Metabolic significance of fatty acid elongation

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Dedicated to the future reader
ABSTRACT

Very long-chain fatty acids (VLCFAs) including polyunsaturated fatty acids (PUFAs) are essential lipids whose functional diversity is enabled by variation in their chain length and degree of unsaturation. Although the simplest form of lipids, the length and pattern of the aliphatic chain of fatty acids is essential for their function, and accordingly, proper elongation and desaturation of fatty acids is important in the maintenance of lipid homeostasis. Fatty acids can either be derived directly from the diet or they can be synthesized de novo through lipogenesis. Fatty acids up to 16 carbons in length are synthesized in the cytosol by the multifunctional protein fatty acid synthase (FAS). Further elongation into VLCFAs is catalysed by the elongase enzymes referred to as elongation of very long-chain fatty acids (ELOVLs), residing in the ER. To date, seven ELOVL proteins (ELOVL1-7) have been identified, with ELOVL1, ELOVL3, ELOVL6 and ELOVL7 preferring saturated and monounsaturated fatty acids as substrate; and ELOVL2, ELOVL4 and ELOVL5 being selective for PUFAs. Recent findings have highlighted the necessity for proper lipid composition in the maintenance of normal whole-body energy homeostasis. Therefore, there is a drive in understanding the tissue-specific contribution of fatty acid modifying enzymes. The enclosed papers discuss issues regarding the regulation, function and contribution to lipid composition of the Elovl genes with special emphasis on Elovl2 and Elovl3.

The regulation of ELOVL3, which is suggested to control the synthesis of saturated and monounsaturated fatty acids of up to 24 carbons in length, was investigated using primary cultures and animal studies. In primary brown adipocytes the gene was shown to be regulated by all three PPAR isoforms, involving both transcriptional activation and mRNA stability. In an attempt to clarify the role of ELOVL3 in liver and whole-body lipid homeostasis, I have investigated the metabolic effects associated with Elovl3-ablation and impaired synthesis of saturated and monounsaturated VLCFAs in mice. Elovl3-ablated mice were lean and showed markedly reduced triglyceride and leptin levels in serum. In addition, the mice were completely resistant to diet-induced obesity, associated with a reduced hepatic lipogenic gene expression and triglyceride content.

Over-expression of Elovl2 in cells promoted accumulation of lipid droplets, associated with enhanced fatty acid uptake and induction of PPARγ target genes. To further assess the in vivo function of ELOVL2, the Elovl2 gene was disrupted by homologous recombination in mouse. Elovl2-ablated mice exhibited an almost complete abolishment of the elongation products of C24:5n-6; that is C26:5n-6, C28:5n-6 and C30:5n-6, indicating a novel role of ELOVL2 in the formation of very-long-chain PUFAs (VLCPUFAs) ≥C26. Elovl2+/− male mice displayed both pre- and post-meiotic deficiency of spermatogenesis. These results specify that ELOVL2-derived fatty acids are indispensable for normal spermatogenesis and give new insights in nutritional intervention as an aid in assisting male fertility problems.
This thesis is based on the following papers, which are referred to in the text by their respective Roman numerals.

Norepinephrine and rosiglitazone synergistically induce Elovl3 expression in brown adipocytes.
Am. J. Physiol. Endocrinol. Metab. 293(5): 1159-68

Ablation of the very long chain fatty acid elongase ELOVL3 in mice leads to constrained lipid storage and resistance to diet-induced obesity
Submitted

Concealed metabolic effects in adipose depots and liver of Elovl3−/− mice
Manuscript

ELOVL2 overexpression enhances triacylglycerol synthesis in 3T3-L1 and F442A cells.

Dominant negative effect on male fertility and sperm maturation by haploinsufficiency of ELOVL2 in mouse
Manuscript

VI. Zadra vec D and Jacobsson, A. (2010)
Physiological regulation of fatty acid elongase and desaturase expression in mouse liver and brown adipose tissue.
Manuscript
1. INTRODUCTION.....................................................................................................................11
2. ORIGIN OF FATTY ACIDS ....................................................................................................11
3. FATE OF FATTY ACIDS ........................................................................................................13
  3.1. Adipose tissue .....................................................................................................................14
  3.2. Energy and fatty acid homeostasis ....................................................................................15
4. FATTY ACID SYNTHESIS .....................................................................................................16
5. MAMMALIAN ELONGASES .................................................................................................18
  5.1. Elongases involved in the elongation of saturated and monounsaturated fatty acids........20
    5.1.1. Elovl6 ...........................................................................................................................21
    5.1.2. Elovl1 ...........................................................................................................................22
    5.1.3. Elovl3 ...........................................................................................................................22
      5.1.3.1. Elovl3 regulation in brown adipose tissue ............................................................23
      5.1.3.2. Elovl3 expression in white adipose tissue ............................................................24
      5.1.3.3. Elovl3-ablated mice are resistant to diet-induced weight gain .............................24
    5.1.4. Elovl7 ...........................................................................................................................26
  5.2. Elongases involved in the elongation of PUFAs ...............................................................27
    5.2.1. Elovl5 ...........................................................................................................................27
    5.2.2. Elovl2 ...........................................................................................................................29
      5.2.2.1. Attenuated PUFA synthesis in Elovl2/−/− mice .......................................................30
      5.2.2.2. Impaired fertility in Elovl2+/− male mice ..............................................................31
      5.2.2.3. Necessity of ELOVL2-derived fatty acids during embryogenesis .......................32
    5.2.3. Elovl4 ...........................................................................................................................32
6. DESATURATION OF FATTY ACIDS ...................................................................................34
  6.1. Acyl chain desaturation performed by stearoyl-CoA desaturases (SCDs) .......................34
  6.2. Acyl chain desaturation of PUFA performed by fatty acid desaturases (FADS) ............35
7. DISCUSSION ............................................................................................................................36
8. ACKNOWLEDGEMENTS .......................................................................................................41
9. REFERENCES ..........................................................................................................................41
ABBREVIATIONS

ACC    Acetyl-CoA carboxylase
BAT    Brown adipose tissue
DGAT   Diacylglycerol acyltransferase
ELOVL  Elongation of very long chain fatty acids
ER     Endoplasmic reticulum
FADS   Fatty acid desaturase
FAS    Fatty acid synthase
gWAT   Gonadal white adipose tissue
HADC   3-hydroxyacyl-CoA dehydratase
iWAT   Inguinal white adipose tissue
LXR    Liver X receptor
PPAR   Peroxisome proliferator-activated receptor
PUFA   Polyunsaturated fatty acid
SCD    Stearoyl-CoA desaturase
SREBP  Sterol regulatory element-binding protein
STGD3  Stargardt-like macular dystrophy
TG     Triacylglycerol
UCP1   Uncoupling protein-1
WAT    White adipose tissue
VLCFA  Very long-chain fatty acid
VLCPUFA Very long-chain polyunsaturated fatty acid
VLDL   Very low density lipoprotein
1. INTRODUCTION

Very long-chain fatty acids (VLCFAs) including polyunsaturated fatty acids (PUFAs) are essential lipids whose functional diversity is enabled by variation in their chain length and degree of unsaturation. With the big variety in lipid molecules it is not surprising that they have received more attention recently, as more pathophysiological syndromes are being ascribed to impaired lipid homeostasis. Except for obesity and its subsequent complications, several additional diseases are associated with disturbances in lipid metabolism such as X-linked adrenoleukodystrophy, retinopathy and infertility (Igarashi et al., 1976; Safarinejad et al., 2009; Tikhonenko et al., 2009).

As a major source of energy and as structural components of membranes, fatty acids are essential for life. They are components of a wide variety of molecules, including wax esters, sterol esters, glycerophospholipids, sphingolipids and triacylglycerol (TG). In addition, certain fatty acids function as signaling molecules and thus perform key biological functions, such as regulation of lipid metabolism, cell division and inflammation (Cao et al., 2008; Duplus and Forest, 2002; Zhang and Rock, 2009).

This diversity of biological functions is reflected in the fact that most cells have the ability to synthesize and modify fatty acids. Although the simplest form of lipids, the length and pattern of saturation/desaturation of the aliphatic chain of fatty acids is essential for their function. Accordingly, proper elongation and desaturation of fatty acids is important for the maintenance of lipid homeostasis and disruption of these processes may have devastating consequences.

In this thesis I will focus on the functional significance of mammalian elongases with special emphasis on metabolism and physiological effects due to gene ablation.

2. ORIGIN OF FATTY ACIDS

Fatty acids can either be derived directly from the diet or they can be synthesized de novo through lipogenesis, a key event in the energy storage system. Lipids, carbohydrates and amino acids can all be metabolized into acetyl-CoA, thus serving as substrate for lipogenesis (Figure 1). The main organ for uptake, de novo synthesis and release of fatty acids into the circulation is the liver. However, de novo lipogenesis also occur in white adipose tissue in both rodents and humans were it contributes for ~80% and ~20% of the total fatty acid pool,
respectively (Strawford et al., 2004; Turner et al., 2003). Fatty acids can further be incorporated into more complex molecules, used for energy production through $\beta$-oxidation in the mitochondria or stored as an energy reservoir in the form of TGs in lipid droplets.

Figure 1. Metabolic pathways in the synthesis of fatty acids into triacylglycerol. In the postprandial state, insulin levels rise and glucose is taken up by the cell. Inside the cell glucose is either, in the case of the liver, converted to glycogen or shunted into the glycolysis pathway by being phosphorylated by glucokinase (GK). The end-product of glycolysis is pyruvate, which enters the Krebs cycle in the mitochondria and subsequently leaves the mitochondria as citrate. Citrate is converted to acetyl-CoA and acetyl-CoA carboxylase (ACC) then catalyses the ATP dependent carboxylation of acetyl-CoA to yield malonyl-CoA. Fatty acid synthase (FAS) then uses malonyl-CoA as the two-carbon donor, to an original acetyl-CoA primer, in the sequential synthesis of palmitic acid (C16:0). C16:0 can then be further elongated, by the ELOVL (elongation of very long chain fatty acids) protein family, and/or desaturated by stearoyl-CoA desaturases (SCDs) before it is coupled to one of the three carbon positions of the glycerol molecule by distinct proteins in the TG synthesis pathway. The TGs can then either be stored in lipid droplets or released into the circulation in the form of very-low-density lipoprotein (VLDL) particles. Adapted from (Postic and Girard, 2008).
3. FATE OF FATTY ACIDS

A primary role of fatty acids in cellular function is the formation of the permeability barrier of cells and subcellular organelles in the form of a lipid bilayer. These membranes constitute the main storage compartment of lipids in most cells. Although the major types of lipids defining the bilayer are glycerol-based phospholipids, other species such as cholesterol and sphingolipids are key components in biological membranes (Verkleij et al., 1973).

Besides being major components of cellular membranes, fatty acids are also subjected to esterification to other lipid species. Wax esters are major constituents of the sebum, an oil secreted from the sebaceous and meibomian glands, which is essential to lubricate skin, hair and eyes. Maintenance of cholesterol levels within the liver is achieved by the formation of cholesterol esters from cholesterol and fatty acids (Buhman et al., 2000). Unlike free cholesterol, the cholesterol esters can be stored in the cells without being toxic and subsequently used for bile acid and steroid synthesis. In addition, sphingolipids such as ceramides, which have a long fatty acid coupled to a sphingosine molecule, are bioactive molecules involved in several pathways including cellular growth, differentiation and apoptosis (Bartke and Hannun, 2009).

Storage of energy predominantly occurs in the form of TGs, consisting of three fatty acids esterified to a glycerol molecule, which is also the major constituent of the lipid droplet. Synthesis of TGs is a highly regulated event taking place at the endoplasmic reticulum (ER; Figure 2).

**Figure 2. Synthesis of triacylglycerol.** A fatty acid is coupled at each of the three carbon positions of the glycerol molecule, by distinct proteins at the ER.. The first fatty acid is coupled by GPAT producing LPA and the second fatty acid is coupled by AGPAT to form PA. The phosphate at the sn-3 position has to be hydrolyzed before the final fatty acid can be coupled by DGAT to form TG. glycerol-3-phosphate acyltransferase (GPAT); 1-acylglycerol-3-phosphate-O-acyltransferase (AGPAT); phosphatidic acid phosphathase (PAP); diacylglycerol acyltransferase (DGAT); glycerol-3-phosphate (G3-P)
In mammals, degradation of fatty acids occurs in the mitochondria as well as the peroxisomes. Mitochondrial β-oxidation is responsible for the breakdown of fatty acids up to 18 carbons in length and supports the production acetyl-CoA which is forwarded to the Krebs cycle, supplying reducing equivalents to the respiratory chain for ATP production through oxidative phosphorylation (Figure 1). β-oxidation of VLCFAs (>18 carbons in length), PUFAs and eicosanoids, is exclusively carried out in the peroxisomes. In addition, partial peroxisomal degradation of C24:5n-6 and C24:6n-3 is an absolute prerequisite in the biosynthesis of docosapentaenoic acid (C22:5n-6, DPAn6) and docosahexaenoic acid (C22:6n-3, DHA) respectively (Voss et al., 1991).

The direction of fatty acids metabolism depends in large on the nutritional status. In the fed state, carbohydrates are converted to fatty acids which are stored in TGs as energy reservoirs, while in the fasted state TG breakdown and fatty acid oxidation predominates. Clearly there exists a reciprocal relationship between fatty acid synthesis and oxidation.

As signaling molecules, fatty acids and derivatives thereof influence whole-body energy homeostasis by, e.g. modulating gene transcription. Specifically, as ligands, fatty acids have been shown to activate several nuclear receptors including the peroxisome proliferator-activated receptor family (PPARα, PPARγ, PPARδ), liver X receptor (LXRα and LXRβ), and retinoid X receptor-alpha (RXRα) (Chakravarthy et al., 2009; de Urquiza et al., 2000; Gottlicher et al., 1992; Jump, 2002; Kliewer et al., 1997). In addition, both fatty acids and cholesterol are found to, in an indirect way, control the activity of the transcription factors sterol regulatory element binding protein-1c (SREBP-1c) and -2, respectively, which are major components in controlling genes involved in fatty acid and cholesterol synthesis (Ou et al., 2001; Sakai et al., 1996; Xu et al., 1999).

3.1. Adipose tissue

The ability to store energy as TGs in lipid droplets is a fundamental process found in almost all organisms. In mammals, adipocytes are the main storage site within the body; however, liver, heart, kidney, intestine, sebaceous and meibomian glands, steroidogenic cells found in adrenal cortex, ovarian follicles and testis, are examples of tissues containing significant amounts of lipid droplets.

Adipose tissue is a heterogeneous tissue composed of different cell types, predominantly adipocytes, but also non-adipocytes such as macrophages, blood cells and fibroblasts. It can be found in various locations throughout the body and can generally be
divided into two major fat depots: subcutaneous and visceral fat (Cinti, 2005). Subcutaneous fat is situated directly under the skin while visceral fat is contained within the peritoneal cavity surrounding internal organs.

There are two distinct types of adipose tissue found in mammals: white adipose tissue (WAT) and brown adipose tissue (BAT). The adipocytes composing the different tissues have distinct morphological and physiological functions. In addition, the two tissues also originate from two distinct cell lineages (Seale et al., 2008; Timmons et al., 2007). WAT is the primary tissue for the storage (lipogenesis) and release (lipolysis) of energy in the form of fatty acids into the circulation for further usage, thus playing a fundamental role in maintaining energy homeostasis of the organism. The adipocytes within WAT are mostly unilocular when fully mature while the adipocytes from BAT have multilocular fat droplets and are rich in mitochondria (Nechad et al., 1983).

BAT is responsible for the adaptive non-shivering thermogenesis upon cold exposure, mostly used for hibernating animals (Nicholls and Locke, 1984). This heat production is dependent on the uncoupling protein-1 (UCP1)-mediated dissipation of the mitochondrial proton gradient, which is built up by the oxidation of fatty acids within the brown adipocytes (Enerback et al., 1997). It is well recognized that BAT is present in most mammals including humans, especially in neonates, but recent evidence also suggests that BAT is present and functional in adult humans (Cypess et al., 2009; Nedergaard et al., 2007; Virtanen et al., 2009).

3.2. Energy and fatty acid homeostasis.

Maintenance of whole-body lipid homeostasis is of essence for normal physiological functions. Besides the well-characterized negative effects of increased adipose mass, lipodystrophy is also associated with several complications related to those found in obese subjects; for example, insulin resistance is likely due to accumulation of ectopic fat in non adipose tissues, such as liver and skeletal muscle in affected patients (Kim et al., 2000; Moitra et al., 1998). Importantly, then, lipid homeostasis is controlled locally by the cell-specific expression of fatty acid metabolizing enzymes, as well as globally, by central regulation of the arcuate nucleus within the hypothalamus.

Adipose tissue furthermore affects energy homeostasis, including food intake, energy expenditure and metabolic efficiency via numerous secreted hormones, termed adipokines. Adiponectin is solely expressed in adipocytes and is found to be low in obese subjects (Arita...
et al., 1999). Administration of adiponectin to obese animal models reverses insulin resistance (Yamauchi et al., 2001).

Leptin acts as a satiety factor that is secreted from the adipose tissue and, acting via leptin receptors in the hypothalamus, causes reduced food intake and increased energy expenditure (Balthasar et al., 2004). The expression of leptin is linearly correlated to the adipose size and lipid content and is subsequently, contrary to its effect, elevated in obese subjects (Maffei et al., 1995). In addition, several other adipokines, such as resistin, visfatin and tumor necrosis factor \( \alpha \) (TNF\( \alpha \)) have been associated with various physiological functions, which is reviewed in (Wozniak et al., 2009). Recently, palmitoleic acid (C16:1n-7) was identified as an adipose tissue-derived hormone (lipokine), which serves as a lipid signal that mediates communication between adipose tissue and other tissues, such as liver and muscle (Cao et al., 2008). C16:1n-7 was found to beneficially influence several metabolic activities including insulin action and hepatic lipogenesis.

As mentioned before, the liver is the major organ for carbohydrate and lipid metabolism, including gluconeogenesis, glycogenesis, cholesterol biosynthesis and lipogenesis (Figure 2). These metabolic events in the liver are tightly controlled by several pancreatic hormones including insulin and glucagon. In addition, liver itself is an endocrine organ, secreting numerous factors involved in the regulation of systemic glucose and lipid homeostasis. In the past several years, a number of liver-derived factors have been identified including, bone morphogenetic protein-9 (BMP-9) (Chen et al., 2003), fibroblast growth factor 21 (FGF21) (Kharitonenkov et al., 2005) and adropin (Kumar et al., 2008). TGs resulting from hepatic lipogenesis can either be stored in the liver through incorporation into lipid droplets or packed into very-low-density lipoprotein (VLDL) particles for further distribution to peripheral tissues. Lipoproteins consist of a hydrophobic core, comprised mainly of TG and cholesterol esters, surrounded by a hydrophilic monolayer consisting of phospholipids and unesterified cholesterol. Upon reaching their appropriate target tissue, the TGs within the VLDL particles are hydrolyzed by lipoprotein lipase and rapidly taken up by the cell.

4. FATTY ACID SYNTHESIS

The carboxylation of acetyl-CoA by acetyl-CoA carboxylase (ACC) to yield malonyl-CoA is considered to be a significant event in de novo synthesis of fatty acids. Two isoforms have been identified, ACC1 and ACC2, with ACC1 being principally expressed in lipogenic
tissues such as adipose tissue and liver, while ACC2 is predominantly expressed in oxidative tissues like heart and skeletal muscle (Kim, 1997). Besides being used for fatty acid synthesis, malonyl-CoA also acts as an inhibitor for carnitine palmitoyltransferase 1 (CPT1) which transports acyl-CoA into the mitochondrion for β-oxidation (McGarry et al., 1977). Fatty acids up to 16 carbons in length are synthesized in the cytosol by the multifunctional protein fatty acid synthase (FAS), which utilizes acetyl-CoA as the priming substrate and malonyl-CoA as the two-carbon donor in the sequential synthesis of palmitic acid (C16:0) (Wakil et al., 1983; Wakil, 1989). The reaction steps are condensation, reduction, dehydration and one further reduction (Figure 3).

**Figure 3. Enzymatic steps performed by fatty acid synthase (FAS).** Fatty acids up to 16 carbons in length are synthesized by the multifunctional enzyme FAS in the cytosol.

As in the case of FAS, the activities of the enzymes involved in both the elongation and desaturation of fatty acids longer than 16 carbons in length, appears to be regulated primarily at the transcriptional level, rather than by posttranslational protein modifications. The presence and levels of relevant transcription factors, ligands and cofactors in a given tissue at any given time thus contribute to this regulation. At the same time, overall lipid homeostasis is regulated at a higher level by, e.g., hormones, circadian rhythms and food intake (Gachon et al., 2004; Gil-Campos et al., 2006; Sul and Wang, 1998). Moreover, there is a pronounced sexual dimorphism in the control of fatty acid homeostasis (Priego et al., 2008). The interactions between these various factors are clearly quite complex and, to date, relatively little is known about them. Nonetheless, it is clear that the expression of both desaturases and the enzymes involved in the elongation process is tightly controlled by tissue-specific factors, indicating that these enzymes are involved in a variety of synthetic pathways that may alter the levels of different fatty acid pools in response to various stimuli.
5. MAMMALIAN ELONGASES

Fatty acids, synthesized by FAS or derived from the diet, can be further elongated and desaturated into VLCFAs by distinct membrane-bound enzymes residing in the ER. As is also the case with the reactions performed solely by the cytosolic FAS, microsomal fatty acid elongation involves the addition of two-carbon units to a fatty acyl-CoA, employing malonyl-CoA as the donor and NADPH as the reducing agent. The process of elongation requires four separate enzymatic reactions: condensation between the fatty acyl-CoA and malonyl-CoA to yield 3-ketoacyl-CoA; reduction of 3-ketoacyl-CoA to generate 3-hydroxyacyl-CoA; dehydration of 3-hydroxyacyl-CoA to produce trans-2-enoyl-CoA, and; reduction of trans-2-enoyl-CoA to form the two-carbon elongated acyl-CoA (Figure 4). The mammalian reduction steps are performed by 3-ketoacyl-CoA reductase (KAR) and trans-2,3,-enoyl-CoA reductase (TER), respectively (Moon and Horton, 2003). Recently, four mammalian 3-hydroxyacyl-CoA dehydratase (HADC1-4) isoforms, homologous to the yeast Phs1p enzyme have been identified (Denic and Weissman, 2007; Ikeda et al., 2008).

In mammals, the initial, and rate-controlling, condensation reaction is catalysed by the elongase enzymes referred to as elongation of very long-chain fatty acids (ELOVLs) (Denic and Weissman, 2007; Moon et al., 2001; Oh et al., 1997). To date, seven ELOVL proteins (ELOVL1-7) have been identified, with ELOVL1, ELOVL3, ELOVL6 and ELOVL7...
preferring saturated and monounsaturated fatty acids as substrate; and ELOVL2, ELOVL4 and ELOVL5 being selective for PUFAs (Figure 5) (Agbaga et al., 2008; Kitazawa et al., 2009; Leonard et al., 2000; Moon et al., 2001; Tamura et al., 2009; Tvrdik et al., 2000; Wang et al., 2005). Interestingly, co-immunoprecipitation has revealed selective interactions between the different HADC and ELOVL proteins, which may be designed to control the enzymatic reactions in a more specific fashion (Ikeda et al., 2008).

![Figure 5](image)

**Figure 5. Long chain and very long-chain fatty acid biosynthesis in mammalian.** The long chain saturated fatty acids and unsaturated fatty acids of the n-10, n-7 and n-9 series can be synthesized from palmitic acid (C16:0) produced by the fatty acid synthase (FAS). Very long-chain fatty acids of the n-6 and n-3 series can only be synthesized from precursors obtained from dietary precursors (DIET). The specific mouse genes likely to contribute to elongation (Elov 1-7) and desaturation (SCDs and Fads) steps are indicated in these pathways. Adapted from (Guillou et al., 2009).

All ELOVL proteins contain several conserved amino acid consensus sequences, such as the KxxExxD T, HxxHH and HxxMYxYY motifs, which are fully conserved between mouse,
rat and human elongases (Leonard et al., 2004; Tvrdik et al., 2000). The overall sequence similarity is approximately 30 % and this similarity is preserved when comparing other homologous elongases in different species, such as human, nematode and yeast (Leonard et al., 2004).

Early data suggested that there are several separate elongation systems responsible for VCLFA synthesis and that these systems contain chain-length-specific elongating enzymes (Goldberg et al., 1973; Luthria and Sprecher, 1997). In addition, the finding that one specific Elop, a yeast homolog to ELOVL, can convert substrates of different chain lengths to one single specific fatty acid specie of the same chain length and, conversely, that different Elops allow for the elongation of the same substrate to end-products of varying chain lengths, suggests that the end-product specificity is not a “fixed” number of carbon additions but rather an intrinsic signal determining the chain length (Paul et al., 2006). In an elegant study Denic and Weissman showed that the catalysis of the condensation reaction takes place at the cytosolic face of the ER membrane and that the chain length is determined by a lysine residue on the luminal side (Denic and Weissman, 2007). By varying the distance between the active site and the juxta-luminal lysine, one helical turn at a time, they managed to modulate the chain length of the end-product. They also showed that the HxxHH motif is an absolute prerequisite for the formation of 3-ketoacyl-CoA.

Of the different Elovl genes, Elovl1, Elovl5 and Elovl6 are ubiquitously expressed while Elovl2, Elovl3, Elovl4 and Elovl7 display a more distinct tissue-specific expression. The physiological consequence of the tissue-specific expression pattern for the different elongases is not known, however, it is clear from in vivo studies and the emergence of several transgenic Elovl animal models, that this is related to the tissue specific demand for the VLCFA produced by the different elongases. As stated above, the elongases can be divided into two classes based on their substrate specificity, i.e. those who have saturated and monounsaturated fatty acids as a substrate and those who have PUFAs as a substrate (Figure 5). Since the substrate specificity and product formation is related to the metabolic significance of the elongases, I will divide the presentation based on these two classes.

5.1. Elongases involved in the elongation of saturated and monounsaturated fatty acids

In contrast to the PUFAs, saturated and monounsaturated VLCFAs have long been associated with undesirable effects on health, including obesity, heart failure and
atherosclerosis. Moreover, there are a number of human disorders known to be related to abnormal levels of saturated and monounsaturated VLCFAs such as peroxisomal disorders involving the adrenal cortex and the nervous system (Moser et al., 1999; Singh et al., 1984; Wanders et al., 1987). Despite this, one cannot neglect the importance of these fatty acids as essential barrier components of the plasma membrane. Noticeably, several of the most common non-essential fatty acids have recently been shown to exert a strong influence on lipogenesis and lipolysis, as well as on fatty acid uptake. For example, C16:1n-7 exerts lipokine effects on lipogenesis and fatty acid uptake, while phosphatidylcholine containing C16:0 and oleic acid (C18:1n-9) is an agonist for PPARα which up-regulates fatty acid oxidation (Cao et al., 2008; Chakravarthy et al., 2009). Longer saturated and monounsaturated fatty acids such as C20 and C22 are important constituents of the skin and have been implicated in lipid droplet formation (Paper II) (Cameron et al., 2007; Li et al., 2007a; Westerberg et al., 2004; Westerberg et al., 2006). In addition, sphingolipids and ceramides containing saturated and monounsaturated VLCFA up to 26 carbon atoms are thought to stabilize membrane rafts and act as second messengers by activating membrane proteins through direct binding (Brown, 2006).

5.1.1. Elovl6

The end-product of FAS, C16:0, and the desaturated product, C16:1n-7, can be further elongated by ELOVL6 to yield stearic acid (C18:0) and vaccenic acid (C18:1n-7), respectively. ELOVL6 was first identified following enhanced expression in the liver of transgenic mice that over-express SREBP-1c and SREBP-2, and was subsequently identified as responsible for the rate-limiting reduction step producing 3-ketoacyl-CoA (Moon et al., 2001). Further regulation by SREBPs was confirmed by Matsuzaka and colleagues who identified Elovl6 using DNA microarray, naming the enzyme, fatty acyl-CoA elongase (FACE) (Matsuzaka et al., 2002b). The expression of Elovl6 is highly up-regulated both in the liver and white adipose tissue in the refed state, indicative of the enzyme playing an important role in the synthesis of long-chain fatty acids.

Recruitment of BAT is correlated with an increase in the elongation of saturated VLCFAs (Tvrdik et al., 2000). For instance, a fivefold increase is seen in the elongation of C16:0. To investigate if the increase in the elongation activity of C16:0 was correlated with an increase of Elovl6 expression, we analyzed BAT from mice subjected to cold exposure at 4°C
for three days. Indeed, there was a clear induction of Elovl6 in response to cold exposure suggesting that the increase in C16:0 elongation could be attributed to ELOVL6 (Paper VI).

Elovl6\textsuperscript{-/-} mice are resistant to the development of diet-induced insulin resistance, without amelioration of obesity or hepatosteatosis (Matsuzaka et al., 2007). Thus, re-establishment of whole-body insulin resistance in Elovl6\textsuperscript{-/-} mice is attributed to restoration of hepatic insulin sensitivity through an IRS-2/Akt signaling pathway. This effect is suggested to be mediated by the increased ratio between C16:1\textsubscript{n-7} and C16:0 in the liver. In addition, breeding the Elovl6\textsuperscript{-/-} mice on an ob/ob genetic background, reduced hyperglycemia and improved insulin resistance as compared to ob/ob mice, suggesting that the improved insulin sensitivity is independent of leptin signaling. In addition, Elovl6\textsuperscript{-/-} mice exhibit partial embryonic lethality, with maintenance of fertility in the surviving male and female offspring, further emphasizing the importance of endogenous C18 fatty acids synthesis.

5.1.2. Elovl1

ELOVL1 was first discovered on the basis of its sequence similarity to ELOVL3, and is suggested to be involved in the production of saturated fatty acids up to 26 carbons in length (Oh et al., 1997; Tvrdik et al., 2000). Yeast complementation studies revealed that ELOVL1-derived fatty acids are important components in sphingolipid synthesis. Likewise, two myelin-deficient animal models, Jimpy and Quaking, display reduced elongation activity of C20:0 and C22:0 in the brain, which is, in numbers, exactly paralleled by a decrease in Elovl1 expression, further supporting a role for ELOVL1 in the formation of sphingomyelin (Suneja et al., 1991; Tvrdik et al., 2000). In the case of the liver, expression of Elovl1 is not directly regulated by PPAR\textalpha, LXR or SREBP-1c and is not subject to nutritionally induced changes (Wang et al., 2006b). Nor does Elovl1 display any diurnal variation in either liver or BAT (Paper VI). However, a small induction of Elovl1 expression was detected in BAT as a result of a high-fat diet.

5.1.3. Elovl3

In contrast to ELOVL1 and ELOVL6, the ELOVL3 enzyme, which is suggested to control the synthesis of saturated and monounsaturated fatty acids of up to 24 carbon atoms, is expressed in a highly tissue-specific manner (Jakobsson et al., 2006; Kitazawa et al., 2009).
The gene was originally identified as cold inducible since its expression is highly upregulated in the BAT of mice upon the adaptation to the cold (Tvrdik et al., 1997). However, significant amounts of Elovl3 expression can also be found in WAT, skin and liver (Paper III) (Tvrdik et al., 1997). In the liver, Elovl3 displays a diurnal expression that is significantly higher in male mice, where the expression is almost absent at the beginning of the dark phase (Brolinson et al., 2008). Interestingly, Elovl3 expression is induced in the liver of male mice when they reach an age of about eight weeks, a phenomenon that is absent in castrated mice. Elovl3 expression is neither regulated by fasting nor refeeding; however, conditional feeding exclusively during the light phase for one week, inverts the diurnal rhythm of Elovl3 expression. This suggests that Elovl3 is under differential transcriptional regulation and has a different functional role in liver metabolism as compared to traditional lipogenic genes, which are normally induced by increased food intake and insulin signaling. Thus, in liver, expression of Elovl3 is regulated by the coordinated release of different gender-specific steroid hormones such as glucocorticoids, androgens and oestrogens (Brolinson et al., 2008). The finding that female ERα-ablated mice have high levels of Elovl3 expression in the liver, further supports the involvement of gender-specific factors in the regulation of Elovl3 (Bryzgalova et al., 2006). In addition, mice with a double targeted mutation in the circadian regulators cryptochromes, Cry1 and Cry2, have a feminized hepatic gene expression as a consequence of altered fluctuations in circulatory growth hormone levels (Bur et al., 2009). This was accompanied by a reduction and absence of diurnal expression of Elovl3 in the mutated male mice. Administration of growth hormone at 12 hour intervals for one week restored hepatic expression of Elovl3.

5.1.3.1. Elovl3 regulation in brown adipose tissue

Recruitment of BAT is mediated by increased sympathetic drive and release of norepinephrine resulting in induced mitochondriogenesis, hyperplasia, Ucp1 and Elovl3 expression, and subsequently an augmented thermogenic capacity for the tissue, reviewed in (Cannon and Nedergaard, 2004). The induction of Elovl3 can be mimicked in vivo by the release of norepinephrine from microosmotic pumps at thermoneutrality (Tvrdik et al., 1997). With the use of primary brown adipocytes, we have shown that Elovl3 expression is regulated by all three PPAR isoforms and that maximal expression of Elovl3 is achieved by a combined mixture of norepinephrine, dexamethasone and rosiglitazone (Paper I) (Jorgensen et al., 2007). This effect was shown to be synergistic, involving both transcriptional activation.
and mRNA stability. In the liver of male mice, diurnal rhythm of Elovl3 expression is independent of PPARα, whereas in BAT the expression is partially PPARα dependent (Brolinson et al., 2008; Jakobsson et al., 2005). In addition, norepinephrine-induced Elovl3 expression is only evident in BAT and not in liver (Jörgensen, unpublished data), suggesting different roles of nuclear receptor regulation of Elovl3 expression in liver and BAT.

5.1.3.2. Elovl3 expression in white adipose tissue

When subjected to cold, mice defend their body temperature by channeling fatty acids to BAT and muscles for the production of heat by mitochondrial uncoupling and shivering, respectively. Congruent with the role of ELOVL3 in BAT during increased energy demand and β-oxidation (Jakobsson et al., 2005), increased Elovl3 expression was also seen in inguinal WAT (iWAT; Paper III) and gonadal WAT (gWAT) of females (Zadravec, unpublished results) during cold exposure. Maximal expression of Elovl3 in BAT is evident within 24-72 hours of cold-exposure followed by a gradual decline, a phenomenon also seen for Ucp1 (Paper III) (Westerberg et al., 2006). In contrast, the cold-induced expression of Elovl3 in iWAT is sustained and does not decline with prolonged cold exposure. The existence of cold-stimulated Elovl3 and Ucp1 expression in iWAT is most likely due to infiltration of brown fat cells sequestered within the WAT depot rather than an induced expression in white adipocytes themselves. However, it was recently shown that brown fat cells embedded within the WAT do not share the same origin as interscapular brown adipocytes (Seale et al., 2008). Additionally, as in the case of liver, we detected gender-specific differences in the regulation of Elovl3 expression in gWAT depots, suggesting that the regulation and function of ELOVL3 within the WAT depots could be different from that of the interscapular BAT (Paper III).

5.1.3.3. Elovl3-ablated mice are resistant to diet-induced weight gain

Ablation of Elovl3 results in a mouse strain with a distinct skin phenotype with tousled fur and impaired skin barrier function as a consequence of altered C20-C24 VLCFA synthesis, TG and sebum formation (Westerberg et al., 2004). Despite elevated food intake during cold exposure, Elovl3<sup>−/−</sup> mice have less body fat as compared to control mice (Paper III) (Westerberg et al., 2006). Due to the impaired skin barrier function, Elovl3<sup>−/−</sup> mice require an elevated heat production for the maintenance of body temperature leading to reduced
amounts of TG content in adipose tissue. However, at thermoneutrality when the mice do not require additional energy to maintain the body temperature, BAT is depleted of fat, suggesting that other factors than skin barrier dysfunction are involved in the reduced amounts of fat. Intriguingly, in mice housed at 24°C, Elovl3 ablation gives rise to a diminished body weight when the animals reach an age of about 15 weeks, regardless of gender (Paper II). The reduced body weight is also apparent when feeding the mice a high-fat diet, an effect that is more pronounced in female mice. The Elovl3/ mice display severely reduced adiposity, a marked reduction in hepatic lipid synthesis, low VLDL-TG plasma content and a decreased ratio between food intake and energy expenditure (Figure 6). The resistance to diet-induced obesity is associated with a reduced expression of lipogenic genes in the liver suggesting that ELOVL3-derived fatty acids in the liver might act as signals, thus regulating systemic metabolic homeostasis.

![Figure 6](image.png)

**Figure 6. Increased oxygen consumption during the light period in Elovl3-ablated mice.** Oxygen consumption varies during the day as a consequence of feeding behavior and physical activity. Elovl3-ablated mice have augmented oxygen consumption during the light period of the day as compared to wild-type mice. Interestingly, this coincides with high hepatic expression of Elovl3 in the wild type mice. Therefore, it is tempting to speculate that the absence of ELOVL3-derived fatty acids in the liver of the ablated mice causes systemic changes in metabolic homeostasis. Adapted from (Paper II) and (Brolinson et al., 2008).

The ablation of Elovl3 reduces the amount of specific fatty acids such as C20:0, C22:0, C20:1n-9 and erucic acid (C22:1n-9) in the liver (Paper II), which is consistent with earlier observations of the enzyme in BAT (Jakobsson et al., 2005; Westerberg et al., 2006).
Since C22:1n-9 has been described as an important fatty acid in lipid metabolism and pathology (Charlton et al., 1975), it is tempting to suggest that a reduced amount of endogenously synthesized C22:1n-9 could, in part, be the cause of the disturbed lipid metabolism seen in the Elovl3−/− mice.

It is noteworthy that reduced levels of VLDL-TG in serum, as a result of eight weeks on high-fat diet, is associated with reduced expression of Elovl3 in the liver, while the expression is induced in BAT during the same condition (Paper II, III). As for cold exposure, chronic intake of a high caloric diet is associated with increased sympathetic drive resulting in recruitment of BAT, enhanced thermogenesis (Rothwell and Stock, 1979), and induced Elovl3 expression in BAT; this is not the case in liver where the gene is not regulated by norepinephrine. During high-fat diet, Fas expression is reduced (Paper VI), as fat accumulation in the liver should be avoided. Accordingly, the hepatic expression of Elovl3 should be reduced in mice on a high-fat diet, as the role of ELOVL3 is to promote TGs and lipid droplet formation. However, whether this is a direct (structural) effect or a secondary effect due to fatty acid signaling, is yet not elucidated.

5.1.4. Elovl7

The VLCFA elongase most recently identified is ELOVL7, which, like Elovl3, shows a rather consistent tissue-specific pattern of expression (Tamura et al., 2009). Under normal physiological conditions Elovl7 is expressed at high levels in the kidney, pancreas, adrenal glands and prostate, being further induced in connection with cancer in the latter organ. Under pathophysiological conditions, such as cancer, the ELOVL7 protein appears to be mistargeted from the ER to the cytosol. Knockdown of Elovl7 in carcinoma cell lines reduces their contents of saturated C20, C22 and C24 fatty acids and dramatically attenuates cell growth. In addition, microsomes containing over-expressed levels of ELOVL7 have been utilized to confirm that this enzyme is involved in the elongation of saturated fatty acids with as many as 24 carbon atoms.

Recently, a patient was identified with a mitochondrial encephalomyopathy which was caused by a homozygous triple gene deletion on chromosome 5 (Janssen et al., 2009). The deletion encompassed the NDUFAF2 gene, encoding CI assembly factor 2, the ERCC8 gene involved in the transcription-coupled nucleotide excision repair pathway, and ELOVL7. Due to the use of human skin fibroblast in this study, which did not express Elovl7, no difference was seen in fatty acid composition. However, liver biopsies from the patient revealed lipid
inclusions in the ER, which could be indicative of a disturbance in fatty acid metabolism, suggesting a possible involvement of the *ELOVL7* deletion in this pathology. Unfortunately, due to the lack of research material, the hypothesis was not further investigated.

### 5.2. Elongases involved in the elongation of PUFAs

Because mammals lack the Δ12 and Δ15-desaturases present in plants, they cannot synthesize fatty acids of the n-6 and n-3 series *de novo* (Wallis et al., 2002). Therefore, linoleic (C18:2n-6) and α-linolenic (C18:3n-3) acids, which are required for normal growth, development and function, have to be derived from the diet (Burr and Burr, 1973). C18:2n-6 and C18:3n-3 serve as precursors for the synthesis of longer PUFAs, including arachidonic (C20:4n-6) and docosahexaenoic acid (C22:6n-3, DHA) required for various physiological functions (Jump, 2002; Spector, 1999). Among these functions are regulation of the composition and fluidity of cell membranes, signaling, and gene expression. Moreover, oxygenated metabolites of the various PUFAs such as eicosanoids regulate a myriad of important signaling pathways (Capdevila et al., 2002; Funk, 2001; Serhan et al., 2008). Biosynthesis of PUFAs of the n-6 and n-3 series occurs via sequential desaturation, elongation (Sprecher, 2000) and partial degradation steps (Voss et al., 1991).

#### 5.2.1. *Elov5*

*Elov5* is expressed to some extent in all tissues tested to date, with highest levels in the liver, testis and adrenal glands (Leonard et al., 2000). Gene expression studies on the rat suggest that this enzyme plays an important role in development of the liver during the postnatal period (Wang et al., 2005). In mouse liver, *Elov5* is regulated by LXRα activation of SREBP-1c, which binds to an SRE motif in the proximal region of the *Elov5* promoter (Qin et al., 2009). However, by using the same LXR ligand and over-expressing SREBP-1c, Wang and colleagues did not see an induction of *Elov5* in primary rat hepatocytes (Wang et al., 2006b). Rather, they suggest that *Elov5* is regulated by a PPARα-dependent pathway. The cause of this discrepancy could be due to specie differences as well as the use of liver tissue vs. primary hepatocytes in the latter study.

ELOVL5 has been shown to be involved in the elongation of PUFA of 18 and 20 carbons in length (Moon et al., 2009; Wang et al., 2008). Over-expression of *Elov5* in
primary rat hepatocytes increases the conversion of C20:4n-6 and eicosapentaenoic acid (C20:5n-3) into adrenic acid (C22:4n-6) and docosapentaenoic acid (C22:5n-3) respectively (Wang et al., 2008). In liver microsomes from Elovl5-ablated mice there is a reduction of the elongation activity of γ-linolenic acid (C18:3n-6) and stearidonic acid (C18:4n-3), thereby reducing the hepatic content of C20:4n-6 and C22:6n-3. This results in a failure to suppress the SREBP-1c activity in the liver leading to an increased activation of SREBP-1c regulated genes and, consequently, the mice accumulate hepatic TGs and develop hepatic steatosis (Moon et al., 2009).

Figure 7. Daily patterns in mRNA levels of the elongases in male liver. Diurnal variation of the elongases in male liver were quantified using real-time PCR. Zeitgeber time (ZT). ZT0 is defined as light on and ZT12 as lights off.

As for Elovl3, we have found that the mRNA level of Elovl5 displays a diurnal variation in mouse liver supporting the existence of positive-negative feedback regulation in the synthesis and maintenance of proper fatty acid homeostasis in the liver (Figure 7; Paper VI). Indeed, SREBP-1 protein has been shown to peak at the end of the dark phase, at the same time as Elovl5 expression peaks (Brewer et al., 2005). In this context, it would be interesting to see if, in addition to the increased activity, the diurnal variation of SREBP-1c is changed in the Elovl5-ablated mice. In addition, we have seen a twofold increase of Elovl5 during BAT recruitment (Paper VI), a process which is paralleled by a twofold increase of C20:4n-6 content in the mitochondrial membrane (Ocloo et al., 2007), supporting a role of ELOVL5 in the improvement of fatty acid oxidation.
5.2.2. Elovl2

Elovl2 was identified as Ssc2 (sequence similarity to cig30-2) on the basis of its sequence homology to Elovl3 (Cig30) (Tvrdik et al., 2000). Yeast complementation studies indicated a possible role for the enzyme in PUFA elongation, and subsequent experiments confirmed a substrate specificity for 20:4n-6, docosatetraenoic acid (22:4n-6), eicosapentaenoic acid (20:5n-3; EPA), and 22:5n-3 in order to produce C24:4n-6 and C24:5n-3 (Kitazawa et al., 2009; Leonard et al., 2002; Moon et al., 2001; Wang et al., 2008). The latter two are also recognized as substrates for Δ6-desaturase and precursors for docosapentaenoic acid (C22:5n-6; DPAn6) and C22:6n-3 synthesis, respectively.

Expression of Elovl2 is highest in the testis and liver, but the corresponding mRNA can also be detected at significant levels in the kidney, brain, lung and WAT (Tvrdik et al., 2000; Wang et al., 2005).

Although the regulation of the Elovl2 gene remains to be elucidated, some evidence suggests that the lipogenic transcription factor SREBP-1a, is involved (Horton et al., 2003). Furthermore, over-expression of SREBP-1c in primary rat hepatocytes induces the expression of Elovl2 (Wang et al., 2006b). Nevertheless, in the same study, treatment of the hepatocytes with insulin, glucose or an agonist of LXR did not alter the expression of Elovl2, indicating that regulation by SREBP-1c may not be so pronounced. Recently, we demonstrated that over-expression of Elovl2 in both 3T3-L1 and F442A cells promotes accumulation of lipid droplets, associated with enhanced fatty acid uptake and induction of PPARγ target genes such as fatty acid-binding protein-4 (FABP4/aP2) and diacylglycerol acyltransferase-2 (DGAT2) (Paper IV) (Kobayashi et al., 2007). In contrast to Elovl5, expression of Elovl2 in the liver is not influenced by fasting and refeeding or by dietary supplementation with fish or olive oils (Wang et al., 2005).

C20:5n-3 is known to regulate hepatic gene expression, partially via SREBP-1, as well as through a PPARα-dependent pathway (Sugiyama et al., 2008). Treatment of primary rat hepatocytes with C20:5n-3 induced the expression of cytochrome P450 4A (CYP4A), cytosolic fatty acid thioesterase-1 (CTE1) and mitochondrial hydroxymethylglutaryl-CoA synthetase (mtHMG-CoA synthase), whereas the mRNA levels of SREBP-1c were reduced (Wang et al., 2008). In cells over-expressing Elovl2, induced expression of PPARα-regulated genes was attenuated, with no effect on SREBP-1c regulated genes. These results suggest a possible role for ELOVL2 in modulating the activity of nuclear receptors involved in the control of lipid metabolism.
5.2.2.1. Attenuated PUFA synthesis in Elovl2/+ mice

With the use of homologous recombination we have recently obtained Elovl2/+ mice (Paper V). In accordance to the proposed role of ELOVL2 in the elongation of C20 and C22 PUFAs, serum analysis of the Elovl2/+ mice displayed increased levels of C20:5n-3 and C22:5n-3, while C22:5n-6 was reduced. In the testis, the Elovl2/+ mice exhibited increased levels of C22:4n-6 accompanied by vastly reduced levels of C22:5n-6 and C24:5n-6 products. Astonishingly, the mice exhibited an almost complete abolishment of the elongation products of C24:5n-6; that is C26:5n-6, C28:5n-6 and C30:5n-6 (Figure 8). These results indicate a novel role of ELOVL2 in the formation of very long-chain PUFAs (VLCPUFAs ≥C26). In the Elovl2/+ mice, lipid analysis revealed that PUFA synthesis was differentially affected with no major changes in either brain or liver but with clear effects of C28:5n-6 and C30:5n-6 in the testis.

Figure 8. Testicular PUFA and VLCPUFA biosynthesis. The essential fatty acids 18:3n-3 and 18:2n-6 are obtained from the diet and are subsequently elongated and desaturated by the indicated enzymes into longer PUFAs. ELOVL5 is involved in the elongation of PUFAs up to 22 carbons in length. ELOVL2 elongates 20:5n-3 and 22:5n-3 to form 24:5n-3. Specifically, in the testis, ELOVL2 is involved in the biosynthesis of VLCPUFA (≥C26) up to 30 carbons in length of the n-6 family, gray background.
5.2.2.2. Impaired fertility in Elovl2\(^{+/-}\) male mice

Breeding of Elovl2\(^{+/-}\) mice for further production of Elovl2\(^{-/-}\) mice revealed both severe fertility problems in male Elovl2\(^{+/-}\) mice, as specified by reduced production of offspring, and an impaired Mendelian ratio, suggesting both pre- and post-meiotic deficiency of spermatogenesis in Elovl2\(^{+/-}\) mice (Paper V). Histological analysis of Elovl2\(^{+/-}\) testis showed regular seminiferous tubules displaying normal Sertoli cells; however, the mice displayed abnormal sperm morphology with numerous spermatozoa displaying a rounded condensed head (Figure 9). Elovl2\(^{-/-}\) mice displayed markedly reduced testicular size and abnormal testicular morphology. The mice displayed spermatogonia and primary spermatocytes, but lacked further germinal cells. The spermatogonia and spermatocytes fused with adjacent cells forming multinucleate giant cells in the lumen of the seminiferous tubule. This suggests an absolute prerequisite for ELOVL2-derived fatty acids for normal progression of spermatogenesis.

Figure 9. Testis histology showing mature spermatozoa with elongated head (arrow) in the seminiferous tubules of wild type mice, Elovl2\(^{+/-}\). Elovl2\(^{+/-}\) testis showing numerous spermatozoa displaying a rounded condensed head (arrow). The seminiferous tubules of the Elovl2\(^{-/-}\) mice exhibit only spermatogonia and primary spermatocytes with multinucleate giant cells in the lumen (arrow).
5.2.2.3. Necessity of ELOVL2-derived fatty acids during embryogenesis

Heterozygous breeding does not produce offspring in a normal 1:2:1 Mendelian ratio. Rather, there is a 1:1 ratio between \textit{Elovl2}^{+/+} and \textit{Elovl2}^{+/−} with very few \textit{Elovl2}^{−/−} mice born, suggesting a haplodeficiency and that the two-allele ablation of \textit{Elovl2} is partially embryonically lethal. The reason for the embryonic lethality has not been further investigated; however, it supports an essential requirement for \textit{Elovl2} for normal embryological development. Indeed, indications of a possible role of ELOVL2-derived fatty acids during embryogenesis recently came with the finding that \textit{Elovl2} expression is temporally regulated during embryogenesis in zebrafish, and that the mRNA levels are paralleled by embryonic production of C22:6n-3 (Monroig et al., 2009). Interestingly, the finding that \textit{Elovl2} mRNA was already present at the zygote stage prior to gene activity, suggests that maternal transfer of \textit{Elovl2} mRNA supplies the early embryo with the proper tools for PUFA synthesis. In addition, \textit{Elovl2} is expressed both in the liver and brain (www.genepaint.org) of developing embryos (Wang et al., 2005), suggesting that ELOVL2-derived fatty acids have an imperative role for normal embryonic development.

5.2.3. \textit{Elovl4}

Stargardt-like macular dystrophy (STGD3) and autosomal dominant macular dystrophy (adMD) are two inherited forms of macular degeneration causative of a five-base pair deletion in the \textit{Elovl4} gene. The deletion results in a frame-shift, generating a premature stop codon, which in turn truncates 51 aa from the C-terminal end, including the ER retention signal (Zhang et al., 2001). This was the first finding that biosynthesis of fatty acids is involved in the pathogenesis of at least two related forms of macular degeneration. Subsequently, several different mutations and deletions in the \textit{Elovl4} gene have been associated with dominant macular dystrophy pathogenesis in humans (Bernstein et al., 2001; Maugeri et al., 2004). Mice heterozygous for \textit{Elovl4} ablation do not show any early signs of macular degeneration; however, those heterozygous for the five base pair deletion display the major features of the retinal pathogenesis exhibited by STGD3 patients (Karan et al., 2005b; Li et al., 2007b). This suggests that a dominant negative effect, rather than haploinsufficiency, is the primary mechanism of retinal degeneration in STGD3 patients. The dominant negative effect is supported by the finding that the mutant ELOVL4 forms aggregates with the wild-type protein, mislocalizing it to the cytosol rather than the ER, causing a null phenotype.
Abnormal protein accumulation and protein trafficking defects are potentially damaging for the cell and are the cause of several degenerative diseases. To avoid these situations, the ubiquitin proteosomal system quickly degrades proteins; thus, the level of ubiquitinated proteins is an indicator of cellular stress. Mice carrying the 5-bp deletion knock-in exhibit an accumulation of both the ELOVL4 protein as well as other ubiquitinated proteins in the retina, suggesting that retinal degeneration seen in the pathology could be caused by protein trafficking defects (Vasireddy et al., 2009). Besides retina, *Elovl4* is also expressed in brain, skin, lens and testis (Mandal et al., 2004). The *Elovl4*-ablated mice die within a few hours after birth due to dehydration caused by a reduction of specific ceramides and saturated VLCFA >C26 in the epidermis of the mutant mice (Cameron et al., 2007; Li et al., 2007a). ELOVL4 is essential for the formation of saturated VLCFA from C28 and beyond and, surprisingly, is also involved in the formation of VLC-PUFA such as 32:6n-3, 34:6n3 and 36:6n3 (Agbaga et al., 2008; Tikhonenko et al., 2009). This is the first finding of an elongase having dual substrate specificity for both saturated VLCFAs and PUFAs.

It is noteworthy, that, in a recent publication, *Elov12* and *Elovl4* expression was shown to be reduced in the retina of diabetic rats, which was associated with a reduction of C22:6n-3 and C32:6n-3, suggesting that both *Elov12* and *Elovl4* are involved in the synthesis of VLCPUFAs (≥C26) (Tikhonenko et al., 2009). Since the *Elov12*-ablated mice accumulate C22:4n-6 as a result of attenuated elongation activity, the absence of C24:5n-6, C26:5n-6, C28:5n-6 and C30:5n-6 could be due to inappropriate substrate for further elongation. We cannot neglect that other elongases and desaturases play a role in the lipid composition seen in the testis of these mice. However, the *Elov12*+/− mice did not display any changes in the level of C22:5n-6, but, did have a decrease in the levels of C28:5n-6 and C30:5n-6, suggesting that ELOVL2 plays an imperative role in the elongation of VLCPUFAs. Interestingly, as stated above, ELOVL4 has recently been implicated to be involved in the synthesis of VLCPUFAs of 28 to 38 carbons in length of the n-6 family. Therefore, there might exist an n-3/n-6 specificity were, ELOVL2 is involved in the elongation of fatty acids of the n-6 family and ELOVL4 of the n-3 family. Nevertheless, in order to clarify the mechanistic contribution in the abolishment of VLCPUFAs seen in the *Elov12*-ablated mice, further analysis in respect of fatty acid substrate specificity is necessary.
6. DESATURATION OF FATTY ACIDS

Besides the variation in chain length, the acyl chain of a fatty acid can be desaturated by the introduction of a position specific double bond performed by acyl-CoA desaturases. Mammalian cells express Δ9-, Δ6-, and Δ5-desaturase activities, where the Δ number indicates the carbon position at which the double bond is introduced, counting from the carboxylic acid moiety. The n-6 and n-3 series of fatty acids are based counting from the methyl end. As with elongases, mammalian desaturases can be divided into two distinct families referred to as stearoyl-CoA desaturases (SCDs), (Paton and Ntambi, 2009) and fatty acid desaturases (FADS) (Nakamura and Nara, 2004), based on their substrate preference. SCDs preferred saturated fatty acids as substrate while FADS preferred PUFAs (Figure 5).

6.1. Acyl chain desaturation performed by stearoyl-CoA desaturases (SCDs)

SCDs catalyze the introduction of a cis double bond at the Δ9 position of C16:0 and C18:0, producing the monounsaturated fatty acids C16:1n-7 and oleic acid (C18:1n-9) (Ntambi, 1999). There are four isoforms of SCDs (SCD-1-4) identified in mouse, of which SCD-1 has been most extensively characterized (Kaestner et al., 1989; Miyazaki et al., 2003; Ntambi et al., 1988; Zheng et al., 2001). Mice with a global disruption of Scd-1 have impaired lipid synthesis, reduced fat depots, and are protected against diet-induced obesity (Miyazaki et al., 2001; Ntambi et al., 2002). Moreover, a number of dietary and genetic challenges that induce obesity, hepatic steatosis and insulin resistance in wild-type mice fail to do so in Scd-1⁻/⁻ mice (Flowers and Ntambi, 2008). A complex combination of metabolic and signaling mechanisms in the Scd-1⁻/⁻ mice appear to reduce rates of lipid synthesis (Chu et al., 2006) and enhance lipid oxidation (Dobrzyn et al., 2004), thermogenesis (Lee et al., 2004) and/or insulin sensitivity (Rahman et al., 2003; Rahman et al., 2005) in tissues such as liver, muscle and adipose tissue.

Two mice strains with conditional Scd-1 ablation in liver and skin have revealed the necessity of tissue-specific gene targeting (Miyazaki et al., 2007; Sampath et al., 2009). First, in contrast to the global ablation, liver-specific Scd-1 ablated mice are not protected against high-fat feeding adiposity and liver steatosis; however, they are protected from carbohydrate-induced obesity (Miyazaki et al., 2007). Secondly, skin specific Scd-1 ablation, recapitulates all major hypermetabolic effects associated with global ablation, including increased lipid oxidation and thermogenesis (Sampath et al., 2009). These two mouse strains reveal the
complexity and difficulties in understanding and elucidating metabolic changes due to global gene ablation.

6.2. Acyl chain desaturation of PUFA performed by fatty acid desaturases (FADS)

FADS1 (Δ5-desaturase) and FADS2 (Δ6-desaturase) both catalyze the introduction of a double bond at Δ5 and Δ6 position, respectively (Cho et al., 1999a; Cho et al., 1999b). Recently, Fads3 was identified, establishing that several forms of the FADS3 protein are expressed differentially in various tissues (Pedrono et al., 2009). However, the functions of these various forms remains unknown and no activity has yet been described.

The human FADS1 was cloned (Cho et al., 1999b) on the basis of its sequence homology with the human FADS2, previously identified by the same group (Cho et al., 1999a). Initially, these authors demonstrated that over-expression of FADS1 in CHO cells promotes Δ5-desaturation of C20:3n-6 to yield C20:4n-6. Subsequently, the ability of FADS1 to utilize both C20:3n-6 and C20:4n-3 as substrate was revealed (de Antueno et al., 2001; Matsuzaka et al., 2002a).

FADS2 catalyses the initial step in the biosynthesis of C20:4n-6, C20:5n-3 and C22:6n-3, which are major PUFA constituents of phospholipids and substrates for several biological signaling molecules. Two different FADS2 knockout strains have recently been developed, providing convincing evidence that no other isoform of Δ6-desaturase can act on 18-carbon precursors of PUFA (Stoffel et al., 2008; Stroud et al., 2009). These important findings are consistent with the characteristics of skin fibroblasts isolated from a patient with a deficiency in this enzyme (Williard et al., 2001). The major phenotype in both studies of Fads2 ablation was infertility. Despite a global depletion of PUFAs greater than 18 carbons in length and an inability to synthesize prostaglandins, leukotrienes and thromboxanes, Fads2-ablated mice were reported, by Stoffel and colleagues, to be viable and displayed an normal lifespan (Stoffel et al., 2008). In contrast, Stroud and colleagues revealed that FADS2 deficiency is not associated with total depletion of PUFAs (Stroud et al., 2009). Moreover, they observed that the deficient mice develop severe ulcerative dermatitis and ulceration of the small intestine. These latter two findings might reflect impaired eicosanoid synthesis due to insufficient availability of the appropriate PUFA precursors. In both cases, several, but not all, of the alterations associated with the ablation were rescued by dietary supplementation with C20:4n-6 or C20:5n-3/C22:6n-3.
A Δ8-desaturase activity detected in a variety of mammalian cells (Cook et al., 1991) and tissues (Schenck et al., 1996) may provide an alternative route for the synthesis of C20:4n-6 and C20:5n-3 from C18:2n-6 and C18:3n-3, respectively, that does not involve a Δ6-desaturation (Figure 5). In this case the substrates for the Δ8-desaturation are C20:2n-6 and C20:3n-3, but the elongase involved in the synthesis of these particular fatty acids has not yet been clearly identified. However, since the levels of C20:4n-6 and C22:6n-3 are markedly attenuated in the Elovl5−/− mice, it is tempting to speculate that elongation of C18:2n-6 to C20:2n-6 and of C18:3n-3 to C20:3n-3 is mediated by ELOVL5.

Recently, over-expression of FADS2 cDNA in yeast promoted the Δ8-desaturation of C20:2n-6 and C20:3n-3 to C20:3n-6 and C20:4n-3, respectively (Park et al., 2009), thereby confirming that the Δ6-desaturase encoded by the Fads2 gene also has Δ8-desaturase activity. Consistent with this finding, is the observation that Fads2−/− mice lack the Δ8-desaturation pathway (Stroud et al., 2009).

7. DISCUSSION

Recent findings have highlighted the necessity for proper fatty acid composition in the maintenance of normal whole-body energy homeostasis (Matsuzaka et al., 2007; Moon et al., 2009; Ntambi et al., 2002). Therefore, there is a drive in understanding the tissue-specific contribution of fatty acid modifying enzymes. As key regulators of the aliphatic chain length, the role of the elongases in determining the outcome of cellular fatty acid composition is indisputable. Recently, several animal models have been developed specifying the necessity of the tissue-specific function of saturated, monounsaturated and polyunsaturated VLCFAs.

Fertility and embryological problems associated with fatty acid metabolism

Several animal models related to alterations in the biosynthesis of different VLCFAs, especially PUFAs, have been associated with reproduction and embryonic developmental problems. For instance, Elovl6-ablated mice display partial embryonic lethality, although surviving male and female mice are both fertile (Matsuzaka et al., 2007). Elovl4-ablated mice are born with predicted Mendelian frequency, however, as stated previously, these mice die within a few hours after birth due to dehydration (Li et al., 2007a; Li et al., 2007b). Interestingly, another Elovl4-ablated mouse line was developed by Raz-Prag and colleagues,
which did not produce any $Elovl4^{-/-}$ offspring (Raz-Prag et al., 2006). Instead, the pups died in utero a couple of days before birth. The reason for this discrepancy is not known, however this is not the first report where two different knock-out strains exhibit dissimilar phenotypes.

The significance of PUFAs in fertility has recently been emphasized by the appearance of $Elovl2^{-/-}$, $Elovl5^{-/-}$ and $Fads2$-ablated mice. Breeding of mice heterozygous for $Elovl5$ ablation, produced offspring in a normal Mendelian ratio (Moon et al., 2009). However, the majority of the female $Elovl5^{-/-}$ mice were infertile, whereas the ablation did not have any impact on male fertility. This is in sharp contrast to the $Elovl2$-ablated mice, where the male mice are sterile and the female mice are unaffected.

As previously mentioned, the major phenotype of $Fads2$-ablated mice is infertility (Stoffel et al., 2008; Stroud et al., 2009). The first report described both male and female sterility along with marked hypogonadism (Stoffel et al., 2008), while the second study reported male sterility without hypogonadism (Stroud et al., 2009). In both cases, crossing heterozygous mice yielded pups in a normal Mendelian ratio. In the second report by Stroud and colleagues, the heterozygous mice displayed a fatty acid composition similar to those of wild-type (Stroud et al., 2009). However, cholesterol ester species in the liver from the heterozygous mice tended to fall to a level between those of wild-type and ablated mice, suggesting that a single copy of $\Delta6$-desaturase cannot fully compensate to gain optimal PUFA synthesis. Interestingly, as for the $Elovl2^{+/-}$ mice, $Fads2$-ablated mice displayed tissue-specific effects on PUFA synthesis with markedly reduced levels of C20:4n-6 in the liver and with only mild effects in the brain.

One distinguishing feature between $Fads2$-ablated mouse models and the $Elovl2^{+/-}$ mice is that the fertility is severely affected in $Elovl2^{+/-}$ mice already at a heterozygous stage. The $Elovl2^{-/-}$ mice exhibit multinucleated cells in the lumen of the seminiferous tubules, and the outcome of the heterozygous breeding is close to a 1:1 ratio between wild-type and $Elovl2^{+/-}$ mice, indicative of both pre- and post-meiotic deficiency of spermatogenesis.

The histology of the testis of the $Elovl2^{-/-}$ mice resembles in part the morphology seen in two mouse models with disruption of the gene epimorphin (Akiyama et al., 2008; Wang et al., 2006a). Epimorphin is a member of SNARE family of proteins, which plays an essential role in membrane fusion at the terminal step of cytokinesis and vesicle fusion during exocytosis (Low et al., 2003). Interestingly, epimorphin has been shown to be localized to the sperm head where it mediates fusing events during the acrosome reaction upon reaching zona pellucida (Hutt et al., 2005). Therefore, it is tempting to speculate that the VLCPUFAs produced by ELOVL2 might be key components during specific cellular events such as germ
cell meiosis and acrosome reaction which both involve membrane fusion and cellular curvature stress. Considering the expression of *Elovl2* and the new insights about the possible function of ELOVL2, besides testis, there might be similar cellular events in other tissues, requiring ELOVL2-derived VLCPUFAs. For instance, SNARE proteins have recently been implicated to be involved in lipid droplet fusion (Bostrom et al., 2007) and possibly VLDL formation (Swift et al., 2003). Therefore, it will be interesting to further analyze the involvement of ELOVL2-derived VLCPUFAs in other tissues such as liver.

**Skin specific effects on fatty acid metabolism**

Two different mouse strains with a mutation in the *Elovl3* gene are presently available: *Elovl3* knockout mice obtained by homologous recombination (Westerberg et al., 2004), and the *scraggly* strain which carries a frameshift mutation caused by the drug chlorambucil resulting in a drastic reduction in expression (Herron et al., 1999). The striking features that distinguish both of these strains from wild-type mice are tousled fur and an imbalance in the lipid content of the sebum. The distinct hair phenotype is due to attenuation or elimination of the expression of *Elovl3* in the inner cell layer of the outer root sheath and in the sebocytes of the sebaceous glands. However, the most pronounced difference between the *Elovl3*-knockout mice and the *scraggly* strain is the general hyperplasia of the sebaceous and meibomian glands present exclusively in the former model. The explanation for this difference remains unclear but may involve low-level expression of *Elovl3* in the *scraggly* animals.

Interestingly, in addition to *Elovl3*-ablation, dysfunctional mutations in SCD-1, -2 and -3, DGAT1 and acyl-CoA binding protein (ACBP), all give rise to abnormalities in the composition of sebaceous lipids and defective skin and eye characteristics (Chen et al., 2002; Lee et al., 2007; Miyazaki et al., 2000; Parimoo et al., 1999; Sundberg et al., 2000; Zheng et al., 1999). However, mice with liver specific ablation of *Scd-1* do not display either alopecia, dry eye syndrome or atrophy of the sebaceous glands, indicating that these effects are due to local reduction of monounsaturated fatty acids, rather than a systemic effect (Miyazaki et al., 2007).

**Dietary vs. endogenously-synthesized fatty acids**

Ingestion of dietary fatty acids leads to their distribution throughout the body with effects on membrane composition and function, eicosanoid synthesis, and gene transcription.
However, cell-specific lipid metabolism and the expression of fatty acid-regulated transcription factors likely play important roles in determining how cells respond to changes in lipid homeostasis. It is alleged that fatty acids in the diet can fully compensate for the loss of endogenous synthesis. However, the finding that anti-obesity effects associated with reductions in *Scd-1*, either through ablation or knock-down studies, occur despite high abundances of MUFAs in the diet, suggests that the endogenously synthesized MUFAs are distinct from those derived from the diet (Jiang et al., 2005). Likewise, though dietary supplementation rescued several of the effects associated with *Fasd2* ablation, it did not rescue all (Stoffel et al., 2008; Stroud et al., 2009). Interestingly, dietary supplementation of 20:4n-6 could not fully compensate for the loss of prostaglandin synthesis in the *Fasd2*−/− mice, even though the ablated mice had elevated levels of 20:4n-6 as compared to the wild type. From this, it is clear that some of the endogenously synthesized fatty acids cannot be rescued by dietary intervention and thus, there exist two distinct “pools” of fatty acids with differential physiological functions.

The similarity of the phenotype between different animal models involved in fatty acid metabolism is indicative of an association of these enzymes, not only in cellular metabolic pathways but also possibly structurally. Indeed, first evidence of a coordinated association of proteins involved in fatty acid and TG synthesis, came with the finding that SCD1 and DGAT2 are co-localized in the ER, where SCD1 serves to provide substrate for the final step in TG synthesis (Man et al., 2006). Therefore, it is likely that other proteins also co-localize with each other including elongases, HADCs and FADS in a complex at the ER-membrane, regulating synthesis, secretion and storage of fatty acids.

The results obtained on lipid composition in *Elovl*-ablated mice further specify the difficulties in interpretation of data on the specific function of the enzymes. Besides compensational effects of other elongases and desaturases, the enzymes are promiscuous in their substrate specificity. For instance, besides PUFAs, ELOVL5 has been implicated in the elongation of mono-unsaturated fatty acids as well (Leonard et al., 2000). Also, ELOVL3 was shown to have low elongation activity for C14:0 and C16:0 (Kitazawa et al., 2009). In addition, during dietary restrictions or gene ablation, minor fatty acid species become abundant as a way to compensate for the deficiency (FULCO and MEAD, 1960; Stroud et al., 2009). Therefore, caution is necessary when assessing a specific function of the elongases.

Alterations of fatty acid homeostasis develop as a complex result of genetic, metabolic, dietary and environmental factors. Development of animal models and
experiments where the genetic and environmental variation are more controlled will provide a useful path for both mechanistic exploration and validation of human data. It is noteworthy that the amount and ratios of C16:0, C18:0, C16:1 and C18:1 seem to be of significant importance since tampering with these fatty acids has severe systemic effects (Cao et al., 2008; Chakravarthy et al., 2009; Matsuzaka et al., 2007; Ntambi et al., 2002). The finding that C16:1n-7 secreted from adipose tissue has the potential to act as a “lipokine” and thereby affect global lipid metabolism, has highlighted the potential of fatty acids not only as cellular signaling molecules, but also regulators of systemic energy homeostasis.

Tikhonenko and colleagues found that diabetes induced pronounced changes in the retinal fatty acid profile, whereas liver fatty acid profile was only slightly changed (Tikhonenko et al., 2009). In addition, the Fads2-ablated mouse has severe reduced levels of C20:4n-6 in the liver while the brain is only mildly affected (Stroud et al., 2009). This suggests that alteration of peripheral tissue fatty acid composition is not simply a result of altered liver metabolism per se, but also a result of peripherally altered expression of fatty acid modifying proteins. Therefore, understanding the regulation and activity of elongases in peripheral tissues might be a way to modulate the fatty acid composition in a tissue specific manner, without systemic alteration due to changes in liver lipid metabolism.

Alterations in cellular fatty acid profiles as a consequence of modulation of elongase expression emphasize the importance of these enzymes in maintaining proper fatty acid homeostasis for cellular function and metabolic control. Unfortunately, purification of these membrane-bound enzymes to homogeneity is a daunting task and has thus made the biochemical characterization of these elongation enzymes rather challenging. Recently, however, a derivative of benzoxazinone was identified as a potent inhibitor for human ELOVL6 and, in mouse; the compound was shown to have dual ELOVL3/ELOVL6 inhibitory activity (Mizutani et al., 2009; Sasaki et al., 2009). The compound was shown to reduce the C18/C16 ration in both liver and serum fatty acid profiles after oral dosing demonstrating potent in vivo inhibitory activity. Further studies using this compound, and others, will be of great importance in understanding the therapeutic applications of elongases as potential drug targets.

Although the general function of the Elovl genes is known, the physiological consequence of the tissue-specific demand for the VLCFAs and PUFAs produced by the different elongases is not fully understood. Despite a growing body of evidence supporting the key role that fatty acid metabolism plays in metabolic diseases, the underlying
mechanistic details by which alterations in tissue specific lipid metabolism are integrated into systemic metabolic homeostasis need further assessment. In order to recognize the etiology of fatty acid associated diseases, it is critical to appreciate the nature and diversity of fatty acids. Understanding the fatty acid specific impact on energy homeostasis will provide key insights into the pathogenesis of these diseases. In conclusion, functional diversity and specificity of fatty acid modifying enzymes enables the cell to maintain a dynamic metabolic balance in response to hormonal signals and varying nutritional status. I believe that improved knowledge about modulation of elongase activity will provide important insight to understanding the etiology of fatty acid associated diseases and their treatment.

8. ACKNOWLEDGEMENTS

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51


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