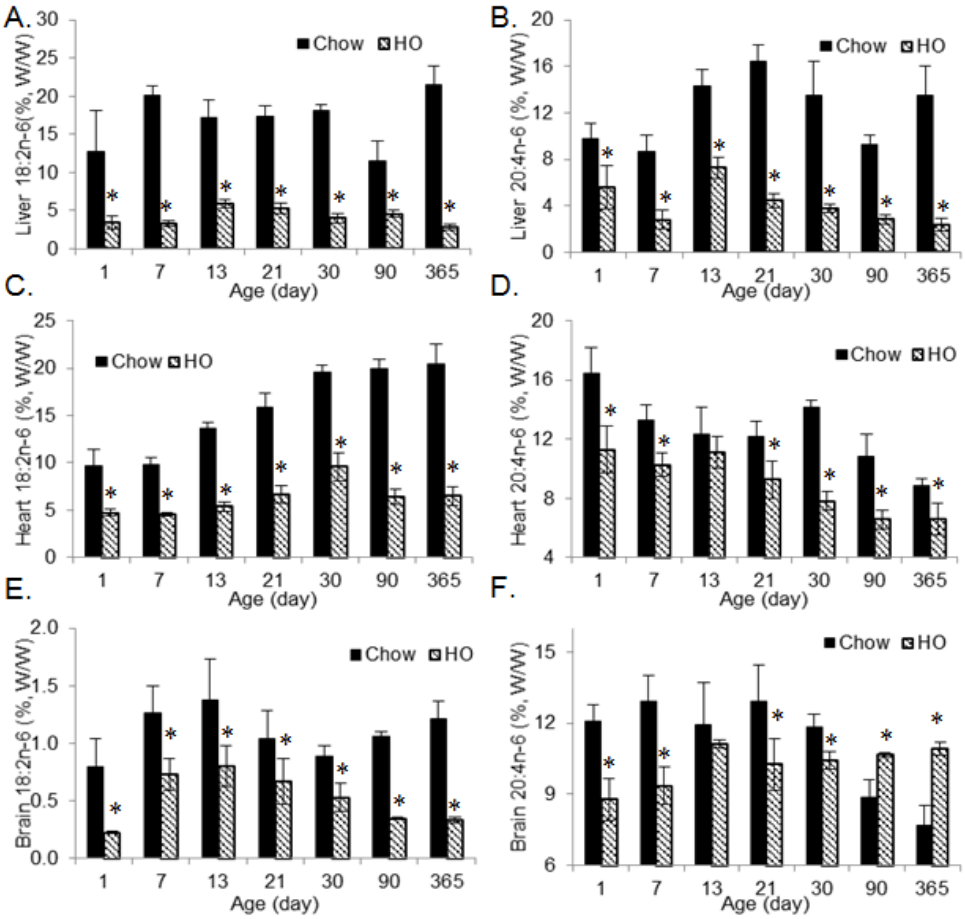

Figure 4.6 Ontogenies of important n-6 polyunsaturated fatty acids from mice tissues. Solid bar represents chow-fed WT mice and the patched bar represents HO-fed WT mice. A) Liver 18:2n-6 from neonates to ages (n=6). Hepatic 18:2n-6 is significantly lower in HO-fed mice livers with growth compared to Chow. B) WT Liver arachidonic acid from neonates to ages (n=6). Hepatic 20:4n-6 is significantly lower in HO-fed mice through postnatal day 1 to 365. C) Heart linoleic acid from neonates to ages (n=6). Heart 18:2n-6 is significantly lower in HO-fed mice cross all the detected time points compared to Chow. D) Heart arachidonic acid from neonates to ages (n=6). Heart 20:4n-6 was significantly decreased in HO-fed mice from P1 to P365 (except for P13) compared to Chow-fed mice. E) Brain linoleic acid from neonates to ages (n=6). The concentrations of brain 18:2n-6 in HO-fed mice were significantly decreased in contrast to Chow-fed mice. F) Brain arachidonic acid from neonates to ages (n=6). The differences of brain 20:4n-6 in HO-fed mice were lower at P1, P7, P21 and P30 but higher at P90 and P365 compared to Chow.

PUFA at P13 and P90 compared to age matched WT. The aging KO hearts contained less total n-3 PUFA than WT when both genotypes were fed by HO diet (Table 4.3). Only at P7 the KO brain 22:6n-3 was significantly lower than WT and no differences of brain n-3 PUFA profiles were detected at the other time points (Table 4.4).

Hepatic 20:5n-3 from HO-fed mice was more than 2 fold higher than Chow-fed mice from P1 to P90 but no difference was observed at the first year after birth (Figure 4.7A). Liver 22:6n-3 levels were relatively constant across ages in Chow-fed group while the levels dropped to 1.92±0.56% at the age of one year in HO-fed group (Supplementary Table 4.1). Compared to Chow-fed mice liver, HO group hepatic 22:6n-3 was significantly lower at P21, P90 and P365 (Figure4.7B). Similar to liver 20:5n-3, heart 20:5n-3 levels in HO-fed mice were three fold of those in Chow-fed mice from P1 to P90 but no differences were found in P365 (Figure 4.7C). Heart accumulated 22:6n-3 with growth (Chow P1 *vs.* P365: 7.16±2.04% *vs.* 23.78±2.78%) shown in supplementary Table 4.2. Different from hepatic 22:6n-3, heart 22:6n-3 was increased from P7 to P90 resulting from HO diet intervention (Figure 4.7D). Brain doesn't contain much of 20:5n-3 thus we tested the levels of 22:5n-3, precursor of 22:6n-3, between Chow and HO group. Levels of brain 22:5n-3 were significantly elevated by HO diet from P1 to P90 (except for P30). No difference of brain 22:5n-3 in old mice between two diet groups was discovered (Figure 4.7E). In Chow-fed mice brains, the level of 22:6n-3 was 12.60±0.76% at P1, reached a peak at the postnatal day 30 (16.42±1.53%)

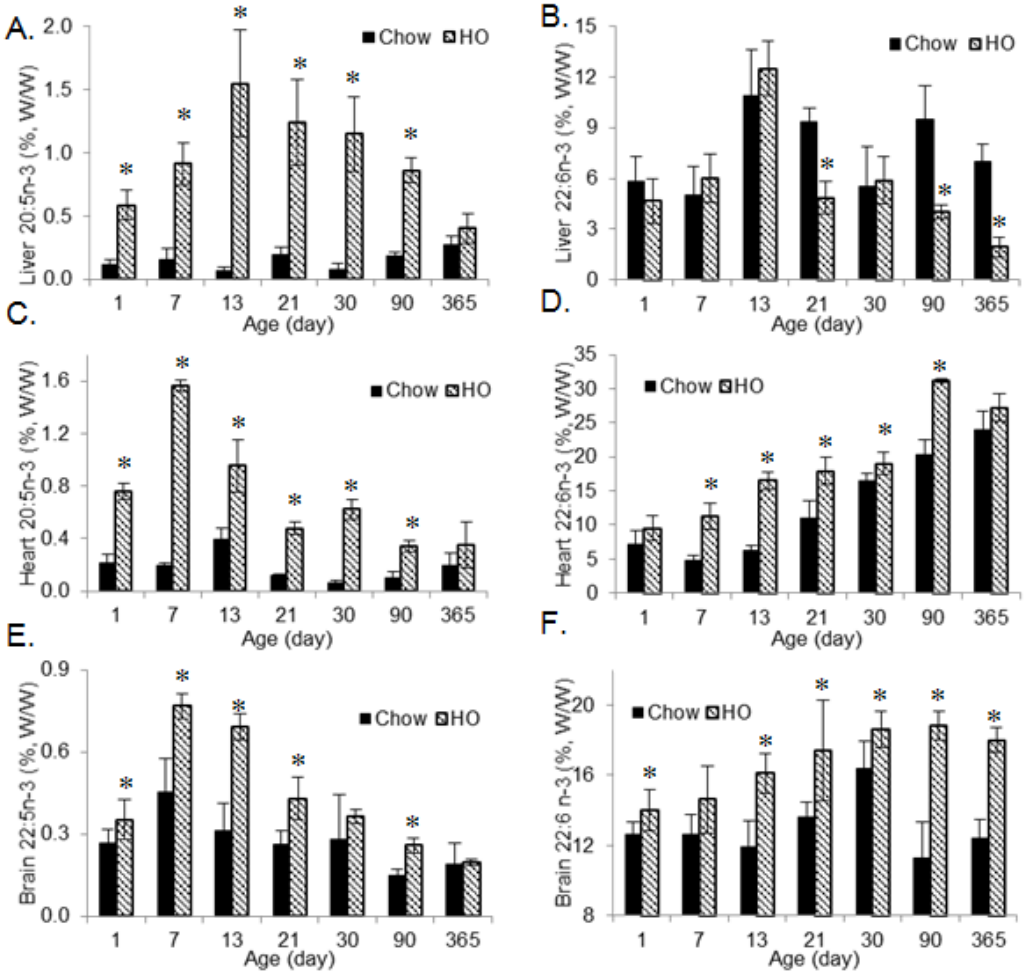

Figure 4.7 Ontogenies of important n-3 PUFA from mice tissues. A) WT Liver eicosapentaenoic acid from neonates to ages (n=6). Hepatic 20:5n-3 from HO-fed mice was more than 2 fold higher than Chow-fed mice from P1 to P90 but no difference was observed at the first year after birth. B) Liver docosahexaenoic acid from neonates to ages (n=6). Compared to Chow-fed mice liver, HO group hepatic 22:6n-3 was significantly lower at P21, P90 and P365. C) Heart eicosapentaenoic acid from neonates to ages (n=6). Similar to liver 20:5n-3, heart 20:5n-3 levels in HO-fed mice were three fold of those in Chow-fed mice from P1 to P90 but no differences were found in P365. D) Heart docosahexaenoic acid from neonates to ages (n=6). Different from hepatic 22:6n-3, heart 22:6n-3 was increased from P7 to P90 resulting from HO diet intervention. E) Brain docosapentaenoic acid from neonates to ages (n=6). Levels of brain 22:5n-3 were significantly elevated by HO diet from P1 to P90 (except for P30). No difference of brain 22:5n-3 in old mice between two diet groups was discovered. F) Brain docosahexaenoic acid from neonates to ages (n=6). Except for P7, brain 22:6n-3 was significantly higher in HO-fed mice compared to the counterpart from neonates to ages.

and with growth the level dropped to 12.41±1.07% at P365 (Supplementary Table 4.3). Differently, brain 22:6n-3 was accreted with growth in HO-fed mice (P1 vs P365: 14.01±1.16% vs 17.99±0.72%). Except for P7, brain 22:6n-3 was significantly higher in HO-fed mice compared to the counterpart from neonates to ages (Figure 4.7F). Similar trends of n-3 PUFA profiles in tissues were observed in KO mice fed by different diets (Supplementary Table 4.4-4.6).

4.3.3.5 n-9 polyunsaturated fatty acids

In the absence of *Fads3* the hepatic concentration of 20:3n-9 was significantly higher than WT mice at P30 (WT vs KO: 0.68±0.14% vs 0.91±0.18%) and the ablation of *Fads3* did not contribute to other differences of brain n-9 PUFA compared to WT (Table 4.2). *Fads3* KO mice had higher amount of heart 20:3n-9 at P13 but lower amount at P90 compared to WT (Table 4.3). Compared to old WT, KO brain had higher level of total n-9 PUFA and no other significant differences of n-9 PUFA profiles were measured (Table 4.4). Mead acid is an indicator of essential fatty acid deficiency. Figure 4.8 showed the comparisons of 20:3n-9 levels between Chow-fed and HO-fed mice in different tissues. HO diet contributed to significant elevations of 20:3n-9 in liver, heart and brain (Figure 4.8). Similar results were also found in KO mice fed by different diets (Supplementary Table 4.4-4.6).

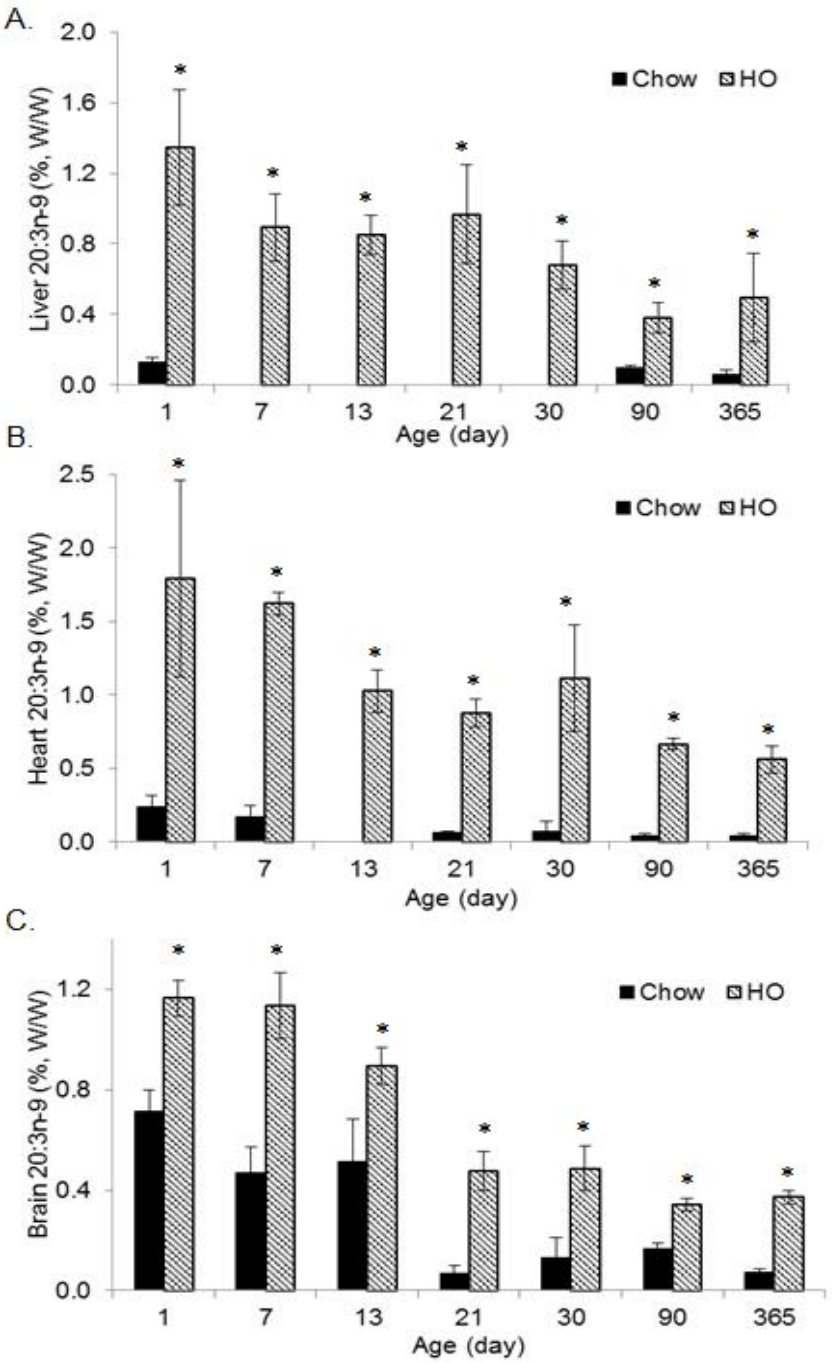

Figure 4.8 Ontogenies of 20:3n-9 from mice tissues. Solid bar represented chow-fed WT mice and the patched bar represented HO-fed WT mice. The differences between two diets were analyzed using Student's *t*-Test (* *P*<0.05). A) WT Liver mead acid from neonates to ages (n=6). Hepatic 20:3n-9 was significantly higher in HO-fed mice at all tested ages compared to Chow-fed mice. B) Heart mead acid from neonates to ages (n=6). Similar to brain, heart 20:3n-9 was significantly higher in HO-fed mice at all tested ages compared to Chow-fed mice. C) Brain mead acid from neonates to ages (n=6). Brain 20:3n-9 was significantly higher in HO-fed mice at all tested ages compared to Chow-fed mice.

4.3.4 mRNA expressions of Fads1 and Fads2 in livers, hearts and brains P1-P30

mRNA expression levels of *Fads1* and *Fads2* in livers, hearts and brains from young mice fed by Chow or HO diet were detected using real time PCR. The results showed that no significant differences of gene expressions were found between WT and KO in the HO-fed group (the comparisons between WT and KO in Chow-fed group were presented in Chapter 2).

After the treatment of HO diet, hepatic *Fads1* expression was upregulated at P13 and no changes of *Fads1* in livers were found at other ages compared to age-matched Chow (Figure 4.9A). Hepatic *Fads2* was upregulated by HO diet at P13 and P21 in contrast to Chow group and no significant difference were found between diets at other time points. (Figure 4.9B). In hearts, expression levels of both *Fads1* and *Fads2* in HO group were more than two fold higher at P1 and significantly lower at P7 and P21 compared to Chow group (Figure 4.9C and 4.9D). Expressions of *Fads1* or *Fads2* in brain were not influenced by dietary treatment (Figure 4.9E and 4.9F).

4.3.5 Histology of heart and liver

Part of freshly dissected heart and liver were imbedded in a thermoform cassette and H&E staining was performed one slide/tissue sample. The results showed that HO group mice possessed large and numerous lipid deposits in old WT mice but the lipid accumulation was not presented in the slide of chow liver (Figure 4.10A, 10B). Additionally, HO-fed young animals (at P30) did not

developed steatosis (Figure 4.10C). The histological slides of hearts from different diets did not show any overt differences (Figure 4.10D-F) suggesting that only liver was the vulnerable tissue responding to environmental changes.

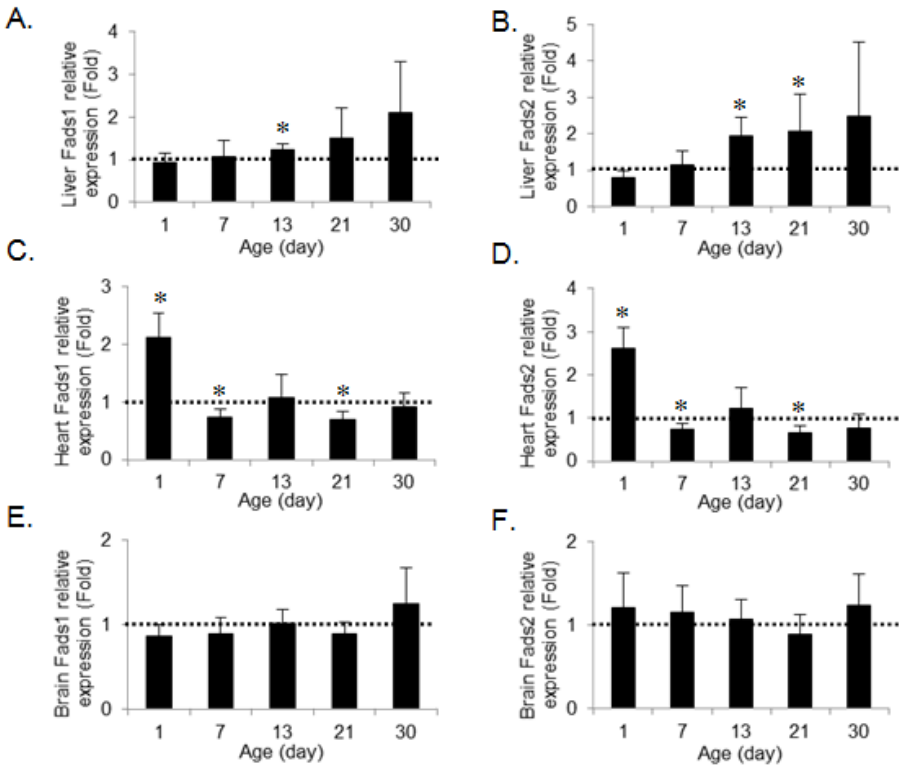

Figure 4.9 Ontogeny of *Fads1* and *Fads2* gene expression levels in tissues. The dash line crossing "1" represented Chow group and the solid bars were mRNA levels of HO-fed mice after normalization to reference genes (Gapdh and ß-actin). A) Liver *Fads1* mRNA expression levels. HO diet increased *Fads1* expression at P13. B) Liver *Fads2* mRNA expression levels. Hepatic *Fads2* was upregulated by HO diet at P13 and P21. C) Heart *Fads1* mRNA expression levels. Expression levels of *Fads1* in HO group were more than two fold higher at P1 and significantly lower at P7 and P21 compared to Chow. D) Heart *Fads2* mRNA expression levels. Expression levels of *Fads2* in HO group were more than two fold higher at P1 and significantly lower at P7 and P21 compared to Chow. E) Brain *Fads1* mRNA expression levels. F) Brain *Fads2* mRNA expression levels. No differences of *Fads1* or *Fads2* expression in brains between two diets were detected.

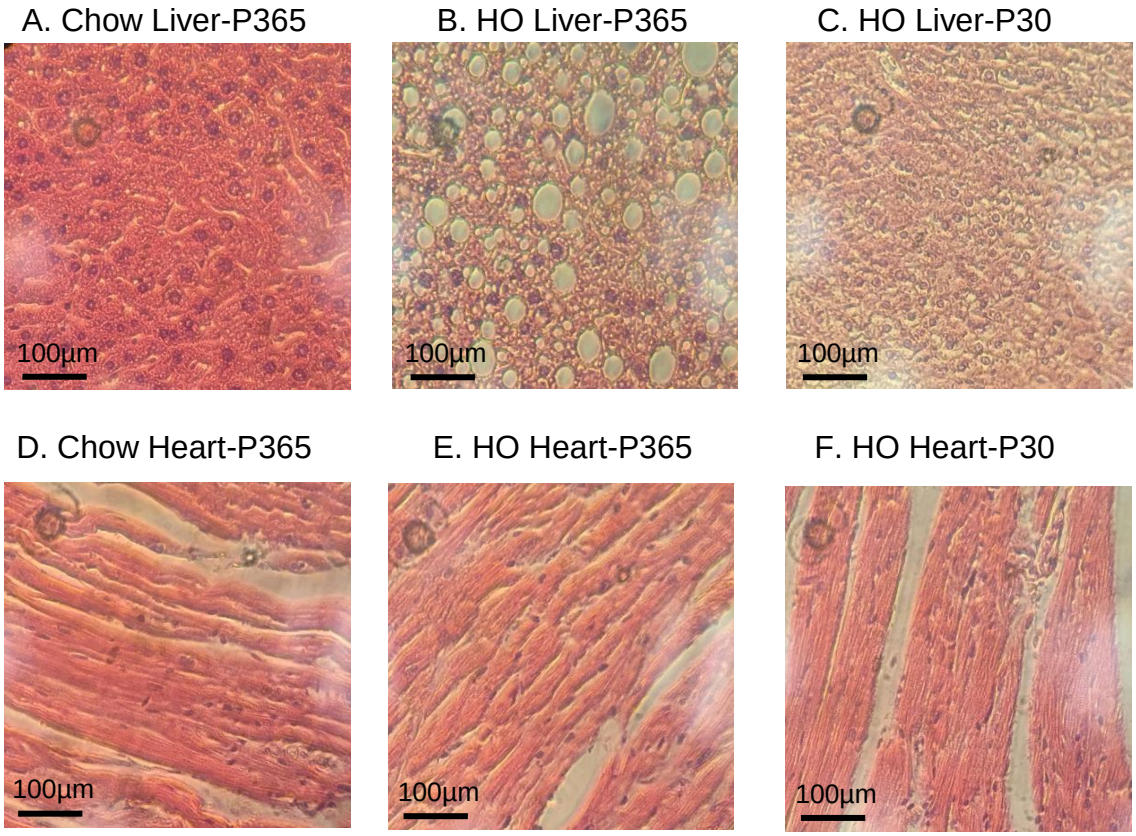

Figure 4.10 E&H staining of mouse liver and heart from different diets. A) Histology slide from one year old WT liver in Chow group. No fat deposit was observed in chow-fed mouse liver. B) Histology slide from one year old WT liver in HO group. With long-term HO consumption, numerous lipid deposits resided in liver. C) Histology slide from P30 WT mouse liver in HO group. Though HO induced lipid accumulation in aging liver, the young liver did not present symptom of steatosis. D) Histology slide from one year old WT heart in Chow group. E) Histology slide from one year old WT mouse heart in HO group. F) Histology slide from P30 WT mouse heart in HO group. Different from liver, neither age nor diet affected heart histological result, indicating that heart was not as vulnerable as liver to environmental change.

4.4 DISCUSSION

It is the first time to elaborate effects of high oleic sunflower oil on fatty acid metabolites of liver, heart and brain from neonates to aging mice. In this study, we substituted soybean oil as the primary fat source of animal with high oleic sunflower oil and determined mouse physical and biochemical changes. Both wild type mice and *Fads3* knockout mice were fed by this customized diet. Similar to the phenomena observed in normal rodent chow study showed previously, ablation of *Fads3* only contributed to the differences at early perinatal age (particular at postnatal day 1) and the differences between two genotypes diminished with growth. Compared to Chow-fed mice, HO group had heavier body weight, larger liver and adipose tissue mass, lower hepatic saturated fat, lower tissue n-6 PUFAs (18:2n-6 and 20:4n-6) but higher heart and brain n-3 PUFAs (20:5n-3, 22:5n-3 or 22: 6n-3) from neonates to old mice. Expressions of *Fads1* and *Fads2*, two genes coding vital enzymes in LCPUFA biosynthesis, were upregulated at P13 in liver and at P1 in heart but were downregulated at P7 and P21 in heart.

Fads3 regulated LCPUFA biosynthesis only at the very early neonatal stage. With HO treatment, KO mice liver had significantly lower amount of palmitic acid (16:0) and stearic acid (18:0) but higher amount of oleic acid (18:1) compared to WT on postnatal day 1 (Table 4.2). With lack of LCPUFA precursors in the diet, KO after birth had higher amounts of n-3 LCPUFAs, such as eicosapentaenoic acid (EPA, 20:5n-3) and docosapentaenoic acid (DPA, 22:5n-3). The differences of liver fatty acid profiles between genotypes

were not significant with growth (Table 4.2). KO heart fatty acid profiles were quite different from WT at P1 and P13. Generally speaking, KO heart tended to have higher amount of 18:1 but lower amount of n-6 and n-3 LCPUFA species compared to WT (Table 4.3). Brain fatty acid profiles between KO and WT were nearly the same (Table 4.4). These findings mentioned above were similar to the results determined in chow-fed animals. It seems that *Fads3* regulated LCPUFA in a tissue dependent manner during the “infancy”. For the sake of conciseness, the following discussion focused on the influences of Chow and HO diet on LCPUFA biosynthesis in WT animal.

The fat percentage of total energy was the same and only the fat source was changed. In order to shift the unbalanced ratio of n-6/n-3 PUFA in modern diet, we designed high oleic sunflower oil diet (Table 4.1) removing 18:2n-6 dense soybean oil and blending flaxseed oil to make a balanced PUFA (but the absolute amount of PUFA in this HO diet was low). Mice from both dietary groups consumed food *ad libitum* and no differences in food intake were detected (Supplementary Figure 4.1). However, starting from 3 month old the body weights of HO-fed mice were significantly higher than Chow-fed mice (Figure 4.1). Despite same food intake in groups, food density of chow and HO diet was 3.1 Kcal/g and 3.8 Kcal/g, respectively (Table 4.1B) which suggested that mice on HO diet consumed more calories than control. After normalization to the body weight, weights of liver, adipose tissues and thymus were increased while brain, heart, kidney, pancreas, lung and spleen were decreased with the treatment of HO diet compared to chow diet. Different

from epidemiological findings that high olive oil intake (high oleic acid intake) is associated positively with weight loss, this HO diet consumption induced obesity by increasing body weight, liver and adipose tissue mass. These results were not in agreement with previous observation that compared to n-6 rich safflower oil, 12 week treatment of iso-energetic high oleic safflower oil diet did not alter body and adipose tissue weight [23].

Pups from HO-fed dam accumulated double amount of oleic acid in liver and heart compared to Chow-fed offspring. However the oleic acid percentage of total fatty acid was highly conserved in brain across all the tested ages (Figure 4.4). Due to 18:1n-9 served as a precursor of 20:3n-9, we found the quantities of 20:3n-9 in all three tissues at all the time points were significantly higher than chow group as expected (Figure 4.8). The triene/tetraene ratio (T/T ratio; 20:3n-9/20:4n-6) in whole plasma higher than 0.2 is considered as essential fatty acid deficiency (EFAD) [24]. Blood was scarce from neonatal mice therefore in this study we only collected and analyzed the T/T ratio in tissues. In supplementary Table 4.7, though heart and brain T/T ratios in HO group were much higher than Chow group, only livers in HO group had the T/T ratios greater than 0.2. A well-known sign of EFAD is severe dermal lesion [25] which was not observed even in mice fed by HO-diet for one year. Another symptom of EFAD is retarded growth [26, 27] which was opposite to body weight of HO-fed mice. It's believed that the diet with less than 0.5% of energy from linoleic acid is considered as EFAD-diet [27]. In HO diet, LA accounted for around 1% of energy (17% of energy

coming from fat and LA is around 6.5% of total fat in table 4.1A). Thus, the HO diet used in this study was not EFAD diet but the fatty acid profiles of tissues were similar to EFAD diet which had increased levels of 18:1n-9 and 20:3n-9 compared to control diet [26].

In addition, blood n-3 index (20:5n-3+22:6n-3) was frequently used as a biomarker for CVD risk. The n-3 index lower than 4% of total fatty acids suggests a high risk for developing CVD [28]. Heart n-3 index influenced by HO diet was increasing during development but hepatic n-3 index was below 4% at age of one year in HO group (Supplementary Figure 4.4).

Saturated fatty acid fractions of total fatty acids in heart and brain were not affected dramatically by dietary fatty acid profiles. Starting from adulthood, the concentrations of 16:0 and 18:0 in HO liver dropped to half of that in Chow liver. It might result from elevated expression of stearoyl-CoA desaturase (SCD) catalyzing conversion of SFA to MUFA with aging [29]. The present study was consistent to this finding that the activity of SCD (16:1/16:0) was increasing with development (Table 4.2).

As mentioned previously, mammals can only utilize polyunsaturated fatty acids longer than 18 carbons as precursors to synthesize other downstream fatty acid species [30]. Thus, tissue levels of linoleic acid (LA) greatly depend on the abundance of LA in the diet. In all tissues at any detected point, the amount of LA was significantly lower in HO-fed mice (Figure4.7A, C, E) due to the limited quantity of PUFA in the designed diet. The lower levels of precursor LA in liver and heart led to lower concentrations

of downstream product arachidonic acid (ARA, 20:4n-6). Human brain accretes LCPUFA (such as ARA, EPA and DHA) during first two years and maintains relatively constant amount during the rest of life [31]. Figure 4.6F showed that during early development when ARA was in a great need for neurocognitive development [32], brain ARA in HO diet was accreted from P1 to P13 and then maintained that level till the age of one year. In the chow group, brain 20:4n-6 was higher during “infancy and adolescence” compared to HO group; however, starting from adulthood (3 month old) their brain ARA dropped from 11.85±0.53% at P30 to 8.86±0.76% at P90, were significantly lower than HO group (Supplementary Table 4.3, Figure 4.6F). The mRNA expressions of brain *Fads1* and *Fads2* coding two key enzymes in LCPUFA biosynthesis were not different between two groups (Figure 4.9E, 4.9F). It suggests that HO diet did not have impact on the activity of desaturation to make 20:4n-6 in brain. Though brain can biosynthesize LCPUFA, the capability is so limited that majority of LCPUFA in brain is transported from liver [33]. It partially explains that in HO diet liver expression of *Fads1* and *Fads2* were upregulated at P13 and P21 (Figure 4.9A, 4.9 B; Fads1 at P21 did not reach statistical significance) but the product of precursors 20:4n-6 was significantly lower compared to Chow group (Figure 4.6B).The synthesized hepatic 20:4n-6 may transported to brain for maintaining its proper function.

Interestingly, the present study demonstrated that with HO intervention, the n-3 LCPUFAs, particularly 20:5n-3 in liver and heart was several fold higher than those in Chow group (Figure 4.7A and 4.7C) which was contrast to

the decreased amount of n-6 LCPUFA in HO group. In LCPUFA biosynthesis pathway, FADS1 and FADS2 compete desaturases for producing n-3 and n-6 LCPUFA but these desaturases prefer using n-3 PUFA to n-6 PUFAs [30]. Therefore, when the quantity of LA and ALA was low in HO diet, the desaturases were used to catalyze conversion of ALA to n-3 products, making significantly higher amount of DHA in Heart. Like brain ARA, brain DHA *de novo* synthesis efficiency is low and it has to be transported from liver to meet physiological requirement [34]. Our results are consistent with this statement that the amount of hepatic DHA was significantly lower at P21, P90 and P365 while the amount in brain in significantly higher than Chow-fed mice (Figure 4.7B and 4.7F). Literature shows that EPA and DHA are cardioprotective nutrients inversely associated with risk of CVD and cardiac death [35, 36]. It is the first time to present that with high oleic intervention, levels of EPA and DHA in heart were significantly elevated (Figure 4.7C and 4.7D) despite downregulated heart *Fads1* and *Fads2* expression at P7 and P13 compared to Chow group (Figure 4.9C and 4.9D).

Though n-3 LCPUFA production and transportation were stimulated by HO diet, the enormous deposit of fat observed in HO-fed liver brought the concern if high oleic oil is safe or even beneficial as it is claimed? In this *in vivo* study, HO-induced steatosis was consistent with previous *in vitro* study that oleic acid accelerates lipid peroxidation and apoptosis via elevation of tumor necrosis factor alpha and suppression of peroxisome proliferator-activated receptors (PPARs) in HepG2 cells [37].

The interesting findings about the increased fractions of beneficial n-3 LCPUFA in brain and heart but heavier body weight, liver and adipose tissue mass with the influence of high oleic sunflower oil triggered the action to detect the systematic inflammation status, insulin sensitivity, levels of CVD risk factors (LDL-c, HDL-c, triglyceride, total cholesterols, etc) and fatty acid profiles in specific lipid species in the future. In addition expression levels of genes and proteins involved in fatty acid and lipid metabolisms, for instance, SCD, sterol regulatory element-binding proteins, PPARs, are needed to further investigate to advance the knowledge of LCPUFA biosynthesis regulation.

4.5 CONCLUSION

It is the first study investigating the liver, brain and heart fatty acid profiles in both rodent chow (rich in soybean oil) and designed diet (rich in high oleic sunflower oil) from birth through adulthood to aging. With the high oleic sunflower oil treatment, tissue fatty acid profiles of *Fads3* KO mice has some differences compared to age-matched WT at P1 and P13 but the differences are not significant with growth suggesting that *Fads3* regulates LCPUFA biosynthesis only at every early stage of life. Mice with HO diet have higher body weight, heavier liver and adipose tissue, fatty liver, but higher amount of beneficial n-3 fatty acids in heart and brain compared to Chow group. The longevity is not impaired by this HO diet but more physiological parameters and fatty acid profiles in lipid classes are needed in order to better understand the impact of oleic acid on lipid metabolisms and health outcome.

Acknowledgements

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CHAPTER 5

SUMMARY

Here we presented the characteristics of the first *Fads3* knockout (KO) mouse colony. No difference in overt phenotypes (survival, growth rate, physical appearance, food intake, metabolic rate) was observed between genotypes. On biochemical level, KO had lower brain DHA than WT on postnatal day 1. KO hepatic expression levels of other desaturase genes (*Fads1* and *Fads2*) were downregulated while the levels of elongase genes (*Elovl2* and *Elovl5*) were upregulated during perinatal age. These suggested that *Fads3* regulated with LCPUFA biosynthesis via interactions with other desaturases and elongases.

Besides eight previously found alternative transcripts of *FADS3*, we discovered another novel alternative transcript (called AT9) in mouse with a complete miss of exon 2. AT9, as well as its classical transcript (CS) was ubiquitously expressed in all the 11 tested mouse tissues. Surprisingly, pancreatic AT9 expression was 10 fold abundant than its CS. Concomitantly, pancreas had highest amount of 20:4n-6 and total n-6 LCPUFA among all the tissues. It indicates that AT9 plays a role in LCPUFA regulation in pancreas, a non-traditional lipid metabolic organ.

Due to the unbalanced n-6/n-3 diet in western diet and the popularity of high oleic oil in market, we treated mice with a high oleic sunflower oil diet to

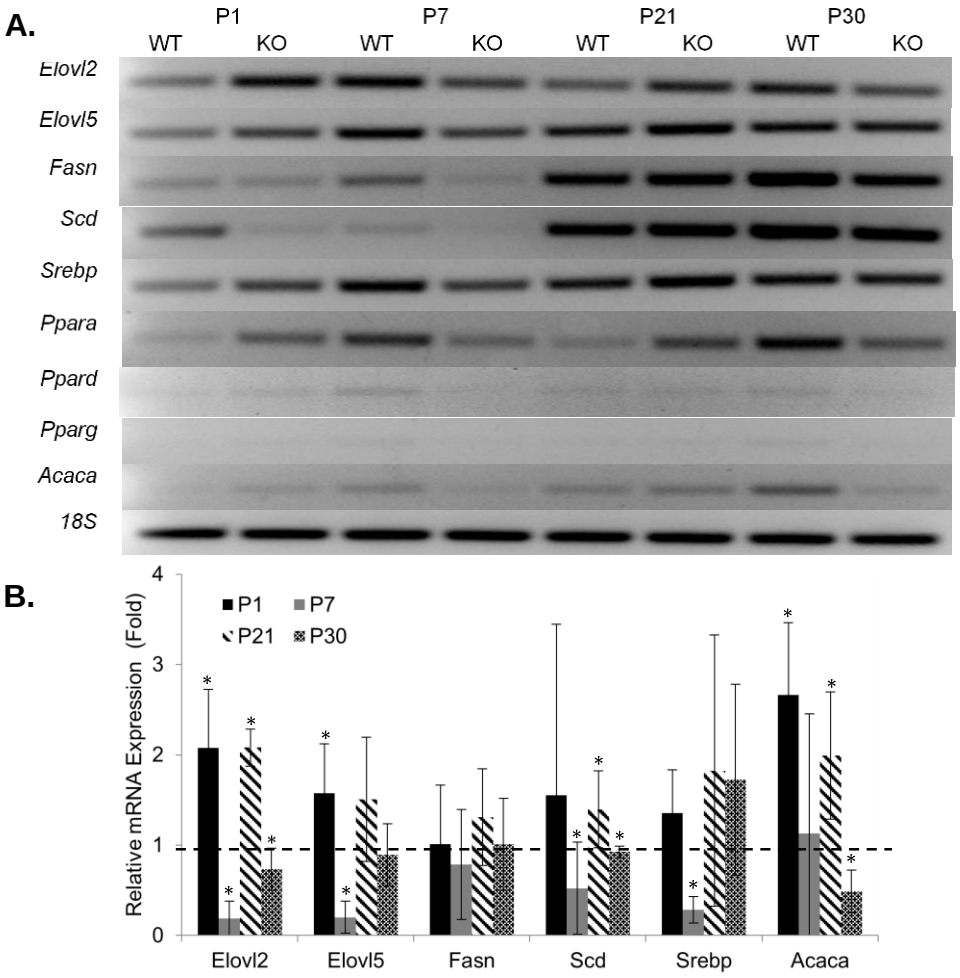
investigate the function of *Fads3* in a condition lack of PUFA precursor. Fads3 did not contribute significantly to LCPUFA biosynthesis under a PUFA-limited condition. Interestingly, we found that mice fed with HO diet had higher n-3 LCPUFA in brain and heart but they developed fatty livers regardless of their genotypes and body weights. The cause of these phenomena was not clear. We speculated that with limited PUFA precursor n-3 LCPUFA biosynthesis was more efficient than n-6 side since the desaturases they competed for preferred n-3 to n-6 PUFA to catalyze the reaction of introducing double bond in carbon chain. In respect of fatty liver, we projected that the HO diet contained some uncertain substances different from the control diet leading to fatty liver since the calorie from fat was well controlled between diets. Literature also suggests that oleic acid is the precursor of mead acid, which is one of risk factors for steatosis.

In the future, application of isotope labeled fatty acid to KO will reveal the impact of *Fads3* on *de novo* LCPUFA synthesis. Since AT9 expressed more abundant than CS in pancreas and pancreas had highest amount of n-6 PUFA, we want to investigate pancreatic primary cells and treat them with different substance so as to find AT9's target. We can also co-transfect KO pancreatic cells with *Fads1*(ATs) or *Fads2*(ATs) to detect interactions between *Fads* genes. More functions of Fads3 will be explored in those preclinical models. Mice fed with HO did not have impaired growth, skin conditions and longevity which indicated that those mice were generally healthy despite overweight with fatty liver. Health conditions, such as inflammation status (IL-

6, NOS, etc) and insulin resistance, should be measured to dig into the mechanism of HO diet on fatty liver development.

SUPPLEMENTARY TABLES AND FIGURES

Supplementary Tables and Figures from Chapter 2



Supplementary Figure 2.1 Liver Ontogeny of expression of genes involved in lipid metabolism.

(A) mRNA expression levels were tested by semi-quantitative reverse transcript PCR, ran on 2% agarose gels and visualized under UV light. (B) Determination of hepatic mRNA expressions of *Elovl2, Elovl5*, *Fasn, Scd, Srebp-1c* and *Acaca* using Image J software. Relative mRNA levels of KO mice were normalized to age-matched WT (represented as the dash line). KO had increased hepatic *Elovl2*, *Elovl5* and *Acaca* on P1 than WT. Values were Mean ± SD (n=4) and **P*<0.05.

Supplemental Table 2.1 Primer sequences.

Primers used for genotyping

Name of Primer	Forward	Reverse	Annealing Temp.	Amplification Cycles
Genotype-Primer 1	5'-CACACCTCCCCCTGAACCTGAAA-3'	5'-GAGAGACGACACAGTGGATCAGAGAG-3'	65	40
Genotype-Primer 2	5'-TGATGAAGCAGACAGGGCATGGTA-3'	5'-GGGCTAGCTCTCCAATCAACA-3'	65	40

Primers used for quantitative real time PCR (conditions are mentioned in the methods section)

Name of Primer	Forward	Reverse
Fads1 classic transcript	5'-TACCTGCTTCACATCCTGCT-3'	5'-TACCTGCTTCACATCCTGCT-3'
Fads2 classic transcript	5'-AGGCCCAAGCTGGATGGCTGC-3'	5'-AGTTGGCTGAGGCACCCTTTA-3'
Gapdh	5'-ATGTCGTGGAGTCTACTGGTGT-3'	5'-TCGTGGTTCACACCCATCACAA-3'
Actb	5'-ATGACGATATCGCTGCGCTGGT-3'	5'-ACATAGGAGTCCTTCTGACCCA-3'

Primers used for semi-quantitative reverse transcription PCR

Name of Primer	Forward	Reverse	Annealing Temp.	Amplification Cycles
Elovl 2	5'-AGCTGGGAAGGAGGTTACAA-3'	5'-TGGAGAAGTAGTACCACCACAA-3'	57	25
Elovl5	5'-GGTGTGTGGGAAGGCAAATA-3'	5'-TGGAGAAGTAGTACCACCAGAG-3'	57	25
Fasn	5'-GGCTCTCTTTCTTCTTCGACTTCA-3'	5'-AGGTTGATCCCACCCACAAG-3'	Touch down: 72 °C 4 cycles; 70°C 4 cycles; 68°C 4 cycles; 66°C 18 cycles.	
Scd	5'-ACCCGGCTGTCAAAGAGAAG-3'	5'-GATGAAGCACATCAGCAGGAG-3'	59	25
Srebp-1C	5'-CTGTGAAGACAGATGCAGGAG-3'	5'-AAGGTACTGTGGCCAAGATG-3'	Touch down: 72 °C 4 cycles; 70°C 4 cycles; 68°C 4 cycles; 66°C 18 cycles.	
Ppara	5'-AAGGCCTCAGGGTACCACTAC-3'	5'-GCAGCTCCGATCACACTTGTC-3'	62	25
Ppard	5'-CAGCCTCAACATGGAATGTC-3'	5'-TCCGATCGCACTTCTCATAC-3'	57	25
Pparg	5'-AAGCATCAGGCTTCCACTATG-3'	5'-TTGTGGATCCGGCAGTTAAG-3'	59	25
Acaca	5'-CCTGGAGGACCCAACAACAA-3'	5'-TTTCGGGTTCTCAGAGGCAT-3'	59	25
18S	5'-GCTACCACATCCAAGGAAGG-3'	5'-CAATTACAGGGCCTCGAAAGA-3'	59	25

Supplemental Table 2.2 Brain Fatty Acid Ontogeny

Name of FA	P1		P3		P7		P13		P21		P30	
	WT	KO	WT	KO	WT	KO	WT	KO	WT	KO	WT	KO
Total SFA	**42.95 ±0.68**	**46.80±1.70***	**48.93 ±3.81**	**47.46 ± 1.94**	**47.71 ± 3.28**	**49.42 ± 3.15**	**49.91 ± 2.58**	**48.63 ±3.22**	**48.27 ± 1.88**	**50.05 ±2.03***	**47.42 ± 1.62**	**46.41 ± 0.72**
14:0	2.21 ± 0.13	2.24 ± 0.24	3.39 ± 1.01	2.71 ± 0.52 *	2.35 ± 0.52	2.59 ± 0.51	1.33 ± 0.13	1.34 ± 0.38	0.72 ± 0.12	0.65 ± 0.11	0.48 ± 0.12	0.40 ± 0.11
15:0	0.10 ± 0.01	0.11 ± 0.01*	0.14 ± 0.07	0.20 ± 0.12 *	0.11 ± 0.03	0.11 ± 0.03	0.13 ± 0.06	0.29 ±0.17*	0.10 ± 0.02	0.10 ± 0.02	0.09 ± 0.02	0.07 ± 0.01 *
16:0	25.19 ±0.74	28.27±1.39*	30.30 ±3.10	27.58 ±1.39*	27.76 ± 1.91	27.79 ± 2.17	27.25 ± 2.08	28.68 ±3.41	24.59 ± 1.76	26.59 ±1.93*	23.84 ± 1.19	23.17 ± 0.73
18:0	15.33 ±0.29	16.10±0.65*	15.14 ±0.52	16.96 ±0.92*	17.49 ± 1.20	18.93 ± 2.52	21.11 ± 0.77	18.08±1.20*	22.61 ± 1.06	22.33 ± 0.44	22.74 ± 0.61	22.64 ± 0.34
20:0	0.12 ± 0.05	0.07 ± 0.03*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.15 ± 0.06	0.27 ±0.08 *	0.34 ± 0.17	0.38 ± 0.17	0.44 ± 0.14	0.37 ± 0.03
Total MUFA	**23.48 ±1.33**	**23.51 ±1.15**	**18.69 ±0.83**	**19.65 ± 1.17***	**19.33 ± 1.57**	**19.33 ± 1.37**	**17.78 ± 2.32**	**18.08 ±2.20**	**18.60 ± 2.55**	**19.14 ± 1.43**	**18.34 ± 1.23**	**18.38 ± 0.80**
16:1n-9	2.86 ± 0.26	2.88 ± 0.17	2.43 ± 0.28	2.40 ± 0.31	2.00 ± 0.23	1.97 ± 0.26	1.16 ± 0.23	1.13 ± 0.18	0.55 ± 0.06	0.51 ± 0.08	0.33 ± 0.04	0.32 ± 0.03
16:1n-7	2.60 ± 0.37	2.61 ± 0.19	2.61 ± 0.50	2.43 ± 0.27	2.20 ± 0.41	2.20 ± 0.25	1.17 ± 0.07	1.28 ± 0.24	0.79 ± 0.13	0.84 ± 0.09	0.72 ± 0.18	0.63 ± 0.04
18:1n-9	14.36 ±0.86	14.42 ±0.78	10.79 ±0.95	11.82 ±1.07*	11.99 ± 1.47	12.11 ± 1.53	12.62 ± 2.03	13.85 ±1.83	13.44 ± 2.01	13.76 ± 1.13	13.21 ± 1.03	14.07 ± 0.74
18:1n-7	3.40 ± 0.17	3.38 ± 0.22	2.49 ± 0.19	2.62 ± 0.22	2.65 ± 0.49	2.67 ± 0.29	2.87 ± 0.51	2.79 ± 0.99	3.21 ± 0.50	3.19 ± 0.28	3.25 ± 0.19	2.87 ± 0.43
20:1n-9	0.05 ± 0.02	0.04 ± 0.02	0.26 ± 0.09	0.25 ± 0.05	0.35 ± 0.15	0.27 ± 0.11	0.35 ± 0.16	0.35 ± 0.12	0.48 ± 0.14	0.54 ± 0.15	0.42 ± 0.19	0.33 ± 0.23
22:1n-9	0.08 ± 0.03	0.07 ± 0.02	0.07 ± 0.01	0.10 ± 0.03 *	0.10 ± 0.06	0.08 ± 0.02	0.08 ± 0.02	0.25 ± 0.11*	0.07 ± 0.04	0.13 ± 0.08	0.07 ± 0.03	0.07 ± 0.03
24:1n-9	0.14 ± 0.03	0.12 ± 0.04	0.08 ± 0.01	0.07 ± 0.03	0.05 ± 0.02	0.04 ± 0.02	0.05 ± 0.02	0.07 ± 0.03	0.06 ± 0.03	0.17 ± 0.06 *	0.39 ± 0.15	0.11 ± 0.07 *
Total n-6 PUFA	**18.83 ±1.26**	**17.66±1.15***	**17.51 ±1.54**	**18.48 ± 1.40**	**19.07 ± 1.71**	**19.60 ± 1.36**	**18.37 ± 1.25**	**18.97 ±2.01**	**18.35 ± 1.52**	**16.82 ± 1.17**	**16.89 ± 0.54**	**17.03 ± 0.80**
18:2n-6	0.74 ± 0.18	0.96 ± 0.13*	1.25 ± 0.21	1.39 ± 0.48	1.27 ± 0.18	1.30 ± 0.19	1.31 ± 0.31	1.47 ± 0.30	1.12 ± 0.29	1.13 ± 0.25	0.85 ± 0.09	0.97 ± 0.10 *
20:2n-6	0.05 ± 0.01	0.04 ± 0.01*	0.04 ± 0.01	0.04 ± 0.02	0.04 ± 0.02	0.03 ± 0.01	0.07 ± 0.03	0.32 ± 0.15*	0.37 ± 0.14	0.33 ± 0.08	0.19 ± 0.02	0.29 ± 0.07 *
20:3n-6	0.40 ± 0.05	0.40 ± 0.04	0.58 ± 0.08	0.59 ± 0.11	0.78 ± 0.14	0.74 ± 0.10	0.68 ± 0.19	0.66 ± 0.22	0.61 ± 0.19	0.53 ± 0.10	0.43 ± 0.05	0.48 ± 0.05
20:4n-6	11.95 ±0.61	11.32±0.54*	11.19 ±1.15	12.00 ± 1.24	12.83 ±1.30	13.66 ± 1.00	12.44 ± 1.30	11.93 ±1.30	12.67 ± 1.51	11.85 ± 0.79	11.85 ± 0.54	11.99 ± 0.53
22:4n-6	3.57 ± 0.27	3.08 ± 0.41*	3.03 ± 0.52	3.00 ± 0.33	3.05 ± 0.48	2.77 ± 0.50	2.96 ± 0.45	3.61 ± 0.95	2.88 ± 0.30	2.39 ± 0.36 *	2.66 ± 0.20	2.70 ± 0.29
22:5n-6	2.12 ± 0.51	1.87 ± 0.47	1.43 ± 0.17	1.47 ± 0.27	1.09 ± 0.12	1.09 ± 0.31	0.93 ± 0.25	0.97 ± 0.31	0.68 ± 0.16	0.58 ± 0.14	0.91 ± 0.14	0.60 ± 0.06 *
Total n-3 PUFA	**13.24 ±1.21**	**10.73±1.03***	**13.61 ±2.66**	**13.29 ± 1.32**	**13.02 ± 1.18**	**10.94 ± 2.00***	**13.29 ± 1.68**	**13.41 ±1.27**	**14.26 ± 1.18**	**13.67 ± 1.06**	**16.89 ± 1.98**	**17.85 ± 0.86**
20:5n-3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
22:5n-3	0.27 ± 0.04	0.22 ± 0.04*	0.47 ± 0.08	0.51 ± 0.17	0.52 ± 0.22	0.37 ± 0.18	0.35 ± 0.10	0.30 ± 0.08	0.29 ± 0.11	0.25 ± 0.08	0.30 ± 0.15	0.51 ± 0.18 *
22:6n-3	12.97 ±1.19	10.52±1.03*	13.14 ±2.60	12.78 ± 1.32	12.50 ±1.25	10.57 ± 1.86*	12.95 ± 1.65	13.22 ±1.28	13.97 ± 1.18	13.42 ± 1.02	16.60 ± 1.90	17.34 ± 0.81
Total n-9 PUFA	**1.30 ± 0.23**	**1.10 ± 0.30**	**0.96 ± 0.32**	**0.81 ± 0.18**	**0.59 ± 0.15**	**0.49 ± 0.21**	**0.50 ± 0.12**	**0.51 ± 0.28**	**0.22 ± 0.11**	**0.14 ± 0.05 ***	**0.25 ± 0.09**	**0.12 ± 0.04 ***
20:2n-9	0.26 ± 0.15	0.32 ± 0.21	0.11 ± 0.03	0.14 ±0.07 *	0.00 ± 0.00	0.00 ± 0.00	0.06 ± 0.01	0.15 ± 0.07*	0.14 ± 0.06	0.09 ± 0.03 *	0.08 ± 0.02	0.08 ± 0.02
20:3n-9	0.73 ± 0.13	0.52 ± 0.10*	0.52 ± 0.27	0.40 ± 0.12	0.44 ± 0.10	0.36 ± 0.17	0.45 ± 0.13	0.22 ± 0.07*	0.06 ± 0.02	0.04 ± 0.01	0.11 ± 0.05	0.05 ± 0.02 *
22:3n-9	0.31 ± 0.06	0.26 ± 0.05*	0.37 ± 0.13	0.27 ± 0.10 *	0.15 ± 0.05	0.13 ± 0.10	0.11 ± 0.07	0.32 ± 0.08*	0.12 ± 0.07	0.04 ± 0.01 *	0.09 ± 0.05	0.03 ± 0.02 *

Data are Mean ± SD (n=14). * represents *P*<0.05

Supplemental Table 2.3 Liver Fatty Acid Ontogeny

Name of FA	P1		P3		P7		P13		P21		P30	
	WT	KO	WT	KO	WT	KO	WT	KO	WT	KO	WT	KO
Total SFA	**42.12 ± 4.99**	**39.48 ± 5.52**	**40.52 ± 3.28**	**37.91±3.06***	**44.82 ±4.48**	**41.88 ± 4.90**	**41.79 ± 2.60**	**41.18 ± 3.38**	**37.54 ± 2.96**	**37.68 ± 2.07**	**38.80 ± 4.43**	**39.96 ± 2.22**
14:0	0.46 ± 0.15	0.78 ± 0.17 *	2.21 ± 0.27	2.42 ± 0.36	2.80 ± 0.54	3.05 ± 0.58	1.22 ± 0.25	1.53 ± 0.30 *	0.87 ± 0.40	0.56 ± 0.20 *	0.54 ± 0.23	0.34 ± 0.11 *
15:0	0.12 ± 0.05	0.13 ± 0.06	0.07 ± 0.01	0.10 ± 0.05*	0.09 ± 0.03	0.07 ± 0.02	0.10 ± 0.08	0.08 ± 0.02	0.17 ± 0.06	0.13 ± 0.02 *	0.11 ± 0.04	0.11 ± 0.02
16:0	24.62 ± 1.75	25.54 ± 1.46	27.10 ± 2.26	25.88 ±1.68	28.99 ±2.67	26.56 ± 3.55	25.74 ± 1.82	24.66 ± 1.93	22.73 ± 1.66	22.78 ± 1.37	22.84 ± 2.37	22.86 ± 1.63
18:0	16.32 ± 4.77	12.33 ±4.41*	10.94 ± 1.24	9.26 ± 1.96*	12.73 ±2.00	12.03 ± 1.57	14.51 ± 1.28	14.75 ± 1.74	13.70 ± 1.55	14.14 ± 0.85	15.10 ± 2.62	16.50 ± 1.07
20:0	0.11 ± 0.05	0.14 ± 0.05	0.18 ± 0.09	0.22 ± 0.10	0.18 ± 0.10	0.14 ± 0.07	0.18 ± 0.10	0.14 ± 0.12	0.11 ± 0.07	0.10 ± 0.05	0.21 ± 0.08	0.15 ± 0.07 *
24:0	0.51 ± 0.21	0.64 ± 0.24	0.07 ± 0.04	0.07 ± 0.03	0.07 ± 0.01	0.09 ± 0.03	0.09 ± 0.02	0.09 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Total MUFA	**25.26 ± 3.26**	**22.53 ±3.93***	**18.77 ± 1.60**	**17.89 ±1.63**	**16.30 ±1.37**	**17.94 ±2.11***	**9.07 ± 0.95**	**8.34 ± 1.46**	**14.69 ± 2.08**	**16.14 ± 2.89**	**18.60 ± 2.19**	**18.39 ± 1.89**
14:1	0.07 ± 0.04	0.10 ± 0.04	0.10 ± 0.02	0.14 ± 0.05*	0.07 ± 0.02	0.11 ± 0.04 *	0.04 ± 0.03	0.03 ± 0.01	0.09 ± 0.07	0.04 ± 0.01	0.04 ± 0.02	0.04 ± 0.03
16:1n-9	0.57 ± 0.12	0.52 ± 0.19	0.30 ± 0.07	0.32 ± 0.05	0.31 ± 0.06	0.31 ± 0.08	0.21 ± 0.05	0.14 ± 0.04 *	0.29 ± 0.09	0.38 ± 0.11 *	0.45 ± 0.09	0.44 ± 0.09
16:1n-7	1.61 ± 0.43	1.92 ± 0.63	2.02 ± 0.26	2.08 ± 0.22	0.92 ± 0.22	1.20 ± 0.30 *	0.34 ± 0.07	0.33 ± 0.05	1.00 ± 0.59	0.89 ± 0.39	1.44 ± 0.33	1.39 ± 0.27
18:1n-9	18.62 ± 2.83	17.29 ± 3.32	13.70 ± 1.39	13.28 ±1.07	12.15 ±1.27	14.05 ±1.57*	6.85 ± 0.87	6.43 ± 1.45	11.27 ± 1.54	12.67 ± 2.29	14.00 ± 1.58	15.58 ±1.36*
18:1n-7	3.78 ± 0.95	2.55 ± 0.56 *	1.83 ± 0.30	1.60 ± 0.23*	1.82 ± 0.21	1.59 ± 0.80	1.19 ± 0.18	1.00 ± 0.17 *	1.54 ± 0.39	1.34 ± 0.35	1.93 ± 0.45	1.07 ± 0.26 *
20:1n-9	0.21 ± 0.04	0.25 ± 0.07	0.24 ± 0.13	0.23 ± 0.11	0.36 ± 0.15	0.26 ± 0.08	0.31 ± 0.08	0.22 ± 0.11 *	0.24 ± 0.11	0.30 ± 0.09	0.30 ± 0.09	0.11 ± 0.05 *
22:1n-9	0.34 ± 0.12	0.36 ± 0.20	0.41 ± 0.12	0.52 ± 0.17*	0.38 ± 0.09	0.28 ± 0.10 *	0.16 ± 0.08	0.22 ± 0.12	0.54 ± 0.25	0.52 ± 0.29	0.31 ± 0.20	0.21 ± 0.08
24:1n-9	0.13 ± 0.04	0.13 ± 0.07	0.09 ± 0.06	0.03 ± 0.02*	0.29 ± 0.22	0.14 ± 0.04 *	0.09 ± 0.03	0.11 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.27 ± 0.20	0.16 ± 0.06
Total n-6 PUFA	**24.96 ± 4.51**	**30.19 ±5.36***	**32.72 ± 2.33**	**34.55±1.78***	**32.22 ±2.70**	**33.40 ± 4.01**	**36.08 ± 1.81**	**37.24 ± 1.38**	**36.94 ± 2.44**	**36.65 ± 1.93**	**35.29 ± 2.60**	**36.93 ± 2.57**
18:2n-6	12.05 ± 4.63	18.23 ±4.37*	18.05 ± 0.92	19.56±1.45*	19.70 ±1.12	21.77 ±2.17*	16.94 ± 1.64	18.55 ±1.96*	16.99 ± 1.44	17.83 ± 0.82	18.63 ± 1.21	19.25 ± 1.01
18:3n-6	0.41 ± 0.27	1.37 ± 0.52 *	0.97 ± 0.24	1.01 ± 0.25	0.62 ± 0.15	0.68 ± 0.18	0.40 ± 0.12	0.30 ± 0.11 *	0.45 ± 0.11	0.42 ± 0.13	0.34 ± 0.10	0.36 ± 0.07
20:2n-6	0.62 ± 0.24	0.57 ± 0.15	0.66 ± 0.05	0.71 ± 0.14	0.91 ± 0.07	0.89 ± 0.09	0.98 ± 0.12	1.01 ± 0.14	0.68 ± 0.17	0.75 ± 0.13	0.49 ± 0.09	0.43 ± 0.06
20:3n-6	0.92 ± 0.25	0.69 ± 0.15 *	1.11 ± 0.11	1.17 ± 0.08	1.05 ± 0.18	1.02 ± 0.28	1.15 ± 0.15	1.13 ± 0.08	1.09 ± 0.11	1.05 ± 0.13	1.21 ± 0.13	0.74 ± 0.59 *
20:4n-6	10.18 ± 1.49	8.19 ± 2.36 *	10.88 ± 1.68	10.94 ±1.71	8.87 ± 1.94	8.06 ± 1.93	15.28 ± 1.51	14.97 ± 1.11	16.40 ± 1.23	15.71 ± 1.76	13.68 ± 2.54	15.64 ±2.29*
22:4n-6	0.70 ± 0.25	1.08 ± 0.43 *	0.67 ± 0.08	0.86 ± 0.28*	0.72 ± 0.19	0.73 ± 0.22	0.82 ± 0.18	0.79 ± 0.15	0.66 ± 0.16	0.54 ± 0.07 *	0.48 ± 0.14	0.35 ± 0.08 *
22:5n-6	0.08 ± 0.04	0.12 ± 0.09	0.39 ± 0.14	0.32 ± 0.07	0.33 ± 0.15	0.27 ± 0.10	0.52 ± 0.12	0.49 ± 0.19	0.67 ± 0.35	0.35 ± 0.08 *	0.46 ± 0.21	0.17 ± 0.05 *
Total n-3 PUFA	**7.30 ± 1.80**	**7.55 ± 2.89**	**7.79 ± 2.00**	**9.48 ± 0.99***	**6.45 ± 2.10**	**6.65 ± 2.40**	**12.90 ± 2.57**	**13.11 ± 1.90**	**10.64 ± 1.35**	**9.46 ± 1.59**	**7.21 ± 2.28**	**5.04 ± 1.08 ***
18:3n-3	0.12 ± 0.09	0.68 ± 0.26 *	0.60 ± 0.12	0.81 ± 0.12*	0.48 ± 0.10	0.59 ± 0.19	0.37 ± 0.11	0.45 ± 0.14	0.40 ± 0.10	0.44 ± 0.10	0.49 ± 0.14	0.55 ± 0.13
20:4n-3	0.04 ± 0.03	0.07 ± 0.05	0.48 ± 0.10	0.35 ± 0.11	0.40 ± 0.14	0.39 ± 0.22	0.37 ± 0.10	0.30 ± 0.15	0.09 ± 0.03	0.10 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
20:5n-3	0.14 ± 0.05	0.09 ± 0.04	0.13 ± 0.05	0.18 ± 0.10	0.16 ± 0.08	0.14 ± 0.07	0.11 ± 0.08	0.16 ± 0.12	0.40 ± 0.27	0.30 ± 0.22	0.12 ± 0.09	0.20 ± 0.17
22:5n-3	0.35 ± 0.20	0.84 ± 0.39 *	0.85 ± 0.23	1.15 ± 0.34*	0.60 ± 0.19	0.84 ± 0.34 *	0.76 ± 0.12	0.93 ± 0.16 *	0.67 ± 0.20	0.77 ± 0.17	0.62 ± 0.10	0.26 ± 0.08 *
22:6n-3	6.72 ± 1.76	6.01 ± 2.60	6.03 ± 1.62	7.16 ± 1.11*	5.09 ± 1.92	4.94 ± 2.14	11.44 ± 2.39	11.49 ± 1.81	9.17 ± 1.18	7.91 ± 1.69 *	5.99 ± 2.12	4.04 ± 0.90 *
Total n-9 PUFA	**0.24 ± 0.10**	**0.21 ± 0.07**	**0.06 ± 0.02**	**0.14 ± 0.02***	**0.11 ± 0.07**	**0.03 ± 0.02 ***	**0.07 ± 0.02**	**0.04 ± 0.01**	**0.09 ± 0.01**	**0.07 ± 0.03**	**0.10± 0.06**	**0.03 ± 0.02 ***
20:2n-9	0.13 ± 0.07	0.15 ± 0.09	0.06 ± 0.02	0.14 ± 0.02*	0.11 ± 0.07	0.03 ± 0.02 *	0.07 ± 0.02	0.04 ± 0.01	0.09 ± 0.01	0.07 ± 0.03	0.06 ± 0.02	0.02 ± 0.01 *
20:3n-9	0.13 ± 0.03	0.17 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.05	0.03 ± 0.02 *

Data are Mean ± SD (n=14). * represents *P*<0.05

Supplementary Tables and Figures from Chapter 3

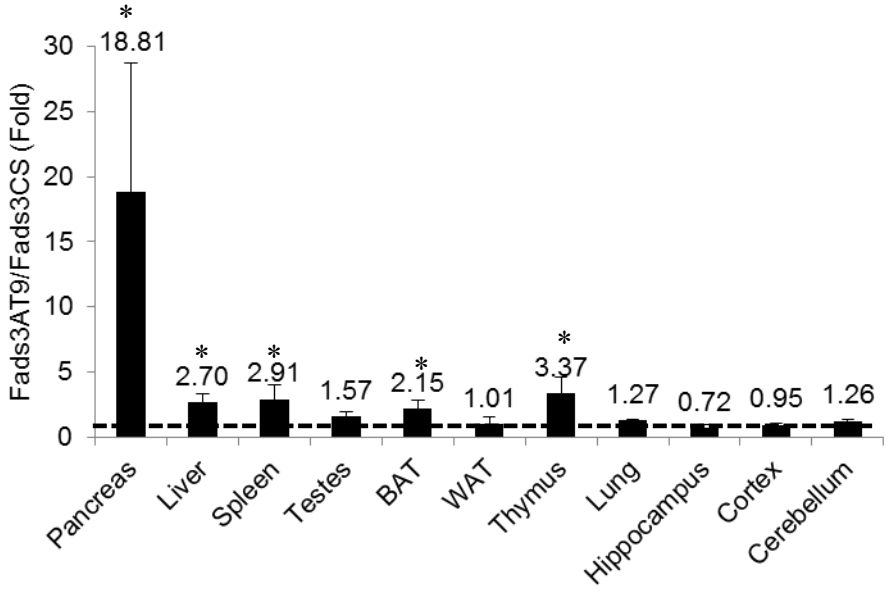
Supplementary Table 3.1 Details of culturing conditions of Brown and White cells

Adipocytes	**Brown**	**White** (3T3-L1 cell line)
Growth Media	DMEM + 20% FBS + 1% P/S	DMEM + 10%FBS + 1% P/S
Induction - Media	DMEM + 20% FBS + 1% P/S + 20 nM Insulin + 0.5 nM IBMX + 5 nM DXM + 1nM T3 + 0.125 mM indomethacin	DMEM + 10%FBS + 1% P/S + 1.7 µM Insulin + 0.25 mM IBMX + 1 µM DXM
Differentiation Media	DMEM + 20% FBS + 1% P/S + 20 nM Insulin + 1 nM T3	DMEM + 10%FBS + 1% P/S + 1.7 µM Insulin
	NOTE: DMEM; Dulbecco's modified Eagles' medium (4.5 g/L glucose & L-glutamine, Invitrogen, 11965-092) FBS; Fetal Bovine Serum P/S; Penicillin Streptomycin (10,000 U/ml, Invitrogen, 15140-122) IBMX; 3-isobutyl-1-methylxanthine (Calbio, 410957) DXM; Dexamethasone (Sigma, D4902) T3; 3,3',5-Triiodo-L-thyromine Indomethacin (Sigma, I7378)	

Supplementary Table 3.2 Details of primer sequences, annealing temperatures and amplification cycles used in the present study

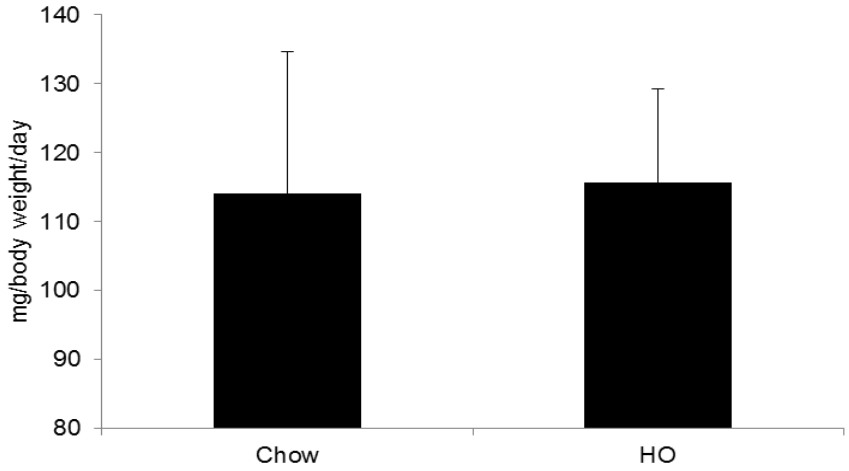
Name of Primer	Forward	Reverse	Annealing Temp. (°C)	Amplification Cycles
Fads3CS	CATGACCTACCAGGCGACAA	GGGCTAGCTCTCCAATCAACA	53	35
Fads3AT9	AGGACGCCACGGCCCAGCTGATCGA	GTAGATGATAAGCCAGGCCAACAA	70	35
18S	GCTACCACATCCAAGGAAGG	CAATTACAGGGCCTCGAAAGA	57	35

Supplementary Figure 3.1 Alignment of amino acid sequences of mice *FADS3CS* and *FADS3AT9* The alignment was constructed by using NCBI protein blast search <http://blast.ncbi.nlm.nih.gov/Blast.cgi>. “HPGG” characteristic of Cytochrome b5 (blue line) and three histidine motifs “HDLGH, HFQHH and QIEHH” (red lines) were conserved. *Fads3AT9* results from loss of exon 2. *FADS3AT9* encoded 412 aa protein with loss of 37 aa compared to *FADS3CS*.

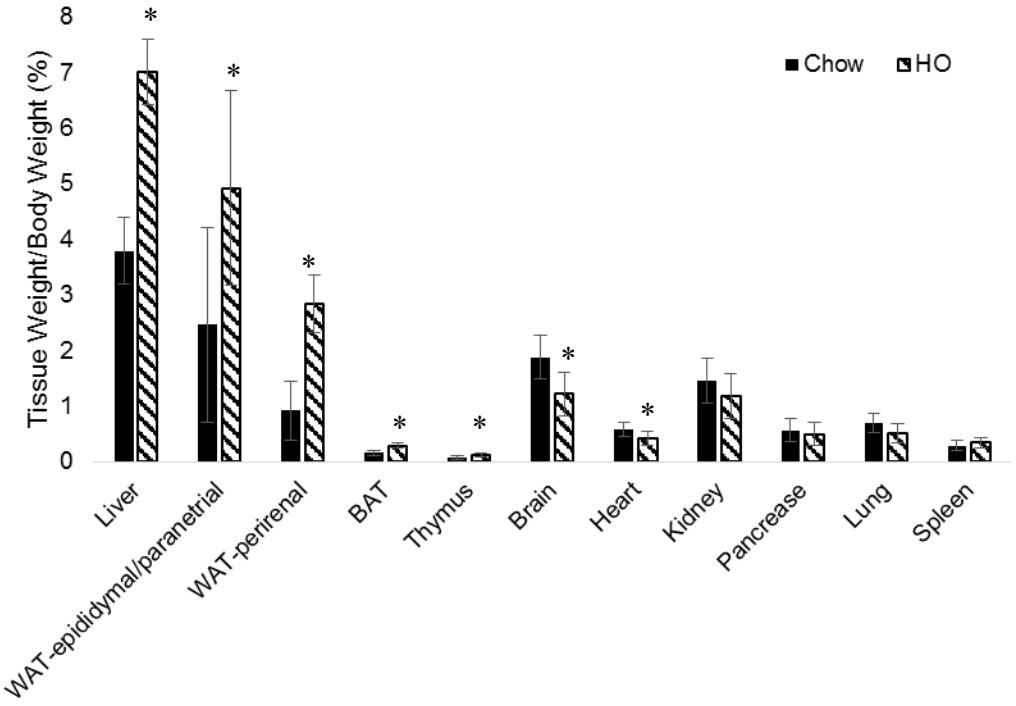


Supplementary Figure 3.2 Determination of mRNA expression of *Fads3AT9* in eleven tissues using Image J software. Relative mRNA levels of *Fads3CS* and *Fads3AT9* were normalized to reference gene 18S. mRNA expression of *Fads3CS* was regarded as "1" (dash line) and the change between CS and AT9 was measured by ratio of AT9/CS. Pancreases, spleen, brown adipose tissue and thymus had higher levels of *Fads3AT9* compared to *Fads3CS*. Data was expressed as Mean ± SD (n=4) and *represents P<0.05.

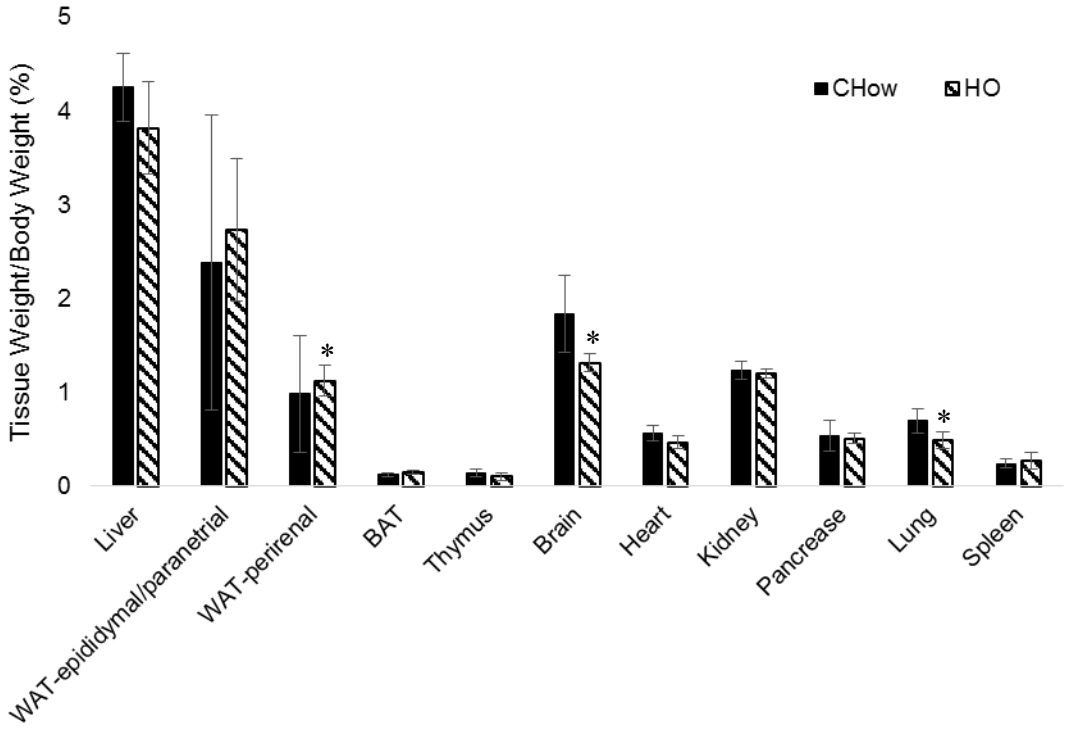
Supplementary Figures and Tables from Chapter 4



Supplementary Figure 4.1 Normalized food intake from diets (n=24). Daily food intake was normalized to body weight. No difference was observed between diets.

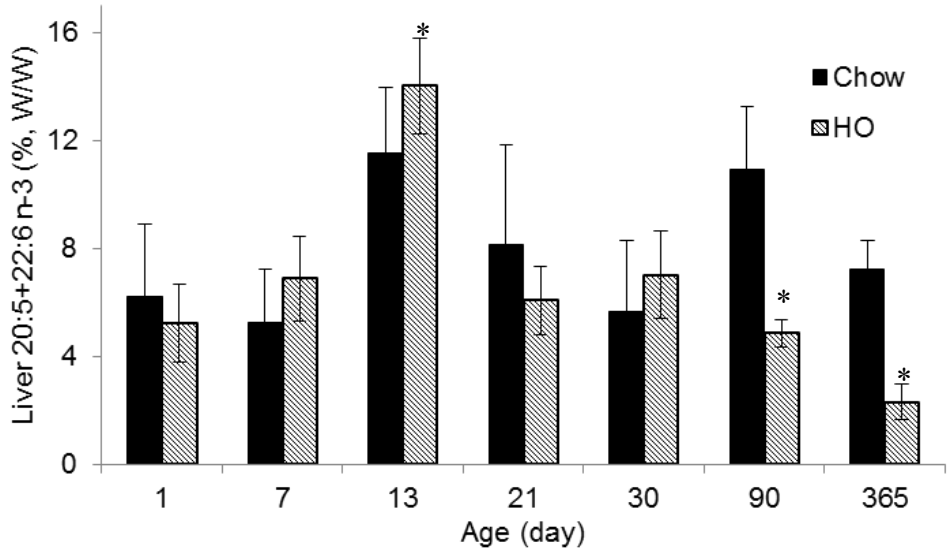


Supplementary Figure 4.2 Normalized tissue weights from Chow- and HO-fed KO mice at the age of one year (n=6). Tissue masses were normalized to total body weight to minimize the biological difference in body size. Solid bar represented chow-fed KO mice and the patched bar represented HO-fed KO mice. The differences between two diets were analyzed using Student's *t*-Test (* $P<0.05$). The weights of liver, adipose tissues, thymus were increased while brain and heart were decreased with the treatment of HO diet compared to chow diet. In comparison to chow diet, HO diet did not have impact on the relative masses of kidney, pancreas, lung and spleen in *Fads3* knockouts.

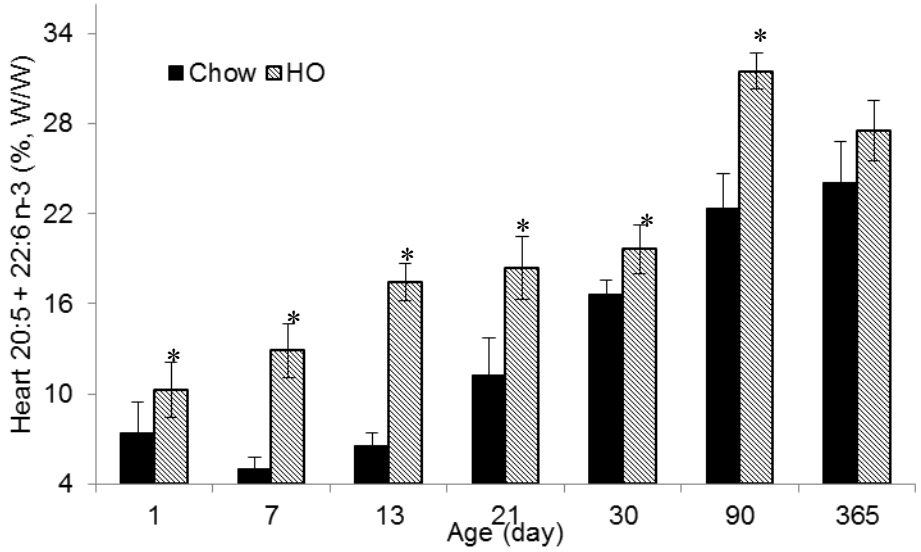


Supplementary Figure 4.3 Normalized tissue weights from Chow- and HO-fed KO mice at the age of three months (n=6). Tissue masses were normalized to total body weight to minimize the biological difference in body size. Solid bar represented chow-fed KO mice and the patched bar represented HO-fed KO mice. The differences between two diets were analyzed using Student's *t*-Test (* $P<0.05$). Epididymis white depot was increased while brain and lung were decreased in HO diet with the comparison of chow-diet fed KO mice.

A.



B.



Supplementary Figure 4.4 n-3 index from WT liver and heart (n=6). Solid bar represented chow-fed KO mice and the patched bar represented HO-fed KO mice. The differences between two diets were analyzed using Student's *t*-Test (* $P<0.05$). A) Hepatic n-3 index in HO group was higher at P13 but significantly lower at P90 and P365 compared to Chow group. At the age of one year, the n-3 index in HO liver was lower than 4%, which is considered as a high risk for CVD. B) Heart n-3 was gradually increased with the influence of HO diet.

Supplemental Table 4.1 Liver fatty acid profiles of WT mice fed by different diets.

	P1		P7		P13		P21	
	Chow	HO	Chow	HO	Chow	HO	Chow	HO
'14:0	0.43±0.16	0.82±0.27*	2.96±0.52	4.59±1.21*	1.30±0.29	3.76±0.54*	0.91±0.53	1.56±0.44*
'15:0	0.10±0.02	0.23±0.04*	0.08±0.03	0.08±0.01	0.07±0.03	0.12±0.02	0.17±0.07	0.13±0.02
'16:0	24.92±1.10	22.20±1.73*	29.23±2.22	23.99±1.65*	26.49±1.83	23.48±1.67*	22.66±1.93	22.86±1.65
'18:0	15.50±5.64	15.88±2.42	12.73±2.27	5.11±0.75*	14.45±1.57	10.77±1.29*	13.46±0.62	9.36±0.85*
'20:0	0.10±0.03	0.17±0.04*	0.15±0.08	0.05±0.04*	0.21±0.12	0.12±0.02	0.07±0.04	0.11±0.03
Total SFA	**41.60±5.97**	**39.79±3.90**	**45.23±3.89**	**35.91±2.84***	**42.59±2.55**	**40.78±1.83**	**37.25±2.48**	**36.16±1.95**
16:1n-9	0.58±0.13	1.13±0.27*	0.31±0.07	0.79±0.08*	0.19±0.05	0.37±0.08*	0.26±0.05	0.91±0.14*
'16:1n-7	1.66±0.47	3.46±0.57*	0.92±0.19	3.37±0.69*	0.37±0.09	1.28±0.21*	0.73±0.25	2.90±0.94*
'18:1n-9	19.48±2.31	33.74±2.88*	11.65±0.30	40.50±2.28*	7.43±0.87	23.09±1.77*	11.15±1.43	35.88±1.91*
'18:1n-7	4.14±1.06	4.31±0.35	1.86±0.13	2.08±0.45	1.26±0.22	1.87±0.13*	1.39±0.33	3.53±0.63*
'20:1n-9	0.21±0.05	0.29±0.05	0.35±0.18	0.58±0.07*	0.26±0.04	0.58±0.09*	0.22±0.15	0.84±0.13*
'22:1	0.29±0.10	0.06±0.00	0.41±0.07	0.03±0.02*	0.13±0.02	0.08±0.02*	0.48±0.23	0.09±0.02*
'24:1	0.13±0.05	0.00±0.00	0.09±0.01	0.26±0.06*	0.09±0.03	0.40±0.05*	0.00±0.00	0.46±0.08*
Total MUFA	**26.54±2.70**	**42.99±3.80***	**15.83±0.47**	**47.68±2.94***	**9.69±0.92**	**27.66±2.19***	**14.11±1.72**	**44.70±1.49***
18:2n-6	12.79±5.28	3.42±0.88*	20.13±1.28	3.30±0.36*	17.19±2.26	5.87±0.52*	17.28±1.52	5.25±0.68*
'18:3n-6	0.20±0.12	0.21±0.04	0.62±0.18	0.10±0.04*	0.37±0.14	0.11±0.03*	0.41±0.07	0.12±0.03*
'20:2n-6	0.62±0.31	0.06±0.03*	0.91±0.06	0.11±0.03*	0.94±0.14	0.34±0.06*	0.69±0.22	0.16±0.03*
'20:3n-6	0.88±0.31	0.38±0.06*	1.04±0.12	0.44±0.06*	1.09±0.20	0.79±0.06*	1.10±0.08	0.64±0.07*
'20:4n-6	9.81±1.25	5.62±1.85*	8.66±1.46	2.81±0.78*	14.31±1.38	7.27±0.92*	16.48±1.38	4.45±0.63*
'22:4n-6	0.64±0.20	0.25±0.10*	0.65±0.10	0.24±0.08*	0.81±0.17	0.22±0.05*	0.66±0.20	0.22±0.08*
'22:5n-6	0.09±0.04	0.13±0.04	0.28±0.08	0.05±0.01*	0.49±0.09	0.11±0.04*	0.81±0.41	0.10±0.03*
Total n6 PUFA	**25.02±5.72**	**10.05±2.88***	**32.28±2.34**	**7.05±1.13***	**35.19±2.43**	**14.71±1.42***	**37.42±2.98**	**10.92±1.29***
18:3n-3	0.06±0.01	0.20±0.05*	0.53±0.13	0.57±0.10	0.38±0.15	0.55±0.08*	0.38±0.08	0.49±0.09
'20:5n-3	0.12±0.04	0.59±0.12*	0.16±0.09	0.91±0.17*	0.07±0.03	1.55±0.42*	0.19±0.06	1.24±0.34*
'22:5n-3	0.49±0.12	0.14±0.04*	0.58±0.17	0.60±0.16	0.76±0.12	0.98±0.16*	0.74±0.25	0.36±0.04*
'22:6n-3	5.85±1.44	4.65±1.33	5.04±1.64	5.99±1.44	10.86±2.74	12.49±1.62	9.38±0.80	4.85±0.96*
Total n3 PUFA	**6.43±1.57**	**5.57±1.51**	**6.46±1.92**	**8.08±1.71**	**12.26±2.89**	**15.57±1.92***	**10.94±.94**	**6.94±1.32***
20:2n-9	0.12±0.05	0.18±0.07	0.10±0.08	0.19±0.02	0.07±0.02	0.22±0.09*	0.09±0.01	0.19±0.06*
'20:3n-9	0.13±0.02	1.35±0.33*	0.00±0.00	0.89±0.19*	0.00±0.00	0.85±0.11*	0.00±0.00	0.97±0.28*
Total n9 PUFA	**0.25±0.07**	**1.67±0.35***	**0.10±0.08**	**1.18±0.22***	**0.09±0.01**	**1.17±0.10***	**0.09±0.01**	**1.26±0.34***

Data are Mean ± SD. * P<0.05.

Supplemental Table 4.1 Liver fatty acid profiles of WT mice fed by different diets (cont.).

	P30		P90		P365	
	Chow	HO	Chow	HO	Chow	HO
'14:0	0.54±0.20	0.51±0.13	0.43±0.05	0.67±0.04*	0.34±0.08	0.45±0.04
'15:0	0.13±0.03	0.10±0.03	0.11±0.01	0.10±0.00	0.13±0.01	0.06±0.01*
'16:0	23.04±2.33	20.31±1.96	21.15±1.23	19.63±0.82	24.52±0.79	20.71±2.09*
'18:0	15.80±2.59	6.39±0.69*	7.05±0.99	3.89±0.65*	10.81±1.77	3.06±1.68*
'20:0	0.19±0.10	0.06±0.03*	0.11±0.04	0.33±0.10*	0.07±0.04	0.08±0.06
Total SFA	**39.69±4.36**	**28.60±1.72***	**29.00±1.07**	**27.58±0.87**	**36.01±1.16**	**24.53±3.46***
16:1n-9	0.44±0.03	1.39±0.45*	1.18±0.14	1.92±0.22*	0.38±0.05	2.77±0.53*
'16:1n-7	1.43±0.23	2.35±0.44*	2.63±0.78	4.17±1.41	1.83±0.51	3.91±0.47*
'18:1n-9	14.36±1.30	47.50±2.06*	38.11±3.76	49.91±0.81*	14.10±1.36	58.11±5.74*
'18:1n-7	2.15±0.38	2.60±0.74	2.19±0.77	2.66±0.30	1.57±0.39	1.15±0.26
'20:1n-9	0.27±0.04	0.63±0.14*	0.56±0.16	0.99±0.17*	0.23±0.04	0.18±0.16*
'22:1	0.20±0.09	0.04±0.02*	0.05±0.02	0.07±0.02	0.00±0.00	0.02±0.02*
'24:1	0.00±0.00	0.15±0.06	0.14±0.03	0.12±0.01	0.07±0.02	0.07±0.01
Total MUFA	**18.95±1.64**	**54.27±2.74***	**44.88±2.49**	**60.18±1.57***	**18.34±1.46**	**66.20±4.97***
18:2n-6	18.15±0.69	4.03±0.63*	8.40±1.39	4.56±0.46*	21.47±2.43	2.84±0.37*
'18:3n-6	0.33±0.14	0.16±0.07*	0.29±0.10	0.13±0.07	0.47±0.14	0.08±0.04*
'20:2n-6	0.51±0.11	0.04±0.01*	0.14±0.05	0.20±0.01	0.30±0.08	0.02±0.01*
'20:3n-6	1.23±0.16	0.56±0.06*	0.64±0.15	0.43±0.11	0.63±0.19	0.39±0.15
'20:4n-6	13.52±2.88	3.76±0.36*	6.81±0.77	2.85±0.42*	13.48±2.53	2.34±0.55*
'22:4n-6	0.44±0.11	0.10±0.04*	0.09±0.01	0.14±0.02*	0.43±0.10	0.23±0.03*
'22:5n-6	0.43±0.19	0.06±0.02*	0.06±0.02	0.10±0.04	0.25±0.08	0.03±0.00*
Total n6 PUFA	**34.61±2.76**	**8.71±1.00***	**16.42±0.68**	**8.30±0.77***	**37.02±0.33**	**5.92±1.06***
18:3n-3	0.45±0.12	0.51±0.08	0.66±0.17	0.67±0.12	0.91±0.25	0.23±0.10*
'20:5n-3	0.08±0.04	1.15±0.29*	1.05±0.38	0.86±0.09	0.28±0.06	0.40±0.12
'22:5n-3	0.58±0.12	0.31±0.12*	0.35±0.09	0.32±0.07	0.42±0.09	0.10±0.02*
'22:6n-3	5.52±2.32	5.89±1.39	7.00±1.62	4.00±0.42*	6.98±1.04	1.92±0.56*
Total n3 PUFA	**6.62±2.38**	**7.86±1.71**	**9.15±2.03**	**5.85±0.57***	**8.58±0.94**	**2.66±0.76***
20:2n-9	0.00±0.00	0.25±0.13*	0.07±0.02	0.20±0.06*	0.04±0.02	0.20±0.06*
'20:3n-9	0.00±0.00	0.68±0.14*	0.45±0.15	0.38±0.09	0.06±0.03	0.50±0.25*
Total n9 PUFA	**0.00±0.00**	**1.01±0.22***	**0.52±0.16**	**0.62±0.15**	**0.10±0.03**	**0.74±0.34***

Data are Mean ± SD. * P<0.05.

Supplemental Table 4.2 Heart fatty acid profiles of WT mice fed by different diets.

	P1		P7		P13		P21	
	Chow	HO	Chow	HO	Chow	HO	Chow	HO
'14:0	0.44±0.07	0.86±0.29*	2.36±0.46	2.04±0.45	1.81±0.61	2.30±0.73	0.60±0.13	1.03±0.31*
'15:0	0.28±0.05	0.34±0.08	0.14±0.06	0.14±0.13	0.11±0.04	0.07±0.03	0.16±0.06	0.07±0.02*
'16:0	20.09±1.82	20.55±0.95	23.83±0.80	19.83±1.78*	19.30±1.18	17.80±1.73	15.68±1.71	13.62±1.51
'18:0	18.50±1.39	15.21±1.88*	19.22±1.05	14.77±0.59*	16.82±0.69	15.99±1.01	19.72±1.20	17.51±0.93*
'20:0	0.19±0.05	0.21±0.04	0.27±0.07	0.34±0.02	0.54±0.09	0.37±0.15*	0.40±0.05	0.32±0.08*
'22:0	0.36±0.11	0.27±0.08	0.21±0.07	0.20±0.01	0.65±0.20	0.25±0.03*	0.37±0.09	0.31±0.06
Total SFA	**39.85±3.08**	**37.44±1.20**	**46.04±1.80**	**37.32±2.31***	**39.61±1.74**	**36.79±1.36***	**36.94±2.56**	**32.86±1.54***
'16:1n-9	0.30±0.08	0.47±0.14*	0.17±0.06	0.24±0.17	0.06±0.01	0.12±0.05*	0.09±0.02	0.22±0.08*
'16:1n-7	1.36±0.19	2.27±0.41*	0.52±0.11	0.85±0.18*	0.26±0.05	0.45±0.10*	0.36±0.17	0.41±0.08
'17:1	0.78±0.10	0.70±0.11	0.63±0.05	0.73±0.57	0.08±0.02	0.92±0.19*	0.59±0.12	1.01±0.32*
'18:1n-9	13.16±1.07	21.76±2.28*	10.32±1.41	19.14±0.32*	12.43±2.04	14.84±1.00*	8.55±1.49	19.29±2.69*
'18:1n-7	3.17±0.40	3.69±0.56	2.86±0.20	3.32±0.05*	1.88±0.62	2.92±0.20*	2.31±0.32	3.42±0.32*
20:1n-9	0.26±0.08	0.44±0.02*	0.44±0.09	1.19±0.02*	0.49±0.12	0.75±0.20*	0.45±0.10	0.84±0.15*
'20:1n-7	0.19±0.06	0.22±0.06	0.43±0.12	0.26±0.01	1.31±0.33	0.21±0.09*	1.38±0.36	0.07±0.03*
'22:1n-9	0.18±0.06	0.28±0.06*	0.16±0.07	0.29±0.02*	0.13±0.02	0.21±0.06*	0.12±0.03	0.13±0.02
Total MUFA	**19.40±1.12**	**29.75±2.22***	**15.53±1.76**	**26.02±0.75***	**16.65±2.14**	**20.43±0.99***	**13.85±1.67**	**25.39±2.81***
'18:2n-6	9.68±1.75	4.70±0.37*	9.83±0.75	4.56±0.14*	13.64±0.68	5.37±0.47*	15.92±1.43	6.66±0.91*
'20:2n-6	1.58±0.41	0.66±0.39*	2.00±0.28	0.30±0.04*	2.67±0.25	0.33±0.03*	2.14±0.33	0.32±0.05*
'20:3n-6	0.88±0.14	0.88±0.10	1.60±0.11	1.69±0.07	1.73±0.19	1.93±0.22	1.48±0.20	1.89±0.23*
'20:4n-6	16.47±1.68	11.28±1.58*	13.29±1.05	10.25±0.79*	12.36±1.81	11.09±1.07	12.20±1.01	9.27±1.26*
'22:4n-6	1.90±0.41	1.06±0.02*	2.43±0.18	0.85±0.14*	2.28±0.32	0.74±0.17*	1.69±0.29	0.50±0.11*
'22:5n-6	1.23±0.40	0.43±0.17*	1.18±0.14	0.39±0.07*	1.20±0.17	0.31±0.10*	1.69±0.36	0.34±0.14*
Total n-6 PUFA	**31.72±1.74**	**19.01±1.23***	**30.32±1.37**	**18.03±0.89***	**33.88±2.92**	**19.78±1.21***	**35.12±1.81**	**18.98±1.91***
'18:3n-3	0.19±0.02	0.33±0.05*	0.25±0.06	0.13±0.08*	0.39±0.12	0.26±0.10	0.28±0.10	0.31±0.13
'20:5n-3	0.21±0.07	0.76±0.06	0.20±0.02	1.56±0.05*	0.39±0.09	0.95±0.20*	0.12±0.02	0.48±.05*
'22:5n-3	1.17±0.22	1.30±0.18	2.57±0.37	3.75±1.89*	2.13±0.22	3.98±0.71*	2.02±0.47	2.97±0.52*
'22:6n-3	7.16±2.04	9.53±1.83	4.80±0.82	11.30±1.94*	6.16±0.84	16.49±1.22*	11.09±2.51	17.91±2.01*
Total n-3 PUFA	**8.66±2.24**	**11.91±1.98***	**7.81±1.13**	**16.75±0.00***	**9.40±0.82**	**21.69±1.21***	**13.51±2.92**	**21.67±2.43***
'20:2n-9	0.19±0.08	0.21±0.02	0.06±0.02	0.22±0.13*	0.26±0.16	0.16±0.03	0.46±0.07	0.16±0.07*
20:3n-9	0.24±0.08	1.79±0.67*	0.17±0.07	1.62±0.08*	0.00±0.00	1.03±0.14*	0.06±0.01	0.88±0.09*
Total n-9 PUFA	**0.30±0.18**	**1.93±0.73***	**0.14±0.08**	**1.80±0.20***	**0.26±0.16**	**1.18±0.15***	**0.49±0.05**	**1.03±0.10***

Data are Mean ± SD. * P<0.05.

Supplemental Table 4.2 Heart fatty acid profiles of WT mice fed by different diets (cont.).

	P30		P90		P365	
	Chow	HO	Chow	HO	Chow	HO
'14:0	0.22±0.11	0.61±0.09*	0.00±0.00	0.11±0.11	0.25±0.08	0.25±0.13
'15:0	0.15±0.04	0.19±0.08	0.29±0.11	0.29±0.03	0.09±0.04	0.08±0.06
'16:0	12.15±0.25	12.73±0.52*	14.60±3.92	12.18±0.61	13.59±0.78	13.60±0.88
'18:0	17.48±0.36	15.49±1.14*	16.50±4.33	14.28±0.93	16.19±0.14	15.34±0.79
'20:0	0.32±0.04	0.29±0.12	0.40±0.20	0.34±0.02	0.34±0.07	0.32±0.05
'22:0	0.04±0.00	0.50±0.10	0.64±0.26	0.02±0.00	0.36±0.07	0.53±0.12
Total SFA	**30.33±0.51**	**29.71±1.42**	**32.42±8.63**	**27.20±0.52**	**30.82±1.00**	**30.12±0.82**
'16:1n-9	0.09±0.02	0.46±0.15*	0.49±0.08	0.26±0.04*	0.15±0.06	0.44±0.15*
'16:1n-7	0.34±0.12	0.61±0.09*	0.98±0.56	0.80±0.12	0.43±0.12	0.86±0.09*
'17:1	0.45±0.06	0.98±0.18*	0.88±0.43	0.78±0.09	0.60±0.06	1.22±0.13*
'18:1n-9	7.39±0.67	21.28±1.70*	32.24±4.01	19.23±1.31*	7.00±1.18	19.38±2.16*
'18:1n-7	2.62±0.19	3.23±0.32*	3.53±0.91	1.81±0.16*	1.82±0.07	2.63±0.27*
20:1n-9	0.29±0.05	0.60±0.06*	1.01±0.27	0.55±0.01*	0.40±0.06	0.55±0.18
'20:1n-7	0.10±0.06	0.18±0.08	0.18±0.09	0.09±0.01	0.12±0.04	0.26±0.15
'22:1n-9	0.02±0.01	0.12±0.03*	0.13±0.06	0.00±0.00	0.06±0.01	0.08±0.01
Total MUFA	**11.30±0.90**	**27.37±1.70***	**39.45±4.83**	**23.51±1.40***	**10.58±1.30**	**25.42±1.74***
'18:2n-6	19.56±0.71	9.58±1.44*	7.99±0.99	6.43±0.84	20.42±2.17	6.51±1.01*
'20:2n-6	1.02±0.06	0.22±0.08*	0.22±0.08	0.05±0.01*	0.55±0.12	0.11±0.07*
'20:3n-6	1.08±0.09	1.61±0.23*	0.84±0.24	1.15±0.11	0.65±0.12	0.98±0.15*
'20:4n-6	14.13±0.47	7.81±0.65*	3.84±1.46	6.59±0.63*	8.83±0.49	6.61±1.05*
'22:4n-6	1.32±0.11	0.26±0.11*	0.21±0.07	0.07±0.04*	0.68±0.19	0.18±0.03*
'22:5n-6	2.20±0.17	0.27±0.05*	0.18±0.15	0.42±0.02*	1.58±0.35	0.27±0.06*
Total n-6 PUFA	**39.30±0.52**	**19.72±1.88***	**13.28±2.11**	**14.71±1.43**	**32.71±2.67**	**14.66±1.87***
'18:3n-3	0.25±0.05	0.45±0.11*	0.21±0.04	0.24±0.02	0.30±0.02	0.26±0.05
'20:5n-3	0.06±0.02	0.62±0.08*	0.00±0.00	0.34±0.04	0.19±0.10	0.35±0.17
'22:5n-3	2.18±0.16	1.81±0.23*	0.93±0.65	1.99±0.18*	1.36±0.66	1.23±0.24
'22:6n-3	16.53±0.98	19.00±1.66*	13.28±10.17	31.15±0.22*	23.91±2.78	27.17±1.99
Total n-3 PUFA	**19.02±1.08**	**21.89±1.69***	**14.42±10.69**	**33.73±0.40***	**25.76±2.68**	**29.02±2.25**
'20:2n-9	0.03±0.00	0.20±0.06	0.00±0.00	0.13±0.02	0.09±0.07	0.19±0.07
20:3n-9	0.07±0.07	1.11±0.36*	0.44±0.15	0.67±0.08*	0.04±0.02	0.56±0.09*
Total n-9 PUFA	**0.06±0.07**	**1.32±0.39***	**0.00±0.00**	**0.80±0.07***	**0.13±0.06**	**0.75±0.14***

Data are Mean ± SD. * P<0.05.

Supplemental Table 4.3 Brain fatty acid profiles of WT mice fed by different diets.

	P1		P7		P13		P21	
	Chow	HO	Chow	HO	Chow	HO	Chow	HO
14:0	2.18±0.09	2.57±0.36*	1.96±0.62	3.08±0.45*	1.41±0.14	1.68±0.34	0.73±0.12	0.74±0.19
15:0	0.10±0.01	0.40±0.04*	0.10±0.01	0.56±0.13*	0.14±0.08	0.71±0.17*	0.10±0.01	0.94±0.24*
16:0	25.27±0.80	26.79±0.69*	26.99±1.72	28.30±1.78	27.40±2.31	26.58±0.71	24.69±1.74	25.07±1.93
18:0	15.41±0.31	15.84±0.96	17.44±1.03	16.21±0.59*	21.31±1.11	18.65±0.91*	22.41±1.33	21.14±1.20
20:0	0.10±0.03	0.28±0.18*	0.00±0.00	0.13±0.02	0.14±0.08	0.19±0.04	0.28±0.11	0.33±0.03
22:0	0.00±0.00	0.00±0.00	0.00±0.00	0.07±0.01	0.00±0.00	0.12±0.03	0.00±0.00	0.22±0.03
Total SFA	**43.05±0.58**	**45.88±2.08***	**0.07±0.01**	**48.34±2.31**	**50.37±3.18**	**47.93±0.63**	**48.21±1.96**	**48.44±3.17**
16:1n-9	2.85±0.31	3.49±0.28*	1.88±0.27	2.81±0.17*	1.20±0.33	1.66±0.30*	0.55±0.03	0.61±0.10
16:1n-7	2.44±0.29	2.73±0.09	2.12±0.70	2.57±0.18	1.21±0.07	1.37±0.24	0.76±0.07	0.78±0.11
18:1n-9	14.45±0.99	15.91±0.55*	12.85±1.92	12.61±0.57	12.80±2.35	12.24±0.25	13.54±2.05	13.58±0.77
'18:1n-7	3.30±0.19	3.87±0.16*	2.84±0.47	2.92±0.32	2.99±0.60	2.83±0.07	3.25±0.48	3.08±0.12
20:1n-9	0.06±0.03	0.40±0.03*	0.32±0.10	0.36±0.05	0.42±0.17	0.37±0.07	0.48±0.11	0.54±0.05
20:1n-7	0.00±0.00	0.04±0.02	0.00±0.00	0.07±0.02	0.00±0.00	0.06±0.01	0.00±0.00	0.14±0.04
22:1n-9	0.07±0.02	0.06±0.02	0.09±0.05	0.07±0.01	0.10±0.03	0.04±0.00	0.07±0.05	0.06±0.02
24:1n-9	0.15±0.04	0.00±0.00	0.05±0.03	0.11±0.02*	0.06±0.02	0.10±0.02*	0.06±0.04	0.27±0.05*
Total MUFA	**23.31±1.50**	**26.47±0.59***	**20.14±1.79**	**21.51±0.75**	**18.75±2.82**	**18.61±0.47**	**18.71±2.54**	**19.01±1.05**
18:2n-6	0.79±0.25	0.22±0.01*	1.27±0.23	0.73±0.14*	1.38±0.36	0.80±0.18*	1.05±0.24	0.67±0.20*
20:2n-6	0.05±0.01	0.16±0.03*	0.05±0.02	0.11±0.04*	0.07±0.04	0.18±0.06*	0.38±0.14	0.16±0.07*
20:3n-6	0.40±0.07	0.22±0.04*	0.85±0.19	0.72±0.07	0.72±0.23	0.83±0.07	0.63±0.24	0.67±0.11
20:4n-6	12.09±0.70	8.77±0.90*	12.94±1.05	9.35±0.79*	11.92±1.79	11.13±0.18	12.92±1.53	10.27±1.10*
22:4n-6	3.62±0.34	1.38±0.35*	3.19±0.38	1.31±0.14*	2.96±0.25	1.45±0.19*	2.99±0.24	1.51±0.25*
22:5n-6	2.27±0.50	0.52±0.06*	1.03±0.12	0.35±0.07*	0.90±0.26	0.35±0.04*	0.68±0.13	0.31±0.06*
Total n-6 PUFA	**19.23±1.56**	**11.28±1.30***	**19.33±1.53**	**12.56±0.89***	**17.93±1.65**	**14.74±0.36***	**18.64±1.21**	**13.57±1.60***
20:5n-3	0.00±0.00	0.19±0.05	0.00±0.00	0.40±0.08*	0.00±0.00	0.39±0.06*	0.00±0.00	0.22±0.09
22:5n-3	0.27±0.05	0.35±0.07*	0.45±0.12	0.77±0.05*	0.31±0.10	0.69±0.05*	0.26±0.05	0.43±0.08*
22:6n-3	12.60±0.76	14.01±1.16*	12.67±1.12	14.62±1.89	11.93±1.49	16.11±1.11*	13.66±0.82	17.40±2.87*
Total n-3 PUFA	**12.87±0.78**	**14.55±1.23***	**13.12±1.10**	**15.80±1.94***	**12.24±1.54**	**17.19±1.14***	**13.92±0.82**	**18.05±2.95***
20:2n-9	0.32±0.20	0.14±0.04	0.00±0.00	0.21±0.03	0.06±0.02	0.19±0.04	0.13±0.03	0.15±0.04
20:3n-9	0.72±0.08	1.16±0.07*	0.47±0.10	1.13±0.13*	0.51±0.17	0.89±0.07*	0.07±0.03	0.48±0.08*
'22:3n-9	0.31±0.02	0.60±0.07*	0.16±0.06	0.38±0.08*	0.13±0.07	0.30±0.02*	0.12±0.08	0.19±0.03
Total n-9 PUFA	**1.34±0.20**	**1.91±0.10***	**0.62±0.15**	**1.72±0.20***	**0.50±0.17**	**1.33±0.12***	**0.25±0.13**	**0.78±0.12***

Data are Mean ± SD. * P<0.05.

Supplemental Table 4.3 Brain fatty acid profiles of WT mice fed by different diets (cont.)

	P30		P90		P365	
	Chow	HO	Chow	HO	Chow	HO
14:0	0.52±0.14	0.38±0.11	0.25±0.03	0.16±0.01*	0.13±0.03	0.16±0.01
15:0	0.10±0.03	0.51±0.10*	0.76±0.09	0.55±0.03*	0.04±0.01	0.55±0.04*
16:0	24.00±0.94	22.87±0.42	26.61±1.49	21.90±0.65*	22.43±0.27	21.60±0.37*
18:0	22.70±0.42	22.17±0.45	24.08±1.31	21.91±0.07*	21.65±0.40	21.87±0.31
20:0	0.48±0.14	0.38±0.03	0.26±0.06	0.21±0.05	0.59±0.05	0.22±0.01*
22:0	0.00±0.00	0.30±0.06	0.28±0.11	0.14±0.08	0.02±0.01	0.15±0.06*
Total SFA	**47.63±1.39**	**46.61±0.74**	**52.34±2.48**	**45.04±0.48***	**45.03±0.59**	**44.72±0.35**
16:1n-9	0.31±0.05	0.30±0.01	0.21±0.04	0.16±0.01	0.12±0.01	0.17±0.01*
16:1n-7	0.82±0.23	0.61±0.04	0.73±0.12	0.64±0.04	0.55±0.06	0.62±0.02
18:1n-9	13.07±0.38	14.63±0.51*	17.64±0.80	15.70±0.97*	21.86±1.73	16.20±0.55*
18:1n-7	3.24±0.13	3.30±0.10	3.67±0.22	3.60±0.07	4.25±0.25	3.71±0.18*
20:1n-9	0.47±0.20	0.75±0.07*	0.95±0.26	0.87±0.25	2.70±0.44	1.04±0.11*
20:1n-7	0.00±0.00	0.33±0.13	0.26±0.09	0.22±0.03	0.51±0.10	0.23±0.02*
22:1n-9	0.06±0.02	0.08±0.02	0.15±0.08	0.03±0.02*	0.13±0.03	0.02±0.02*
24:1n-9	0.36±0.16	0.00±0.00	0.51±0.21	0.22±0.04	0.92±0.02	0.16±0.06*
Total MUFA	**18.32±0.69**	**19.99±0.77***	**24.13±1.50**	**21.44±1.22***	**31.03±2.25**	**22.16±0.81***
18:2n-6	0.89±0.09	0.53±0.12*	0.46±0.04	0.35±0.01*	1.21±0.16	0.33±0.03*
20:2n-6	0.19±0.02	0.09±0.03*	0.11±0.08	0.04±0.01	0.29±0.02	0.06±0.03*
20:3n-6	0.43±0.04	0.64±0.05*	0.36±0.04	0.48±0.02*	0.40±0.02	0.40±0.04
20:4n-6	11.85±0.53	10.44±0.37*	8.86±0.76	10.65±0.12*	7.70±0.83	10.94±0.28*
22:4n-6	2.66±0.11	1.50±0.20*	1.76±0.17	2.17±0.07*	1.52±0.10	2.45±0.17*
22:5n-6	0.88±0.06	0.27±0.05*	0.24±0.04	0.30±0.02	0.07±0.02	0.24±0.03*
Total n-6 PUFA	**16.89±0.58**	**13.48±0.48***	**11.80±0.91**	**13.99±0.16***	**11.18±0.87**	**14.43±0.49***
20:5n-3	0.00±0.00	0.18±0.06	0.00±0.00	0.00±0.00	0.04±0.01	0.00±0.00
22:5n-3	0.28±0.16	0.36±0.03	0.15±0.03	0.26±0.03*	0.19±0.08	0.19±0.01
22:6n-3	16.42±1.53	18.59±1.02*	11.32±1.99	18.83±0.79*	12.41±1.07	17.99±0.72*
Total n-3 PUFA	**16.70±1.59**	**19.13±1.01***	**11.47±2.01**	**19.09±0.82***	**12.64±1.12**	**18.19±0.73***
20:2n-9	0.08±0.01	0.17±0.02*	0.00±0.00	0.12±0.02	0.10±0.02	0.13±0.01
20:3n-9	0.13±0.08	0.49±0.09*	0.17±0.02	0.34±0.03*	0.07±0.01	0.37±0.03*
22:3n-9	0.07±0.01	0.00±0.00*	0.00±0.00	0.15±0.03	0.00±0.00	0.19±0.00
Total n-9 PUFA	**0.24±0.09**	**0.66±0.08***	**0.17±0.02**	**0.61±0.05***	**0.18±0.02**	**0.69±0.03***

Data are Mean ± SD. * P<0.05.

Supplemental Table 4.4 Liver fatty acid profiles of KO mice fed by different diets.

	P1		P7		P13		P21	
	Chow	HO	Chow	HO	Chow	HO	Chow	HO
14:0	0.76±0.15	1.04±0.32	3.11±0.43	4.67±1.04*	1.58±0.38	2.98±0.86*	0.51±0.20	1.37±0.61*
15:0	0.12±0.07	0.12±0.03	0.09±0.02	0.07±0.01	0.08±0.02	0.13±0.03*	0.12±0.03	0.14±0.02
16:0	25.30±0.94	18.43±2.84*	27.67±4.30	22.55±2.00	23.54±2.11	25.37±1.54	22.03±1.78	22.63±1.64
18:0	11.01±2.51	9.92±1.84	12.50±1.04	5.05±0.61*	13.65±1.38	12.47±1.65	14.03±0.87	9.68±1.53*
20:0	0.11±0.07	0.13±0.05	0.19±0.08	0.07±0.04*	0.12±0.06	0.07±0.04	0.08±0.03	0.09±0.04
Total SFA	**38.22±2.86**	**30.16±4.49***	**43.59±4.79**	**34.81±3.42***	**39.02±3.45**	**42.57±3.34**	**36.71±2.73**	**36.01±2.53**
16:1n-9	0.50±0.15	0.94±0.11*	0.29±0.07	0.76±0.09*	0.13±0.04	0.35±0.05*	0.40±0.10	0.92±0.21*
16:1n-7	2.04±0.66	2.84±0.48*	1.13±0.21	3.64±0.68*	0.30±0.03	1.25±0.27*	1.04±0.49	2.52±0.62*
18:1n-9	17.42±3.24	44.74±3.42*	14.17±1.91	41.67±2.76*	6.90±1.42	24.20±1.27*	13.36±2.74	31.61±1.10*
18:1n-7	2.53±0.43	2.95±0.76	1.82±0.81	2.14±0.17	0.97±0.16	2.29±0.27*	1.46±0.53	3.65±0.80*
20:1n-9	0.21±0.04	0.36±0.10*	0.27±0.12	0.55±0.03*	0.18±0.07	0.68±0.05*	0.29±0.06	0.90±0.18*
22:1	0.35±0.19	0.03±0.02	0.28±0.08	0.04±0.00*	0.20±0.07	0.10±0.03*	0.27±0.15	0.10±0.02
24:1	0.14±0.08	0.01±0.01*	0.16±0.04	0.24±0.03*	0.08±0.02	0.72±0.16*	0.00±0.00	0.42±0.12*
Total MUFA	**22.45±4.10**	**51.93±2.55***	**18.23±2.14**	**49.16±2.92***	**8.69±1.36**	**29.62±1.35***	**16.85±3.66**	**40.19±2.20***
18:2n-6	18.77±3.50	3.48±0.85*	21.30±2.20	3.41±0.40*	19.45±1.65	5.67±0.47*	17.69±0.71	6.03±0.91*
18:3n-6	1.28±0.63	0.20±0.04*	0.64±0.17	0.08±0.05*	0.30±0.07	0.12±0.03*	0.38±0.11	0.21±0.07*
20:2n-6	0.52±0.10	0.06±0.02*	0.89±0.11	0.12±0.04*	1.02±0.19	0.23±0.06*	0.78±0.11	0.16±0.06*
20:3n-6	0.67±0.16	0.29±0.03*	1.10±0.33	0.44±0.06*	1.13±0.06	0.94±0.11*	1.10±0.13	0.87±0.11*
20:4n-6	8.03±2.28	4.58±1.06*	7.50±1.59	2.79±0.66*	14.81±0.93	6.64±1.01*	15.92±2.15	5.88±0.99*
22:4n-6	1.05±0.41	0.32±0.11*	0.74±0.17	0.24±0.09*	0.85±0.19	0.22±0.06*	0.54±0.08	0.14±0.07*
22:5n-6	0.07±0.04	0.11±0.04	0.27±0.08	0.06±0.01*	0.56±0.27	0.09±0.01*	0.41±0.01	0.11±0.04*
Total n6 PUFA	**30.36±4.29**	**9.04±1.49***	**31.78±4.57**	**7.14±1.07***	**38.12±1.53**	**13.91±1.27***	**36.83±2.64**	**13.40±1.70***
18:3n-3	0.56±0.24	0.26±0.08*	0.56±0.17	0.57±0.10	0.48±0.14	0.39±0.03	0.37±0.06	0.51±0.17
20:5n-3	0.08±0.03	0.82±0.19*	0.13±0.06	0.84±0.09*	0.15±0.10	1.55±0.44*	0.14±0.02	1.68±0.61*
22:5n-3	0.92±0.32	0.33±0.10*	0.86±0.36	0.55±0.03	0.95±0.22	0.85±0.14	0.76±0.18	0.49±0.11*
22:6n-3	5.39±2.80	6.08±2.69	4.44±1.44	5.66±1.02	12.32±1.46	9.79±1.86*	8.03±2.16	6.07±1.37
Total n3 PUFA	**6.98±3.21**	**7.50±2.87**	**6.29±1.47**	**7.62±0.95**	**14.03±1.47**	**12.58±2.34**	**9.54±2.10**	**8.76±2.08**
20:2n-9	0.10±0.04	0.13±0.03	0.03±0.03	0.20±0.02*	0.04±0.01	0.16±0.07*	0.06±0.03	0.22±0.08*
20:3n-9	0.17±0.06	1.14±0.22*	0.00±0.00	0.84±0.11*	0.00±0.00	0.96±0.07*	0.00±0.00	1.28±0.28*
Total n9 PUFA	**0.23±0.05**	**1.39±0.25***	**0.01±0.02**	**1.13±0.12***	**0.04±0.01**	**1.24±0.07***	**0.06±0.03**	**1.67±0.35***

Data are Mean ± SD. * P<0.05

Supplemental Table 4.4 Liver fatty acid profiles of KO mice fed by different diets (cont.)

	P30		P90		P365	
	Chow	HO	Chow	HO	Chow	HO
14:0	0.29±0.05	0.68±0.14*	0.54±0.16	0.55±0.15	0.37±0.08	0.48±0.04
15:0	0.10±0.01	0.12±0.02*	0.16±0.04	0.12±0.03	0.15±0.02	0.05±0.01*
16:0	21.89±0.49	20.24±1.98	27.65±3.85	18.13±2.66*	23.47±0.99	20.01±1.77*
18:0	16.32±0.77	7.30±1.17*	9.68±1.90	4.00±0.95*	8.38±1.67	2.92±2.02*
20:0	0.16±0.05	0.07±0.04*	0.21±0.13	0.20±0.06	0.13±0.05	0.07±0.06
Total SFA	**38.75±0.53**	**29.96±2.03***	**38.46±3.08**	**26.13±2.27***	**32.52±2.29**	**23.70±0.95***
16:1n-9	0.41±0.12	1.34±0.46*	1.51±0.47	1.91±0.41	0.55±0.16	2.61±1.02*
16:1n-7	1.26±0.27	2.45±0.79*	4.68±2.01	4.13±0.84	2.14±0.38	4.13±0.41*
18:1n-9	14.57±1.21	42.53±3.27*	16.14±2.21	49.14±1.26*	16.43±1.26	59.65±1.31*
18:1n-7	0.73±0.00	2.40±0.69	2.65±0.42	2.38±0.54	1.45±0.62	1.15±0.46
20:1n-9	0.13±0.06	0.90±0.26*	0.64±0.15	0.82±0.29	0.20±0.04	0.18±0.17*
22:1	0.23±0.08	0.06±0.02*	0.07±0.01	0.08±0.01	0.00±0.00	0.02±0.02
24:1	0.17±0.03	0.18±0.07	0.19±0.06	0.15±0.05	0.00±0.00	0.07±0.03*
Total MUFA	**16.88±1.28**	**49.94±4.65***	**25.98±1.43**	**58.82±2.58***	**20.87±1.45**	**67.82±1.48***
18:2n-6	19.84±1.02	4.85±0.71*	13.11±1.61	5.87±1.63*	24.66±5.07	2.55±0.59*
18:3n-6	0.38±0.05	0.13±0.03*	0.43±0.21	0.34±0.05	0.49±0.11	0.08±0.05*
20:2n-6	0.44±0.04	0.07±0.03*	0.13±0.07	0.06±0.04	0.19±0.04	0.02±0.01*
20:3n-6	0.72±0.36	0.68±0.10	0.74±0.16	0.49±0.02*	0.70±0.32	0.30±0.06*
20:4n-6	17.32±1.17	4.42±1.31*	8.53±1.76	3.36±0.63*	12.44±2.65	1.97±0.49*
22:4n-6	0.39±0.07	0.07±0.04*	0.11±0.02	0.17±0.02*	0.24±0.05	0.15±0.04*
22:5n-6	0.17±0.03	0.06±0.02*	0.08±0.01	0.10±0.04	0.07±0.02	0.01±0.00*
Total n6 PUFA	**39.02±1.43**	**10.28±1.97***	**23.13±1.46**	**10.27±2.44***	**38.79±2.49**	**5.08±1.13***
18:3n-3	0.62±0.12	0.52±0.09	1.15±0.37	0.66±0.12	1.19±0.38	0.22±0.12*
20:5n-3	0.18±0.08	1.43±0.51*	1.47±0.25	0.76±0.02*	0.20±0.02	0.36±0.14
22:5n-3	0.28±0.05	0.21±0.06*	0.53±0.11	0.41±0.13	0.40±0.07	0.11±0.02*
22:6n-3	4.65±0.63	6.46±2.24	8.55±2.19	4.82±0.97*	5.94±0.78	2.10±0.70*
Total n3 PUFA	**5.80±0.54**	**8.63±1.96***	**11.83±2.07**	**6.66±1.20***	**7.72±0.51**	**2.79±0.95***
20:2n-9	0.03±0.03	0.21±0.09*	0.08±0.02	0.13±0.06	0.00±0.00	0.19±0.07*
20:3n-9	0.03±0.00	0.91±0.18*	0.53±0.23	0.43±0.08	0.09±0.03	0.42±0.06*
Total n9 PUFA	**0.04±0.03**	**1.23±0.22***	**0.61±0.25**	**0.60±0.10**	**0.09±0.03**	**0.65±0.14***

Data are Mean ± SD. * P<0.05.

Supplemental Table 4.5 Heart fatty acid profiles of KO mice fed by different diets.

	P1		P7		P13		P21	
	Chow	HO	Chow	HO	Chow	HO	Chow	HO
14:0	0.60±0.14	0.74±0.14	1.85±0.51	2.35±0.52	1.72±0.44	2.24±0.42	0.62±0.20	0.72±0.14
15:0	0.22±0.07	0.25±0.06	0.09±0.03	0.07±0.02	0.09±0.05	0.07±0.02	0.21±0.09	0.08±0.04*
16:0	21.73±2.99	20.34±2.60	23.94±2.11	20.79±1.21*	19.39±2.42	18.07±1.22	16.67±1.98	14.26±1.51*
18:0	14.27±1.60	13.22±0.85	18.90±1.21	14.36±1.17*	19.18±1.58	15.39±0.90*	18.09±1.56	17.88±1.63
20:0	0.18±0.07	0.27±0.06	0.23±0.03	0.41±0.12*	0.32±0.05	0.62±0.19*	0.32±0.07	0.35±0.08
22:0	0.26±0.09	0.17±0.05	0.18±0.05	0.23±0.07	0.24±0.02	0.28±0.08	0.87±0.15	0.34±0.11*
Total SFA	**37.27±3.63**	**34.99±2.66**	**45.19±2.99**	**38.21±2.00***	**40.93±3.73**	**36.66±1.78***	**37.79±3.59**	**33.63±2.74***
16:1n-9	0.28±0.06	0.37±0.10	0.14±0.09	0.30±0.09*	0.06±0.02	0.26±0.14*	0.11±0.03	0.26±0.04*
16:1n-7	1.40±0.25	1.79±0.30*	0.55±0.07	1.14±0.33*	0.31±0.13	0.76±0.25*	0.39±0.17	0.48±0.06
17:1	0.64±0.26	0.65±0.12	0.54±0.13	0.60±0.17	0.18±0.07	0.83±0.18*	0.34±0.09	0.78±0.19*
18:1n-9	22.65±2.17	27.57±2.42*	9.72±1.43	21.88±2.43*	8.70±1.44	19.39±2.47*	10.87±2.63	18.98±1.85*
18:1n-7	3.63±0.22	3.50±0.61	2.88±0.17	3.40±0.45*	2.04±0.60	3.31±0.38*	2.17±0.32	3.55±0.38*
20:1n-9	0.46±0.11	0.70±0.15*	0.40±0.05	1.14±0.23*	0.37±0.12	0.92±0.18*	0.48±0.11	0.94±0.13*
20:1n-7	0.34±0.18	0.23±0.08	0.10±0.03	0.17±0.05*	1.15±0.43	0.34±0.10*	1.47±0.31	0.09±0.03*
22:1n-9	0.19±0.05	0.26±0.07	0.14±0.02	0.29±0.04*	0.13±0.03	0.28±0.09*	0.12±0.03	0.16±0.04
Total MUFA	**29.59±2.18**	**35.06±2.62***	**14.46±1.51**	**28.92±2.69***	**12.94±1.99**	**26.09±2.15***	**15.96±2.83**	**25.24±1.82***
18:2n-6	12.07±1.85	4.34±0.41*	10.06±0.85	5.11±1.42*	11.92±0.76	5.13±0.69*	17.36±1.35	6.76±0.88*
20:2n-6	1.56±0.27	0.60±0.28*	1.88±0.28	0.42±0.10*	2.60±0.29	0.55±0.12*	2.19±0.36	0.33±0.07*
20:3n-6	0.78±0.10	0.87±0.22	1.66±0.22	1.59±0.45	1.80±0.24	1.76±0.10	1.27±0.11	2.08±0.29*
20:4n-6	10.69±2.56	10.22±1.71	14.62±1.76	9.33±1.32*	14.53±2.41	9.13±0.88*	10.98±1.38	9.74±0.65
22:4n-6	1.73±0.42	1.10±0.19*	2.51±0.54	0.69±0.08*	2.57±0.57	0.64±0.15*	1.37±0.22	0.49±0.13*
22:5n-6	0.82±0.24	0.32±0.13*	0.96±0.20	0.26±0.09*	1.22±0.19	0.24±0.09*	1.28±0.23	0.25±0.09*
Total n-6 PUFA	**27.65±2.63**	**17.45±1.71***	**31.69±2.07**	**17.41±0.78***	**34.63±3.14**	**17.46±0.81***	**34.44±2.49**	**19.65±0.97***
18:3n-3	0.30±0.10	0.28±0.06	0.18±0.03	0.20±0.08	0.32±0.17	0.37±0.09	0.46±0.12	0.29±0.07*
20:5n-3	0.25±0.07	1.08±0.22*	0.22±0.05	1.19±0.36*	0.20±0.02	1.01±0.20*	0.17±0.04	0.57±0.09*
22:5n-3	1.00±0.26	1.65±0.32*	2.85±0.85	3.22±0.79	2.91±0.74	3.48±0.48	1.52±0.25	3.20±0.93
22:6n-3	3.57±1.30	7.76±1.37*	5.21±1.26	9.22±1.57*	7.82±2.09	13.36±1.40*	8.06±2.61	16.16±2.66*
Total n-3 PUFA	**5.11±1.58**	**10.76±1.72***	**8.47±2.11**	**13.82±2.25***	**11.25±2.68**	**18.22±1.56***	**11.11±2.81**	**20.22±3.56***
20:2n-9	0.15±0.08	0.16±0.02	0.06±0.02	0.22±0.06*	0.12±0.09	0.17±0.05	0.45±0.15	0.23±0.06*
20:3n-9	0.16±0.05	1.63±0.33*	0.08±0.02	1.32±0.46*	0.00±0.00	1.23±0.13*	0.12±0.05	0.99±0.26*
Total n-9 PUFA	**0.29±0.13**	**1.79±0.33***	**0.14±0.03**	**1.54±0.47***	**0.12±0.09**	**1.40±0.16***	**0.49±0.12**	**1.22±0.27***

Data are Mean ± SD. * P<0.05.

Supplemental Table 4.5 Heart fatty acid profiles of KO mice fed by different diets (cont.).

	P30		P90		P365	
	Chow	HO	Chow	HO	Chow	HO
14:0	0.21±0.11	0.89±0.17*	0.00±0.00	0.16±0.03	0.14±0.02	0.23±0.03*
15:0	0.11±0.04	0.11±0.03	0.21±0.10	0.18±0.09	0.17±0.10	0.13±0.07
16:0	12.35±0.60	13.07±0.71	13.86±2.16	12.12±0.97	14.62±0.77	14.44±0.69
18:0	17.29±0.91	15.75±1.52	16.01±2.03	13.11±0.65	17.15±0.71	15.87±0.72*
20:0	0.31±0.03	0.22±0.05*	0.36±0.09	0.51±0.24	0.41±0.03	0.28±0.06*
22:0	0.04±0.01	0.37±0.07*	0.38±0.17	0.30±0.14	0.27±0.03	0.40±0.05*
Total SFA	**30.29±1.12**	**30.41±2.19**	**31.11±2.99**	**26.36±1.40**	**32.76±0.95**	**31.35±0.50***
16:1n-9	0.12±0.03	0.45±0.18*	0.18±0.13	0.50±0.07*	0.16±0.05	0.42±0.13*
16:1n-7	0.28±0.08	0.58±0.11*	0.50±0.39	0.94±0.17	0.44±0.05	1.02±0.14*
17:1	0.41±0.05	0.85±0.13*	0.78±0.27	0.65±0.05	0.63±0.09	1.30±0.11*
18:1n-9	8.91±0.96	22.47±3.41*	7.83±0.87	24.82±3.79*	7.79±1.35	19.52±2.48*
18:1n-7	2.76±0.16	3.41±0.18*	3.31±0.76	1.89±0.71*	2.02±0.16	2.72±0.13*
20:1n-9	0.34±0.04	0.75±0.19*	0.35±0.27	0.66±0.09	0.39±0.11	0.45±0.15
20:1n-7	0.07±0.01	0.18±0.10*	0.06±0.15	0.13±0.05	0.08±0.02	0.12±0.02*
22:1n-9	0.07±0.00	0.14±0.04	0.14±0.09	0.02±0.01*	0.07±0.01	0.07±0.02
Total MUFA	**12.90±0.86**	**28.74±3.59***	**13.08±1.66**	**29.61±3.07***	**11.59±1.55**	**25.62±2.35***
18:2n-6	21.60±1.42	9.43±0.86*	20.16±0.96	6.53±0.86*	23.22±2.41	6.10±0.34*
20:2n-6	1.12±0.15	0.55±0.33*	0.50±0.19	0.06±0.00*	0.39±0.06	0.06±0.02*
20:3n-6	1.08±0.07	1.55±0.09*	0.93±0.24	1.13±0.14	0.70±0.16	0.94±0.24
20:4n-6	12.98±0.60	7.66±0.35*	10.35±1.68	5.68±0.59*	9.10±0.80	6.64±0.32*
22:4n-6	1.08±0.11	0.21±0.05*	0.18±0.06	0.12±0.08	0.51±0.08	0.17±0.04*
22:5n-6	1.68±0.27	0.27±0.06*	0.15±0.09	0.32±0.08*	1.04±0.10	0.24±0.03
Total n-6 PUFA	**39.54±0.57**	**19.68±1.00***	**32.58±2.20**	**13.81±1.00***	**34.96±2.15**	**14.16±0.58***
18:3n-3	0.35±0.15	0.52±0.17	0.24±0.07	0.24±0.05	0.25±0.03	0.26±0.03
20:5n-3	0.10±0.07	0.74±0.21*	0.10±0.02	0.47±0.24*	0.07±0.03	0.25±0.04*
22:5n-3	1.81±0.14	1.67±0.31	0.94±0.52	1.79±0.22	1.28±0.37	1.12±0.38
22:6n-3	14.94±1.42	17.05±1.68	19.59±1.85	27.04±1.33*	19.01±3.25	26.46±1.94*
Total n-3 PUFA	**17.19±1.44**	**19.98±1.71***	**20.71±2.53**	**29.53±1.43***	**20.61±3.19**	**28.09±2.25***
20:2n-9	0.11±0.04	0.16±0.08	0.00±0.00	0.21±0.08	0.03±0.01	0.12±0.01*
20:3n-9	0.04±0.01	1.02±0.12*	0.05±0.02	0.45±0.10*	0.04±0.01	0.64±0.10*
Total n-9 PUFA	**0.09±0.06**	**1.18±0.18***	**0.05±0.02**	**0.66±0.13***	**0.06±0.02**	**0.76±0.10***

Data are Mean ± SD. * P<0.05.

Supplemental Table 4.6 Brain fatty acid profiles of KO mice fed by different diets.

	P1		P7		P13		P21	
	Chow	HO	Chow	HO	Chow	HO	Chow	HO
14:0	2.20±0.24	2.51±0.43	2.75±0.41	3.19±0.20	1.24±0.28	1.75±0.32*	0.62±0.15	0.67±0.08
15:0	0.11±0.01	0.40±0.08*	0.10±0.01	0.38±0.04*	0.30±0.17	0.66±0.11*	0.09±0.01	0.68±0.16*
16:0	28.19±1.62	26.50±1.17	27.80±2.32	28.66±2.46	29.03±3.82	27.17±0.95*	25.83±1.68	24.97±1.49
18:0	15.86±0.52	15.77±0.64	17.98±1.07	16.34±0.87*	17.81±1.52	17.92±0.71	22.29±0.61	20.73±0.79*
20:0	0.06±0.01	0.20±0.03*	0.00±0.00	0.14±0.03	0.28±0.12	0.22±0.04	0.43±0.11	0.36±0.08
22:0	0.00±0.00	0.00±0.00	0.00±0.00	0.06±0.02	0.00±0.00	0.08±0.02	0.00±0.00	0.22±0.07
Total SFA	**46.42±1.74**	**45.37±1.84**	**48.63±3.65**	**48.78±3.05**	**48.67±3.09**	**47.80±1.00**	**49.26±2.03**	**47.62±1.02**
16:1n-9	2.89±0.19	3.52±0.29*	1.98±0.28	2.76±0.16*	1.05±0.16	1.44±0.21*	0.47±0.04	0.57±0.09*
16:1n-7	2.58±0.23	2.85±0.24	2.33±0.18	2.65±0.12*	1.22±0.13	1.32±0.18	0.79±0.07	0.77±0.09
18:1n-9	14.33±0.88	15.92±0.70*	11.77±1.52	13.31±0.85	13.84±2.33	12.39±0.86	14.39±1.34	13.34±0.44
18:1n-7	3.26±0.10	3.85±0.13*	2.78±0.32	2.96±0.15	3.15±1.04	2.82±0.15	3.43±0.22	3.09±0.08*
20:1n-9	0.04±0.03	0.43±0.03*	0.23±0.04	0.34±0.06*	0.41±0.14	0.40±0.08	0.63±0.15	0.59±0.07
20:1n-7	0.00±0.00	0.07±0.01	0.00±0.00	0.05±0.01	0.00±0.00	0.08±0.03	0.00±0.00	0.17±0.03
22:1n-9	0.06±0.02	0.06±0.02	0.07±0.02	0.04±0.03*	0.28±0.12	0.07±0.03*	0.13±0.05	0.08±0.01
24:1n-9	0.12±0.03	0.00±0.00	0.04±0.01	0.09±0.02	0.08±0.04	0.16±0.06	0.19±0.09	0.37±0.08*
Total MUFA	**23.27±1.29**	**26.67±1.12***	**19.21±1.50**	**22.18±0.88***	**17.88±2.84**	**18.60±1.03**	**20.03±1.55**	**18.79±0.67**
18:2n-6	0.92±0.18	0.37±0.07*	1.39±0.30	0.83±0.12*	1.54±0.36	0.95±0.29*	1.27±0.33	0.71±0.25*
20:2n-6	0.04±0.01	0.19±0.04*	0.03±0.02	0.14±0.06*	0.40±0.19	0.18±0.05*	0.38±0.10	0.21±0.10*
20:3n-6	0.39±0.06	0.28±0.04*	0.75±0.15	0.74±0.05	0.67±0.30	0.90±0.05	0.58±0.14	0.80±0.12*
20:4n-6	11.48±0.41	9.04±0.86*	13.75±0.99	9.30±1.15*	12.03±1.24	10.71±0.41*	11.58±0.62	10.76±0.49*
22:4n-6	3.22±0.29	1.56±0.31*	2.90±0.61	1.07±0.32*	3.40±0.94	1.33±0.27*	2.32±0.37	1.60±0.12*
22:5n-6	1.89±0.53	0.47±0.08*	1.23±0.39	0.36±0.13*	0.90±0.28	0.39±0.09*	0.53±0.16	0.36±0.07
Total n-6 PUFA	**17.95±0.86**	**11.91±1.25***	**20.05±0.78**	**12.44±1.52***	**18.94±2.01**	**14.47±0.66***	**16.66±0.85**	**14.43±0.53***
20:5n-3	0.00±0.00	0.25±0.06	0.00±0.00	0.33±0.06*	0.00±0.00	0.44±0.12*	0.00±0.00	0.27±0.13
22:5n-3	0.22±0.04	0.40±0.09*	0.46±0.24	0.67±0.08	0.29±0.09	0.71±0.15*	0.23±0.06	0.52±0.08*
22:6n-3	10.88±0.9	13.44±1.92*	10.89±1.63	13.96±2.32*	13.35±0.86	16.43±1.16*	13.50±1.03	17.60±1.20*
Total n-3 PUFA	**11.10±0.87**	**14.05±2.07***	**11.35±1.87**	**14.96±2.32***	**13.54±0.76**	**17.58±1.19**	**13.74±1.02**	**18.39±1.21***
20:2n-9	0.28±0.18	0.13±0.03	0.00±0.00	0.19±0.08	0.17±0.09	0.23±0.06	0.10±0.03	0.17±0.05*
20:3n-9	0.54±0.12	1.24±0.30*	0.39±0.27	0.98±0.17*	0.24±0.06	0.89±0.13*	0.05±0.02	0.52±0.09*
22:3n-9	0.26±0.06	0.56±0.08*	0.12±0.07	0.31±0.06*	0.30±0.09	0.30±0.07	0.04±0.01	0.20±0.02*
Total n-9 PUFA	**1.08±0.32**	**1.92±0.37***	**0.51±0.29**	**1.48±0.26***	**0.57±0.28**	**1.36±0.18***	**0.17±0.01**	**0.83±0.16***

Data are Mean ± SD. * P<0.05.

Supplemental Table 4.6 Brain fatty acid profiles of KO mice fed by different diets (cont.)

	P30		P90		P365	
	Chow	HO	Chow	HO	Chow	HO
14:0	0.39±0.09	0.32±0.09	0.18±0.04	0.15±0.01	0.15±0.03	0.18±0.06
15:0	0.07±0.02	0.56±0.11*	0.69±0.12	0.47±0.03*	0.47±0.07	0.45±0.02
16:0	22.89±0.93	22.19±0.58	25.69±1.37	21.19±0.45*	22.72±0.45	21.13±0.29*
18:0	22.87±0.30	22.17±0.29*	22.68±0.90	21.44±0.30*	20.09±0.23	21.81±0.33*
20:0	0.37±0.01	0.38±0.10	0.23±0.04	0.20±0.01	0.30±0.04	0.23±0.05
22:0	0.00±0.00	0.37±0.07	0.27±0.09	0.17±0.04	0.24±0.06	0.97±0.66
Total SFA	**46.34±0.82**	**45.99±0.44**	**49.87±1.75**	**43.81±0.45***	**44.07±0.67**	**44.96±1.56**
16:1n-9	0.31±0.02	0.30±0.02	0.20±0.04	0.15±0.01*	0.11±0.00	0.18±0.02*
16:1n-7	0.62±0.03	0.59±0.05	0.73±0.08	0.66±0.02	0.58±0.03	0.65±0.04*
18:1n-9	13.77±0.49	15.17±0.80*	17.56±0.78	16.09±0.52*	17.98±0.70	16.87±1.07
18:1n-7	3.01±0.35	3.40±0.11*	3.72±0.14	3.72±0.10	4.44±0.22	3.87±0.13*
20:1n-9	0.33±0.24	0.86±0.14*	1.02±0.13	0.94±0.16	1.85±0.29	1.12±0.33*
20:1n-7	0.00±0.00	0.31±0.12	0.34±0.15	0.25±0.02	0.30±0.03	0.35±0.13
22:1n-9	0.07±0.03	0.12±0.04*	0.19±0.10	0.04±0.02*	0.04±0.02	0.05±0.02
24:1n-9	0.08±0.04	0.00±0.00*	0.54±0.18	0.12±0.09*	0.03±0.03	0.22±0.03*
Total MUFA	**18.18±0.77**	**20.75±1.04***	**24.30±0.95**	**21.96±0.72***	**25.32±1.06**	**23.31±1.53**
18:2n-6	0.99±0.13	0.49±0.08*	0.56±0.12	0.41±0.06	1.31±0.12	0.36±0.07*
20:2n-6	0.26±0.04	0.09±0.02*	0.14±0.08	0.05±0.01	0.19±0.02	0.04±0.04*
20:3n-6	0.47±0.04	0.70±0.04*	0.39±0.03	0.52±0.03*	0.42±0.02	0.43±0.04
20:4n-6	12.34±0.50	10.28±0.49*	8.89±0.43	10.49±0.30*	8.24±0.37	9.64±1.82
22:4n-6	2.82±0.30	1.60±0.23*	1.77±0.09	2.23±0.04*	0.92±0.15	2.36±0.09*
22:5n-6	0.59±0.06	0.25±0.03*	0.24±0.05	0.32±0.03*	0.07±0.01	0.25±0.03*
Total n-6 PUFA	**17.47±0.81**	**13.41±0.70***	**11.98±0.59**	**14.02±0.23***	**11.18±0.50**	**13.09±1.64**
20:5n-3	0.00±0.00	0.17±0.07	0.00±0.00	0.00±0.00	0.05±0.01	0.00±0.00
22:5n-3	0.45±0.20	0.36±0.04	0.16±0.02	0.30±0.05*	0.20±0.01	0.24±0.04
22:6n-3	17.23±0.54	18.65±1.02*	13.25±1.82	19.51±0.74*	19.02±0.88	17.83±0.95
Total n-3 PUFA	**17.68±0.60**	**19.17±0.96***	**13.41±1.82**	**19.81±0.76***	**19.29±0.89**	**18.07±0.97**
20:2n-9	0.08±0.02	0.13±0.04*	0.00±0.00	0.11±0.01	0.08±0.01	0.16±0.03*
20:3n-9	0.04±0.01	0.41±0.05*	0.15±0.03	0.33±0.06*	0.04±0.00	0.40±0.05*
22:3n-9	0.05±0.04	0.00±0.00	0.00±0.00	0.15±0.02	0.00±0.00	0.20±0.03
Total n-9 PUFA	**0.12±0.05**	**0.54±0.07***	**0.15±0.03**	**0.59±0.08***	**0.13±0.02**	**0.76±0.04***

Data are Mean ± SD. * P<0.05.

Supplementary Table 4.7 Tissue triene/tetreane ratio (20:3n-9/20:4n-6).

	WT				KO			
	Liver							
	Chow		HO		Chow		HO	
Age	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	0.01	0.00	0.26	0.09	0.02	0.01	0.25	0.06
7	0.00	0.00	0.33	0.06	0.00	0.00	0.32	0.10
13	0.00	0.00	0.12	0.03	0.00	0.00	0.15	0.02
21	0.00	0.00	0.23	0.08	0.00	0.00	0.23	0.07
30	0.00	0.00	0.18	0.05	0.00	0.00	0.22	0.08
90	0.06	0.02	0.13	0.03	0.06	0.02	0.13	0.03
365	0.00	0.00	0.21	0.07	0.01	0.00	0.22	0.06
	Heart							
	Chow		HO		Chow		HO	
Age	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	0.01	0.01	0.17	0.08	0.02	0.00	0.16	0.04
7	0.01	0.01	0.17	0.07	0.01	0.00	0.14	0.06
13	0.00	0.00	0.09	0.02	0.00	0.00	0.14	0.02
21	0.00	0.00	0.10	0.02	0.00	0.01	0.10	0.03
30	0.00	0.00	0.14	0.05	0.00	0.00	0.13	0.02
90	0.11	0.03	0.10	0.01	0.10	0.01	0.08	0.01
365	0.00	0.00	0.09	0.03	0.00	0.00	0.10	0.01
	Brain							
	Chow		HO		Chow		HO	
Age	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	0.06	0.01	0.13	0.02	0.04	0.01	0.14	0.04
7	0.03	0.01	0.12	0.01	0.03	0.01	0.11	0.03
13	0.04	0.01	0.08	0.01	0.02	0.01	0.08	0.01
21	0.00	0.00	0.05	0.00	0.00	0.00	0.05	0.01
30	0.01	0.01	0.05	0.01	0.00	0.00	0.04	0.00
90	0.02	0.00	0.03	0.00	0.02	0.00	0.03	0.00
365	0.01	0.00	0.03	0.00	0.01	0.00	0.04	0.00