Progress in Lipid Research 49 (2010) 186–199

Contents lists available at ScienceDirect

Progress in Lipid Research

journal homepage: www.elsevier.com/locate/plipres

Review

The key roles of elongases and desaturases in mammalian fatty acid metabolism: Insights from transgenic mice

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ARTICLE INFO

Article history:
Received 19 November 2009
Received in revised form 9 December 2009
Accepted 10 December 2009

Keywords:
Elongase
Desaturase
VLCFA
PUFA
Knockout mice

ABSTRACT

In mammalian cells, elongases and desaturases play critical roles in regulating the length and degree of unsaturation of fatty acids and thereby their functions and metabolic fates. In the past decade, a great deal has been learnt about these enzymes and the first part of this review summarizes our current knowledge concerning these enzymes. More recently, several transgenic mouse models lacking either an elongase (*Elovl3*$^{-/-}$, *Elovl4*$^{-/-}$, *Elovl5*$^{-/-}$, *Elovl6*$^{-/-}$) or a desaturase (*Scd-1*$^{-/-}$, *Scd-2*$^{-/-}$, *Fads2*$^{-/-}$) have been developed and the second part of this review focuses on the insights gained from studies with these mice, as well as from investigations on cell cultures.

Contents

Abbreviations: CoA, acyl-coenzyme A; DGAT, diacylglycerol acyltransferase; ELOVL, elongation of very-long-chain fatty acids; ER, endoplasmic reticulum; FACE, fatty acyl-CoA elongase; FADS, fatty acid desaturases; FAS, fatty acid synthase; HADC, 3-hydroxyacyl-CoA dehydratase; KAR, 3-ketoacyl-CoA reductase; MUFA, mono-unsaturated fatty acid; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acid; SCD, stearoyl-CoA Desaturase; SREBP, sterol regulatory element binding protein; TER, trans-2,3,-enoyl-CoA reductase; VLCFA, very-long-chain fatty acid (⩾20 °C); VLC-PUFA, very-long-chain polyunsaturated fatty acid.

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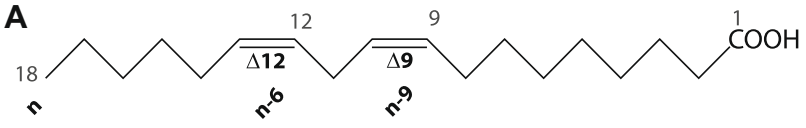
doi:10.1016/j.plipres.2009.12.002

1. Introduction

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 oils, waxes, sterols, glycerophospholipids, sphingolipids and triacylglycerols. 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 [1–3].

This diversity of biological functions is reflected in the fact that most cells have the ability to synthesize fatty acids. Although the simplest form of lipids, the length and pattern of saturation/desaturation of the aliphatic chain of fatty acids (Fig. 1) is essential for their functions. Accordingly, proper elongation and desaturation of fatty acids are essential to the maintenance of lipid homeostasis and disruption of these processes may have devastating consequences.

The well-characterized multisteps of *de novo* synthesis of fatty acids by fatty acid synthase (FAS) is virtually identical in all biological systems [4]. At the same time, during the course of evolution from archaebacteria to mammals, a number of enzymes related to FAS (and which produce a broad spectrum of fatty acid compounds) have developed [5]. In the eukaryotic cell, fatty acids either synthesized in the cytosol by FAS or derived from the diet can be further desaturated and/or elongated into long-chain (16C, 18C) and very-long-chain fatty acids (VLCFAs) ($\geqslant$C20) by specific membrane-bound enzymes localized in the endoplasmic reticulum (ER). As an increasing number of pathophysiological syndromes are being ascribed to impaired lipid homeostasis, VLCFAs are receiving more and more attention. Much of the recent activity in this area, both in our laboratory and in others, has concerned the *in vivo* functions of desaturases and elongases. In the present review we attempt to integrate observations on both of these enzymatic processes into pathways with potentially significant physiological roles in mammals.



n= chain length = 18 carbons

x= 2 double bounds

Cn:x(n-y) → C18:2(n-6)

n-y= n-6
position of the first double bound from the methyl end

B

Symbol	Common Name	
C16:0	Palmitic acid	Saturated fatty acids
C18:0	Stearic acid	
C20:0	Arachidic acid	
C22:0	Behenic acid	
C24:0	Lignoceric acid	
C16:1(n-7)	Palmitoleic acid	Non essential unsaturated fatty acids
C16:1(n-10)	Sapienic acid	
C18:1(n-9)	Oleic acid	
C20:1(n-9)	Gadoleic acid	
C22:1(n-9)	Erucic acid	
C20:3(n-9)	Mead acid	
C18:2(n-6)	Linoleic acid	n-6 and n-3 polyunsaturated fatty acids
C20:4(n-6)	Arachidonic acid	
C22:5(n-6)	Docosapentaenoic acid	
C18:3(n-3)	α-Linolenic acid	
C20:5(n-3)	Eicosapentaenoic acid	
C22:6(n-3)	Docosahexaenoic acid	

Fig. 1. *Fatty acid nomenclature*. (A) As an example, the structure of linoleic acid (C18:2*n* − 6) is given. (B) This table summarizes the common names of various fatty acids which are widely referred to in this review.

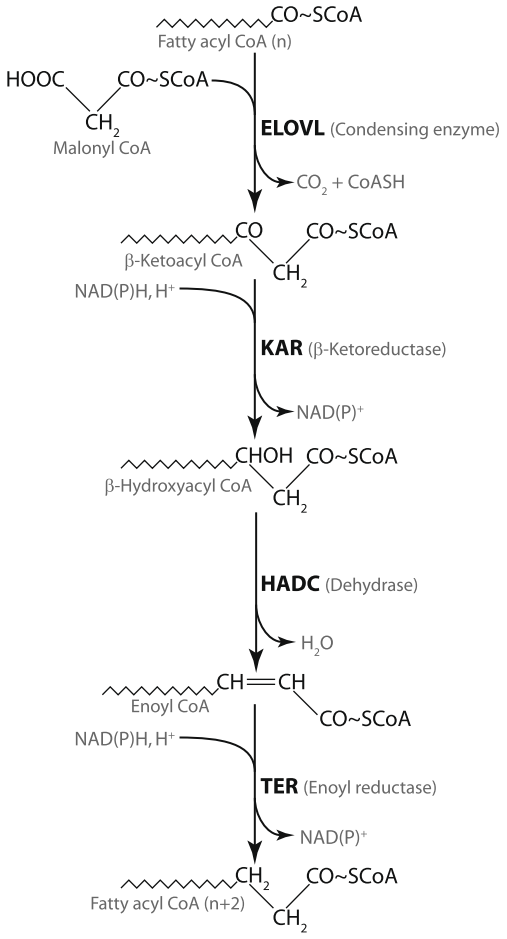


Fig. 2. *Enzymatic steps in long-chain fatty acid elongation*. Enzymatic steps of microsomal fatty acyl chain elongation. ELOVL, elongation of very-long-chain fatty acids; KAR, 3-ketoacyl-CoA reductase; HADC, 3-hydroxyacyl-CoA dehydratase; TER, trans-2,3,-enoyl-CoA reductase.

2. Mammalian elongases and desaturases

As in the case of FAS, the activities of the enzymes involved in both the elongation and desaturation of fatty acids 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. Moreover, there is a pronounced sexual dimorphism in the control of fatty acid homeostasis. The interactions between these various factors are obviously quite complex and, to date, relatively little is know 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 diversity of synthetic pathways that may alter the levels of different fatty acid pools in response to various stimuli. Below, we discuss the individual proteins known to be involved in the control of fatty acid elongation and desaturation.

2.1. Elongases

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. Elongation is achieved with four separate enzymatic reactions (Fig. 2): 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, finally, reduction of trans-2-enoyl-CoA to form the elongated acyl-CoA [6–8]. In mammals, the two reductions are performed by 3-ketoacyl-CoA reductase (KAR) and trans-2,3,-enoyl-CoA reductase (TER), respectively (Fig. 2) [9]. Recently, four mammalian 3-hydroxyacyl-CoA dehydratase (HADC1–4) isoforms, homologous to the yeast Phs1p enzyme have been identified [10,11].

In mammals, the initial and rate-controlling condensation reaction is catalyzed by the elongase enzymes referred to as Elongation of very-long-chain fatty acids (ELOVLs) [10,12,13]. To date, seven ELOVL proteins (ELOVL1–7) have been identified, with ELOVL1, ELOVL3, ELOVL6 and ELOVL7 preferring saturated and mono-unsaturated fatty acids as substrate and ELOVL2, ELOVL4 and ELOVL5 being selective for polyunsaturated fatty acids (PUFAs) [12,14–19]. 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 [11].

All ELOVL proteins contain several stretches of amino acids that are fully conserved in mice, rats and humans [14,20]. For example, the HxxHH motif is absolutely necessary for the formation of 3-ketoacyl-CoA [10]. The overall sequence similarity between these proteins, as well as between the homologous elongases in species as different as nematode and yeast, is approximately 30% [21].

Evidence suggesting the existence of several separate elongation systems with different chain-length specificities was obtained at an early stage [22,23]. One specific Elop, a yeast homolog to ELOVL, can convert fatty acids with different chain lengths into the same elongated fatty acid and, conversely, several different Elops elongate the same fatty acid, to end-products of varying chain lengths. These observations suggest that end-product specificity involves a distinct chain length, rather than a certain number of two-carbon additions [24]. In an elegant study Denic and Weissmann [10] demonstrated that while catalysis of the condensation reaction occurs at the cytosolic surface of the ER membrane, the chain length of the end-product is determined by a lysine residue at the luminal surface. By varying the distance between the active site and this juxta-luminal lysine by one helical turn at a time, these investigators managed to increase the chain length of the end-product by four carbon atoms.

Of the different *Elovl* genes, *Elovl1*, *Elovl5* and *Elovl6* are expressed ubiquitously whereas *Elovl2*, *Elovl3*, *Elovl4* and *Elovl7* display more distinctly tissue-specific levels of expression. Although the physiological consequences of such tissue-specific patterns of expression are not yet known, *in vivo* studies, including those involving several novel transgenic strains of mice, indicate that this is related to tissue-specific demands for the VLCFA synthesized by the different elongases.

As described above, the elongases can be divided into those that preferentially utilize saturated and mono-unsaturated fatty acids as substrate and those that are more selective for PUFAs. This substrate specificity, along with the product formed is related to the metabolic roles of the elongases, as will be discussed below in relation to specific desaturases.

2.2. Desaturases

Acyl-coenzymeA (CoA) desaturases introduce a double bond at a specific position on the acyl chain of long-chain fatty acids, thereby influencing several of the key biological properties of the fatty acid itself and of more complex lipids containing this acyl chain. Mammalian cells express Δ9, Δ6 and Δ5-desaturase activities

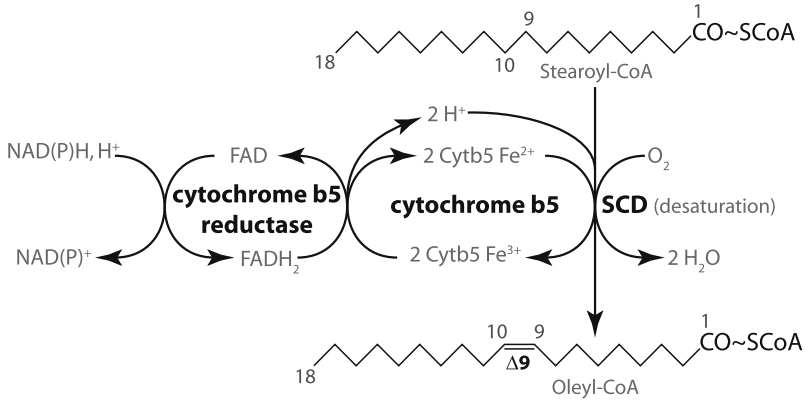


Fig. 3. Δ9-desaturation of stearic acid (C18:0) to oleic acid (C18:1$n-9$) by stearoyl-CoA desaturase (SCD).

where the Δnumber indicates the position at which the double bond is introduced (see Fig. 1). The desaturases can be divided into two distinct families referred to as stearoyl-coA desaturases (SCDs), [25] and fatty acid desaturases (FADS) [26].

2.2.1. Isoforms of SCD

SCDs introduce a single double bond at position Δ9 (Fig. 3) counting from the carboxylic acid moiety of saturated fatty acids such as palmitic and stearic acid. Rat liver Δ9-desaturase (SCD-1) was the first to be purified [27] and has been most extensively, characterized, so that our current view of desaturase properties is based largely on the studies of this protein. In the ER, Δ9-desaturase functions together with NADH, the electron donor cytochrome b_5 and its flavoprotein reductase, and with molecular oxygen (Fig. 3) [25,28–30].

The enzyme exhibits three histidine-rich stretches (His Boxes) containing residues essential for its catalytic activity, whereas there is only one such motif in the ELOVL proteins. These three regions are thought to bind the iron within the catalytic center [31]. The total amino acid sequence indicates that the desaturase has four membrane-spanning regions, with the *N*- and *C*-termini exposed at the cytosolic surface of the ER. Determination of the amino acid sequence of rat liver Δ9-desaturase allowed the *Scd-1* gene to be cloned from this species [32] and, subsequently, from mouse as well [33]. Regulation of *Scd-1* transcription by hormonal, nutritional and pharmacological stimuli has been extensively investigated. In contrast to its partners, cytochrome b_5 and NADH-cytochrome b_5 reductase, the SCD-1 protein demonstrates a rapid turnover, making it likely that proteolysis [30] also contributes to regulation of its activity. A detailed model for the membrane topology of mouse SCD-1 has recently been proposed [34]. Furthermore, SCD-1 is co-localized with diacylglycerol acyl transferase 2 (DGAT2) in the ER [35], which may explain why mono-unsaturated fatty acids (MUFAs) produced endogenously are incorporated more efficiently into non-polar lipids than are dietary MUFAs.

SCD-1 and the three other isoforms (SCD-2, -3 and -4) identified in the mouse to date [36–38] show distinct pattern of tissue distribution. SCD-1 is expressed at high levels in the adipose tissue and liver of animals fed a diet high in carbohydrates [33]. SCD-2 is abundant in the brain and neuronal tissues [36]. SCD-3 is expressed specifically in sebocytes in the skin, as well as in the harderian and preputial glands [37]. Finally, SCD-4 is expressed only in the heart.

Interestingly, humans have a gene highly homologous to *Scd-1* that is ubiquitously expressed but do not express genes highly homologous to the other three mouse isoforms [39]. However, a novel human isoform, Scd-5, that is expressed at high levels in the brain and pancreas has been identified [40].

2.2.2. The FADS family

While Δ9-desaturases are referred to as SCDs, the Δ6 and Δ5-desaturases genes were designated as FADS after being cloned [41–43] and have subsequently been shown to belong to the same cluster of genes [44]. Although their existence has long been known [45,46], the Δ6- and Δ5-desaturases, unlike the Δ9-desaturase, have never been purified in a reproducible fashion.

Utilizing an approach based on a gain-of-function, the Δ6-desaturase was first cloned from the cyanobacterium *Synechocystis* [47] and, later, from *Caenorabditis elegans* [48] and *Borage officinalis* [49]. Comparison of these DNA sequences to mammalian cDNA banks then allowed mouse, human and rat Δ6-desaturase to be successfully cloned [41,43]. The open reading frame of these genes encodes 444 amino acids with a mass of 52 kDa, and when expressed in eukaryote cells, these enzymes can introduce a double bond at position Δ6 on the acyl chain of linoleic (C18:2$n-6$) and α-linolenic (C18:3$n-3$) acids (essential fatty acids of the $n-6$ and $n-3$ series, respectively).

The Δ5-desaturase was subsequently cloned in humans [42,50], rat [51] and mouse [52] by various investigators. The length of this protein, as predicted from the open reading frame, is also 444 amino acids. de Antueno and coworkers [53] have shown that like Δ6-desaturase, Δ5-desaturase utilizes members from both the $n-6$ (C20:3) and $n-3$ (C20:4) series as substrates.

The Δ6- and Δ5-desaturase genes exhibit 75% sequence similarity and are located in the same region of chromosome 11 in humans [50]. Interestingly, a third gene highly homologous to both the Δ6- and Δ5-desaturase genes has been identified within the same region of chromosome 11 [44] and also cloned from the mouse, baboon [54] and rat [55]. In 2000, Marquardt and colleagues suggested the names for FADS1 for the Δ5-desaturase, FADS2 for the Δ6-desaturase and FADS3 for this third putative desaturase of the cluster, for which no activity has yet been described. A recent report concerning several forms of this FADS3 expressed in rats [55] is consistent with the various alternative transcripts detected in the baboon [54].

Although FADS and SCDs share certain common structural features, including transmembrane regions and the presence of conserved histidine-rich domains, there are clear phylogenic differences between these two families of desaturases. When they first cloned FADS2 (Δ6-desaturase), Clarke and colleagues [43] found that it contained an *N*-terminus region homologous to that of cytochrome b_5, a region which is present in FADS1 and FADS3 as well. On the basis of this observation these investigators proposed that NADH-cytochrome b_5 reductase transfers the electrons required for Δ6-desaturation directly to the catalytic site of this enzyme, without the involvement of cytochrome b_5.

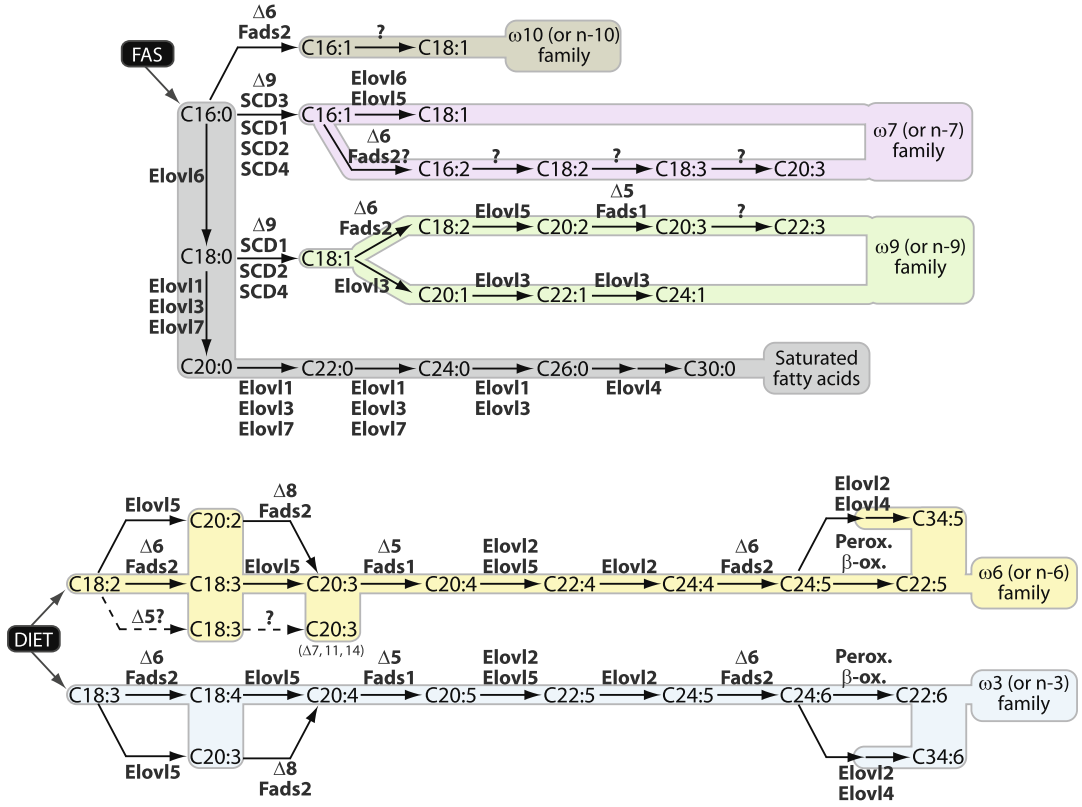


Fig. 4. *Long chain and very long-chain fatty acid biosynthesis in mammals.* The long chain saturated fatty acids and unsaturated fatty acids of the $n-10$, $n-7$ and $n-9$ series can be synthesized from palmitic acid (C16:0) produced by the fatty acid synthase (FAS). Long-chain fatty acids of the $n-6$ and $n-3$ series can only be synthesized from precursors obtained from dietary precursors (DIET). The name of the mouse genes likely to contribute to elongation (Elovl 1–7) and desaturation (SCDs and Fads) steps are indicated in these pathways.

In agreement with this hypothesis, when overexpressed in mammalian cells, rat Δ6-desaturase/FADS2 is targeted to the ER, where it interacts physically with the NADH-cytochrome b_5 reductase [56]. In addition, when expressed in a yeast strain that lacks cytochrome b_5 [57], the rat Δ6-desaturase is still active [58]. Finally, the typical HPGG motif of the cytochrome b_5-like domain of Δ6-desaturase is essential for its activity [59] and cannot be replaced by free cytochrome b_5 [56].

In addition to their high degree of sequence homology the organization of the introns and exons within the *Fads* genes is highly similar, suggesting evolution from a common ancestor [44]. Indeed, the presence of a single protein with both Δ6- and Δ5-desaturase activities in the zebrafish supports such a common origin [60]. Finally, more recently a naturally occurring antisense transcript for Δ5-desaturase encoded by the same Fads cluster has been isolated and shown to inhibit the transcription of the *Fads1* gene as well as Δ5-desaturase enzymatic activity [61].

3. Insights gained from transgenic mice

In the previous paragraphs we have briefly introduced the various elongase and desaturase genes and the biochemical functions of the corresponding proteins in mammals. In this part of the review we have made an attempt to summarize the cellular and physiological relevance of these enzymes by extracting data obtained from transgenic mice in which one or another of the corresponding genes have been mutated, as well as from cell culture studies. Fig. 4 summarizes our current view of the implications of mouse elongases and desaturases isoforms in the pathways that allow the synthesis of very-long-chain fatty acids. During the past decade, cloning of genes coding for elongases and desaturases as well as the development of cell models and transgenic mouse strains has considerably advanced our understanding of their substrate specificity and of the biological significance of both non-essential and essential fatty acid metabolism.

3.1. Metabolism of non-essential fatty acids

In contrast to the PUFAs, saturated and mono-unsaturated 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 mono-unsaturated VLCFAs such as peroxisomal disorders involving the adrenal cortex and the nervous system [62–64]. 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, palmitoleic acid (C16:1$n-7$) exerts lipokine effects on lipogenesis and fatty acid uptake, while phosphatidylcholine (PC) containing palmitic acid (C16:0) and oleic acid (C18:1$n-9$) is an agonist of the nuclear peroxisome proliferator-activated receptor-alpha (PPAR) which up-regulates fatty acid oxidation [2,65]. In addition, sphingolipids and ceramides containing saturated and mono-unsaturated very-long-chain fatty acids up to 26-carbon atoms are thought to stabilize membrane rafts and act as second messengers by activating membrane proteins through direct binding [66].

3.1.1. Biosynthesis of $n-10$ fatty acids in mice and men

Although C16:0 is either elongated or Δ9-desaturated under normal physiological circumstances this fatty acid can also be converted to $n-10$ fatty acids via a minor pathway only expressed in

sebocytes. Interestingly, Δ6-desaturation of C16:0 was detected in the preputial gland of *Scd-1*$^{-/-}$ mice [67] and shown to be catalyzed by FADS2, the same Δ6-desaturase that acts on PUFAs [68,69]. On the basis of what has been reported concerning mammalian cells it can be speculated, in agreement with the observations on *Scd-1*$^{-/-}$ mice, that when both Δ9-desaturase activity and the PUFA substrates for Δ6-desaturase are absent, biosynthesis of sapienic acid (C16:1$n-10$) may occur.

Interestingly, whereas C16:1$n-10$ has not been detected in the skin of wild-type mice, this fatty acid is the most abundant fatty acid in human sebum and may therefore be highly important in maintaining homeostasis in human skin. In support of such a possibility is the report of several skin problems in a patient born with very low expression of FADS2 due to a mutation in the *Fads2* promoter [70], problems that could not be ameliorated by dietary treatment with PUFAs of the $n-6$ and $n-3$ series [70].

In addition to C16:1$n-10$, another fatty acid of the $n-10$ series, sebaleic acid (C18:1$n-10$), is synthesized in isolated human sebocytes [71], strongly suggesting the existence of as-yet-unidentified elongases capable of elongating C16:1$n-10$.

3.1.2. Elongation of C16: insights from *Elovl6*$^{-/-}$ mice

The C16:0 product of FAS, and its metabolite produced by SCD-1 desaturation, C16:1$n-7$, can both be further elongated by ELOVL6 to yield stearic acid (C18:0) and vaccenic acid (C18:1, $n-7$), respectively. ELOVL6 was first identified following enhanced expression in the liver of transgenic mice that overexpress the sterol regulatory element binding protein-1c (SREBP-1c) and 2 (SREBP-2) [12]. Regulation of this protein by SREBPs was further confirmed by Matsuzaka and collegues [72], who identified *Elovl6* (which they named fatty acyl-CoA elongase or FACE) on the basis of microarray analysis. The expression of *Elovl6* is highly up-regulated both in the liver and white adipose tissue in the refed state indicative of an important role in the synthesis of long-chain fatty acids.

Deletion of *Elovl6* in mouse prevents the development of diet-induced insulin resistance, without amelioration of obesity or hepatosteatosis [73]. This maintenance of whole-body insulin sensitivity in *Elovl6*$^{-/-}$ mice is attributed to continuous signaling through the hepatic IRS-2/Akt pathway, an effect suggested to be mediated by the increased ratio of C16:1$n-7$ to C16:0 in the liver. Furthermore, breeding *Elovl6*$^{-/-}$ with *ob*/*ob* mice yielded offspring with attenuated hyperglycemia and improved insulin resistance compared to *ob*/*ob* mice, suggesting that the improvement in insulin sensitivity is independent of leptin signaling. In addition, *Elovl6* knockout mice exhibit partial embryonic lethality, with maintenance of fertility in the surviving male and female offspring, further emphasizing the importance of C18 fatty acids synthesized endogenously.

3.1.3. Desaturation of C18: insights from *SCD-1*$^{-/-}$ mice

In order to investigate their substrate specificities, the four SCD isoforms isolated from mice have been expressed in *Saccharomyces cerevisiae* [74]. It is highly noteworthy that, unlike the other isoforms, SCD-3 cannot desaturate C18:0, but only C16:0. These observations suggest that SCD-3 should actually be considered as a palmitoyl-CoA desaturase, while SCD-1, SCD-2 and SCD-4 are both palmitoyl- and stearoyl-CoA desaturases with a preference for stearic acid.

Studies on transgenic animals lacking SCD-1 [75] or SCD-2 [76] demonstrate clearly the significance of endogenous MUFA synthesis. Firstly, lack of SCD-1 is associated with severe impairment of the biosynthesis of many lipids including triacylglycerols, cholesterol esters [77], wax esters [75] and alkylgycerols rich in MUFAs [78]. Moreover, *Scd-1*$^{-/-}$ mice [75] exhibit sebaceous gland hypoplasia, depletion of sebaceous lipids and alopecia, phenomena similar to those observed in asebia mice, which carry a genomic deletion that disrupts expression of SCD-1 [79]. SCD-1 deficiency also impairs the function of the meibomian gland and synthesis of meibum, leading to the "dry eye" syndrome [75]. Moreover, SCD-1 has been suggested to produce lipids that play an important role in protecting the colon from inflammation [80], although this appears to be controversial [81]. Very recently, it has also been reported that the loss of SCD-1 attenuate adipocyte inflammation [82].

The mice lacking SCD-2 developed by Ntambi and coworkers [76] have revealed that this protein plays a key role in the synthesis of MUFAs required for the synthesis of other lipids during development of the skin and liver. Most of the *Scd-2*$^{-/-}$ pups that survive until birth die within a day, so, little investigation of adult *Scd-2*$^{-/-}$ mice has been possible. However, it has been shown that in adipocytes expression of SCD-2 is correlated to the expression of the adipogenic nuclear receptor PPARγ [83], suggesting that one important role for SCD-2 might be to provide fatty acid signals to PPARγ-dependent pathways.

Apparently, no animal models lacking SCD-3 or SCD-4 have yet been developed. Moreover, the substrate specificities and physiological significance of the various isoforms in different tissues remain to be further elucidated. For instance, a better understanding of the involvement of SCDs in the biosynthesis of C16:1$n-7$, a potential lipokine derived from adipose tissue that contributes to the regulation of global lipid homeostasis [2] is of considerable importance.

At the same time, extensive investigations by Ntambi and colleagues employing global SCD-1 knockout mice [84], asebia mice and, more recently, mice lacking SCD-1 specifically in the liver [85] or skin [86], have already indicated that endogenous MUFA synthesis makes a major contribution to whole-body energy homeostasis. Mice lacking SCD-1 are lean and protected against induction of obesity by diet [84] or leptin deficiency [87]. Moreover, a number of dietary, genetic and pharmacological challenges that induce obesity, hepatic steatosis and insulin resistance in wild-type mice fail to do so in *Scd-1*$^{-/-}$ mice [88]. A complex combination of metabolic and signaling mechanisms in these *Scd-1*$^{-/-}$ mice appear to reduce rates of lipid synthesis [89] and enhance lipid oxidation [90], thermogenesis [91] and/or insulin sensitivity [92,93] in tissues such as the liver, muscles and adipose tissue. The results of liver-specific disruption of the *Scd-1* gene [85], strongly indicate the important role played by this protein in carbohydrate-induced adiposity.

Finally, it has recently been reported that mice lacking SCD-1 in their skin [86] exhibit the same hypermetabolic phenotype as *Scd-1*$^{-/-}$ mice, while, unlike in the latter strain, their livers respond normally to a high-carbohydrate diet. These mice lacking SCD-1 in their skin are characterized by enhanced energy expenditure and basal thermogenesis, display cold intolerance and are resistant to obesity induced by a diet rich in fat. Consequently, most of the hypermetabolic phenotype of these mice is due to SCD-1 deficiency in the skin, not in the liver. Thus, local synthesis of MUFAs in the skin is not only important in maintaining the intactness of the skin and hair, but also strongly influences whole-body energy metabolism.

3.1.4. Elongation of saturated and mono-unsaturated fatty acids above C18: Elovl1, -3, -4 and -7

Further elongation of saturated and mono-unsaturated C18 fatty acids is catalyzed by the ELOVL1 and ELOVL3 enzymes.

3.1.4.1. Elovl1. ELOVL1 was initially discovered as a mammalian homologue to the Elo3/sur4 protein in yeast, which is suggested to catalyze the formation of saturated fatty acids containing as many as 26-carbons [13,14]. Complementation studies in yeast

have revealed that such fatty acids are important substrates for the synthesis of ceramides and other sphingolipids. The demonstration that yeast VLCFA elongases play important roles in connection with several aspects of cellular growth [94,95] suggests that the corresponding mammalian enzyme may be equally important with respect to membrane-related functions involving sphingolipids. Although ELOVL1 has been proposed to be a "housekeeping elongase" on the basis of its ubiquitous pattern of expression, the requirements for saturated VLCFA within an animal vary with both location and time. During the period when myelin develops in mice, around 20 days after birth, C22:0 elongation activity is particularly high [96].

The two mutant mice strains Jimpy and Quaking are deficient in nerve myelination throughout the central nervous system, which results in severe problems with movement and an early death [97,98]. Together with their mutations in the genes encoding the myelin-associated glycoprotein (*Mag*) and the proteolipid protein (*Plp*), respectively, these mice also demonstrate a pronounced decrease in the activity of C20 and C22 elongation in the brain, which is closely associated with a reduction in the expression of *Elovl1*. This provides further support for a role of ELOVL1 in the synthesis of sphingolipids and formation of myelin [14,99].

In the case of the liver, expression of *Elovl1* is not directly regulated by PPARα, Liver X Receptor (LXR) or SREBP-1c and is not subject to nutritionally-induced changes [100].

3.1.4.2. Elovl3. In contrast to ELOVL1 and ELOVL6, the ELOVL3 enzyme, which is suggested to control the synthesis of saturated and mono-unsaturated fatty acids with as many as 24-carbon atoms, is expressed in a highly tissue-specific manner [18,20]. The gene was originally identified as cold-inducible since its expression is highly up-regulated in the brown adipose tissue of mice upon the adaptation to the cold [101]. Further investigations, both *in vivo* and in primary cultures of brown adipocytes, revealed that this *Elovl3* expression is under the control of glucocorticoids, norepinephrine and the three isoforms of PPAR, i.e., PPARα, PPARδ and PPARγ [102,103]. Significant expression of *Elovl3* also occurs in white adipose tissue, the sebaceous glands of the skin and the liver [101,104], with a pronounced diurnal variation in the latter organ of adult male mice regulated by coordinated release of various steroid hormones, including glucocorticoids, androgens and oestrogens [105–107].

Two different mouse strains with a mutation in the *Elovl3* gene are presently available: *Elovl3* knockout mice obtained by homologous recombination [104] and the *scraggly* strain which carries a frameshift mutation caused by the drug chlorambucil resulting in a drastic reduction in expression [108]. 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. This distinct hair phenotype might be 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 in the former model. The explanation for this difference remains unclear but may involve low-level expression of *Elovl3* in the scraggly animals.

Analysis of the entire epidermis and hair lipids of the *Elovl3* knockout mice employing thin layer chromatography in combination with gas chromatography has shown that the fatty acid composition of the non-polar lipids, such as triacylglycerols, wax and sterol esters, but not that of ceramides is abnormal. The most striking anomaly was the reduced content of C22 and longer fatty acids in the triacylglycerols of the hair lipids. The presence of an enormous amount of eicosenoic acid (20:1$n-9$) instead indicates that *Elovl3* may be involved in elongation of C20:1 to provide fatty acids normally incorporated into certain triacylglycerols required for proper functioning of the skin barrier, which cannot repel water efficiently in the knockout animals.

Interestingly, in addition to *Elovl3*$^{-/-}$, dysfunctional mutations in both SCD-1 and -3 and DGAT1, which are involved in the synthesis of saturated and mono-unsaturated fatty acids and of triacylglycerols, respectively, give rise to abnormalities in the composition of sebaceous lipids and defective skin characteristics [77,109, 79,110,111].

Along with their abnormal compositions of sebum, the SCD-1, ELOVL3 or DGAT knockout mice also exhibit metabolic irregularities in their adipose tissue and liver. However, in contrast to several of the enzymes involved in fatty acid and triacylglycerol synthesis in the liver, expression of *Elovl3* is not influenced by either fasting or refeeding. These evidence indicate that the function played by *Elovl3* is to replenish the intracellular pool of triacylglycerol in order to maintain lipid homeostasis [112]. Consistent with such possibility, *Elovl3* expression is elevated in mice lacking the peroxisomal ABC transporter, ABCD2, and attenuated in mice that overexpress this same transporter, suggesting a tight coupling between VLCFA synthesis and peroxisomal fatty acid oxidation [106].

3.1.4.3. Elovl4. Although ELOVL4 is thought to be involved in the elongation of PUFAs (as discussed below in Section 3.2), Agbaga and coworkers [19] have recently shown that ELOVL4, using cultured cells over-expressing this protein, is also involved in the synthesis of saturated C28 and C30 VLCFA. This is the first elongase known to act on both saturated, such as C28:0 and C30:0, and very-long-chain PUFAs (VLC-PUFAs).

In addition, the skin of *Elovl4* knockout mice displays a pronounced depletion of saturated and mono-unsaturated VLCFA containing 26-carbon atoms or more. These mice die within a few hours after birth due to the dehydration resulting from the reduced levels of specific ceramides present in the epidermis [113]. Despite this early mortality and skin abnormalities, the internal organs appear to be normal [113–116].

3.1.4.4. Elovl7. The VLCFA elongase identified most recently is ELOVL7, which, like Elovl3, shows a rather consistent tissue-specific pattern of expression [17]. 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, this 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 overexpressed levels of *Elovl7* have been utilized to demonstrate that this enzyme is involved in the elongation of saturated fatty acids with as many as 24-carbon atoms.

Recently, a patient was found to have a mitochondrial encephalomyopathy caused by a homozygous deletion encompassing the *NDUFAF2* gene (which encodes the CI assembly factor 2), the *ERCC8* gene (whose product is involved in nucleotide excision repair coupled to transcription), and the *ELOVL7* gene, all located on chromosome 5 [117]. Liver biopsies from this patient exhibited lipid inclusions in the ER, which could be indicative of a disturbance in fatty acid metabolism. However, due to a lack of tissue, this hypothesis could not be examined further.

To conclude on this part; alterations in cellular fatty acid profiles as a consequence of ablation of one of the "non-essential" elongase or desaturase genes in mice clearly illustrates the importance of these enzymes in maintaining proper fatty acid homeostasis and, thereby, cellular functions and metabolic control. The concept of "essential" fatty acids needs to be considered in the sense of what

sort of lipids have to be synthesize via an endogenous pathway, and for what purpose and how is this regulated, and what impact do dietary fatty acids have on this regulation [170].

Whereas ELOVL1, 4 and 7 may act as enzymes controlling the synthesis of VLCFA as membrane components for optimal barrier function; the ELOVL3, 6 and the SDC enzymes are part a more complex system controlling cellular and whole body lipid homeostasis.

Our current view on the importance of the common saturated and mono-unsaturated C16 and C18 fatty acids has been re-evaluated based on the recent data gained by disruption of either an elongase or desaturase. By increasing the ration between C16:0, C18:0 and C16:1, C18:1 fatty acids by ablation of *Scd-1*, or between C16:0, C16:1 and C18:0, C18:1 fatty acids seen in *Elovl6* knockout mice, it is shown that *de novo* fatty acid synthesis is affected strongly in mouse hepatocytes which in turn influence the development of insulin resistance and liver steatosis. Considering this, it is noteworthy that Cao and coworkers in a recent study [2] imply that adipose tissue uses C16:1$n-7$ -palmitoleate as a "lipokine" communicator to regulate systemic metabolic homeostasis and that the phospholipid phosphatidylcholine (containing 16:0 and 18:1 moieties) acts as a potent PPAR ligand.

It is also possible that disruption of enzymes involved in metabolic activities further downstream of SCD-1 and/or ELOVL6, such as ELOVL1, ELOVL3, ELOVL7 and DGAT2 may influence the cellular level of saturated and mono-unsaturated C16 and C18 fatty acids. For example, the similarities seen in the skin of mice carrying mutations in SCDs, ELOVL3 and DGAT2 indicate that these enzymes participate sequentially in a process that is necessary for the production of triacylglycerols of sebum. However, the histopathological differences seen in the hair follicles and sebaceous glands of *Scd-1*$^{-/-}$, *Elovl3*$^{-/-}$ and *Dgat2*$^{-/-}$ mice suggests that it is not exclusively an impaired level of C16 and C18 fatty acids that explain the mechanism behind the phenotype of these mice.

It is also worth mentioning the possible role of ELOVL3 in the endogenous synthesis of C22 fatty acids such as erucic acid C22:1$n-9$. Although it is considered as a rare fatty acid in the body, it is easily accessible and described as a potential cardiopathic molecule when obtained from the diet [118,119]. The mechanism behind this is described as C22:1$n-9$ being a potent regulator of the peroxisomal -oxidation enzyme system in both heart and liver [120,121]) effecting the level of VLCFA in these tissues. Despite this, the data obtained from studies on the *Elovl3*$^{-/-}$, *Elovl6*$^{-/-}$ and *Scd-1*$^{-/-}$mice clearly show that endogenous synthesis of certain saturated and mono-unsaturated C16-C22 fatty acids are a prerequisite for normal function of several tissues such as sebaceous, meibomian, harderian glands as well as for the liver to control whole body lipid homeostasis.

3.2. Metabolism of essential PUFAs

Because they lack the Δ12 and Δ15-desaturases present in plants, mammals cannot synthesize fatty acids of the $n-6$ and $n-3$ series [122]. Therefore, C18:2$n-6$ and C18:3$n-3$ must be provided by the mammalian diet and these so-called essential fatty acids then serve as precursors for the synthesis of longer PUFAs, including the arachidonic (C20:4$n-6$) and docosahexaenoic acids (C22:6$n-3$) required for various physiological functions [123,124]. 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 [125–127].

Biosynthesis VLC-PUFAs of the $n-6$ and $n-3$ series occurs via sequential desaturation, elongation [128] and partial degradation step [129]. On the basis of substrate competition studies [45] and analyses of the fatty acid compositions of the tissues of animals fed $n-6$ or $n-3$ fatty acids, it has been proposed that the $n-6$ and $n-3$ biosynthetic pathways involve the same desaturases and elongases [130].

3.2.1. FADS2 Δ6-desaturation and $n-6$ and $n-3$ elongation by ELOVL5

The Δ6-desaturase FADS2 catalyzes the initial and rate-limiting desaturation of C18:2$n-6$ and C18:3$n-3$ for the production of longer-chain PUFAs [41,43]. Studies on the two different FADS2 knockout strains of mice recently developed [131,132] have provided convincing evidence that no other isoform of Δ6-desaturase can act on 18-carbon precursors of PUFA. These important findings are consistent with the characteristics of skin fibroblasts isolated from a patient with a deficiency in this enzyme [70].

The first report on *Fads2*$^{-/-}$ mice to appear showed that the viability and lifespan of these mice is normal but they are sterile [131], possibly as a consequence of disruptions of sertoli cell polarity, blood-testis barrier and gap junctions between the granulosa cells of ovarian follicles. The second report [132] also described a major deficiency in male and female reproduction due to FADS2 deficiency. However, the work by Stroud and collegues [132] revealed that FADS2 deficiency is not associated with total depletion of VLC-PUFAs. 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.

Elovl5 is expressed to some extent in all tissues tested to date, with highest levels in the liver, testis and adrenal glands [15]. Gene expression studies on the rat, suggest that this enzyme plays an important role in development of the liver during the postnatal period [16]. ELOVL5 has been proposed to be involved in the elongation of 18- and 20-carbons polyunsaturated fatty acyl-CoA substrates (Fig. 4) [16,133,134] Adenoviral overexpression of this protein in primary rat hepatocytes promotes elongation of 20:4$n-6$ and eicosapentaenoic acid (20:5$n-3$) to adrenic acid (22:4$n-6$) and docosapentaenoic acid (22:5$n-3$), respectively [135], resulting in altered lipid and carbohydrate compositions.

Recently, *Elovl5* knockout mice were found to develop hepatic steatosis, with elevated levels of hepatic triacylglycerols due to enhanced activities of SREBP-1c and the products of its target genes, such as the insulin-sensitive glucose transporter type 4 (GLUT4), acetyl-CoA carboxylase (ACC), FAS and ELOVL6 [136]. The underlying mechanism can be explained on the basis of the reduced levels of 20:4$n-6$ and 22:6$n-3$ present, since these PUFAs attenuate the level of active (nuclear) SREBP-1c [137]. Interestingly, in the liver of wild-type mice the expression of *Elovl5* is regulated by SREBP-1c, which binds to a SRE motif in the proximal region of the *Elovl5* promoter [138]. In addition, PUFAs suppress *Elovl5* expression, as would be expected for the product of an SREBP-1 target gene [16], which suggests the existence of a complex positive–negative feedback loop in which SREBP-1c-induced lipogenesis and *Elovl5* expression are sequentially suppressed by elevated levels of $n-3$ and $n-6$ PUFAs.

Finally, like in *Fads2*$^{-/-}$ fertility is altered in *Elovl5*$^{-/-}$ female mice, whereas no infertility is observed in the males.

3.2.2. FADS2 Δ8-desaturation in an alternative pathway for PUFA synthesis

A Δ8-desaturase activity detected in a variety of mammalian cells [139] and tissues [140] may provide an alternative route for the synthesis of C20:4$n-6$ and C20:5$n-3$ from C18:2$n-6$ and C18:3$n-3$, respectively, that does not involve a Δ6-desaturation (Fig. 4). In this case the substrates for the Δ8-desaturation are C20:2$n-6$ and C20:3$n-3$, but the elongase involved in the synthesis of these particular fatty acids has not yet been clearly identified. However, since the levels of C20:4$n-6$ and C22:6$n-3$ in *Elovl5*$^{-/-}$ mice are attenuated markedly, it is tempting to speculate

that elongation of C18:2$n-6$ to C20:2$n-6$ and of C18:3$n-3$ to C20:3$n-3$ is dependent on ELOVL5.

Recently, overexpression in yeast of a FADS2 cDNA isolated from baboon was shown to promote the Δ8-desaturation of C20:2$n-6$ and C20:3$n-3$ to C20:3$n-6$ and C20:4$n-3$, respectively [141], 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 [132]. Although competition experiments [141] indicate that FADS2 exhibits considerably higher Δ6-desaturase than Δ8-desaturase activity, even in the presence of its preferred substrates (i.e., C18:2$n-6$ and C18:3$n-3$) Δ8-desaturation of C20:2$n-6$ and C20:3$n-3$ does occur. Altogether, these investigations indicate that C20:2$n-6$ and C20:3$n-3$ should not be considered to be end-products of PUFA metabolism, but may be precursors for a number of other physiologically important PUFAs.

3.2.3. FADS1 Δ5-desaturation and $n-6$ and $n-3$ elongation by ELOVL2

The human Δ5-desaturase (FADS1) was cloned [42] on the basis of its sequence homology with the human Δ6-desaturase previously identified by the same group [43]. Initially, these authors demonstrated that overexpression of FADS1 in CHO cells promotes Δ5-desaturation of C20:3$n-6$ to yield C20:4$n-6$. Subsequently, the ability of human Δ5-desaturase to utilize both C20:3$n-6$ and C20:4$n-3$ as substrate was revealed by overexpression in yeast [53].

Like FADS2, FADS1 is involved in the metabolism of both $n-6$ and $n-3$ PUFA. Surprisingly, unusual Δ5-desaturase activity has been revealed in mice after oral gavage with radio-labeled C18–2$n-6$. Both in wild-type and *Fads2*$^{-/-}$ mice a Δ5-desaturation and subsequent elongation of C18:2$n-6$ led to the hepatic accumulation of labeled C20:3$n-6$ (Δ7,11,14) [132]. While this synthesis occurs in both genotypes the C20:3$n-6$ fatty acid is only incorporated into the phospholipids of *Fads2*$^{-/-}$ mice.

Mouse Δ5-desaturase (FADS1) was first identified as an EST clone whose expression is potently enhanced in the liver of transgenic mice that overexpress the transcription factor SREBP-1a [52]. This same investigation demonstrated that both Δ5- (FADS1) and Δ6- (FADS2) desaturation are up-regulated in the liver of this mouse strain and, moreover, that agonists of PPAR elevate hepatic expression of both enzymes. Although this induction is dependent on PPAR in mice [142], several groups have reported that this process is delayed relative to the up-regulation of prototypical peroxisome proliferator-responsive genes [143]. PPAR [144] and SREBP-1 [145,146] are well-characterized sensors of PUFA status and hepatic expression of both FADS1 [42] and FADS2 [43] is readily induced by dietary PUFAs. Thus, in the liver, PPAR and SREBP-1 make important contributions to PUFA homeostasis through coordinated regulation of FADS1, FADS2 and ELOVL5 [26,100,147].

Elovl2 was identified as *Ssc2* (sequence similarity to cig30–2) on the basis of its sequence homology to *Elovl3* (*Cig30*) [14]. Complementation studies in yeast indicate that this enzyme may participate in PUFA elongation and, indeed, it can utilize (C20:4$n-6$, C20:5$n-3$, and C22:5$n-3$ as substrates [12,18,135,148]. More specifically, ELOVL2 produces C24:4$n-6$ and C24:5$n-3$, the substrates for Δ6-desaturase and precursors for C22:5$n-6$ and C22:6$n-3$, respectively [135,148].

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 white adipose tissue [14,16].

Although the regulation of the *Elovl2* gene remains to be elucidated, some evidence suggests that the lipogenic transcription factor SREBP-1a, is involved [149]. Furthermore, overexpression of SREBP-1c in primary rat hepatocytes induces the expression of *Elovl2* [100]. However, treatment of these same hepatocytes with insulin, glucose or an agonist of LXR does not alter the expression of *Elovl2*, indicating that regulation by SREBP-1c may not be so pronounced. Recently, we demonstrated that overexpression of *Elovl2* in both 3T3-L1 and F442A cells promotes accumulation of lipid droplets, together with enhanced fatty acid uptake and induction of PPARγ target genes such as fatty acid-binding protein-4 (FABP4/aP2) and DGAT2 [150]. In contrast to the case of *Elovl5*, expression of *Elovl2* in the rat liver is not influenced by fasting or refeeding or by dietary supplementation with fish or olive oils [16].

C20:5$n-3$ is known to regulate hepatic gene expression, partially via SREBP-1, as well as through a PPARα-dependent pathway [151]. Exposure of primary rat hepatocytes to eicosapentaenoic acid (EPA) (20:5$n-3$) enhances the expression of cytochrome P450 4A (CYP4A), cytosolic fatty acid thioesterase-1 (CTE1) and mitochondrial hydroxymethylglutaryl-CoA synthetase (mtHMG-CoA synthase), while at the same time reducing the level of SREBP-1c mRNA [135]. In cells over-expressing *Elovl2*, induced expression of PPARα-regulated genes was attenuated, with no effect on SREBP-1c regulated genes. Nonetheless, the liver of *Elovl5* knockout mice exhibits elevated elongation activity of the ELOVL2 substrates 20:4$n-6$ and 20:5$n-3$, suggesting the existence of a compensatory mechanism whereby ELOVL2 endeavor to counteract the reduction in 22:6$n-3$ seen in these mice. These findings indicate that not only PUFA products solely produced by ELOVL5, but also those obtained by ELOVL2-catalyzed elongation may significantly influence SREBP-1c-regulated lipogenesis.

3.2.4. FADS2 Δ6-desaturation of C24 PUFAs and elongation by ELOVL4

In connection with the biosynthesis of VLC-PUFAs, the identity of the enzyme that catalyzes the final desaturation required for conversion of C22:4$n-6$ to C22:5$n-6$ and of C22:5$n-3$ to C22:6$n-3$ has been controversial. One possibility was that this conversion involves a Δ4-desaturase [152]. However, Sprecher and coworkers [129] found that elongation of C22:4$n-6$ and C22:5$n-3$ produces 24-carbon intermediates that are subsequently Δ6-desaturated prior to chain-shortening through partial β-oxidation, ultimately yielding C22:5$n-6$ and C22:6$n-3$.

Additional findings by several other groups lend support to the involvement of the Δ6-desaturase encoded by *Fads2* in this metabolic pathway. For example, human cells that lack FADS2 are unable to synthesize C22:6$n-3$, even when provided with the appropriate precursor [70]. Moreover, yeast [53] and mammalian [153] cells that overexpress the human and the rat form of FADS2, respectively, are able to desaturate both 18- and 24-carbon fatty acids of the $n-6$ and $n-3$ series.

Analyses of the fatty acid compositions of the tissues of two different *Fads2*$^{-/-}$ mouse strains have produced somewhat different results. For instance, in one case [131], C22:6$n-3$ was found to be totally absent from the liver and testis, whereas in another investigation [132], residual C22:6$n-3$ was detected in all tissues analyzed. Despite such discrepancies that still remain to be explained, both findings are consistent with the conclusion that FADS2 is the sole Δ6-desaturase required for the desaturation of 18- and 24-carbon fatty acids in connection with the metabolism of $n-6$ and $n-3$ PUFAs. Indeed, Stroud et al. [132] suggested that such residual level of C22:6$n-3$ observed in the *Fads2*$^{-/-}$ mice was similar to those obtained in mice with experimental diets lacking $n-3$ PUFAs.

Moreover, a highly interesting finding is that in both of these mouse strains lacking FADS2, reproduction is impaired [131,132]. In addition, in both cases significant improvement in reproduction can be obtained by providing VLC-PUFAs in the diet [131]. Dietary C22:6$n-3$ is particularly efficient in this respect, whereas C22:4$n-6$ formed from dietary C20:4$n-6$ can act as an inferior

substitute [154]. One interesting possibility discussed by Roqueta-Rivera and coworkers is that the structure and function of the sperm head in FADS2$^{-/-}$ mice are disturbed, due to the absence of VLC-PUFAs in its ceramides and sphingomyelin. Indeed, significant levels of VLC-PUFAs such as C32:6$n-3$ are present in the ceramides and sphingomyelin of sperm from various animal species [155,156].

ELOVL4 is thought to be involved in the elongation of PUFAs in the retina, brain, skin and testis [157]. Employing cultured cells that overexpress this protein, Agbaga and coworkers [19] have recently shown that ELOVL4 is involved in the synthesis of saturated C28 and C30 VLCFA, as well as of polyunsaturated C28–C38 VLC-PUFAs such as C34:6n3 and C36:6n3.

Diabetes is known to be associated with retinopathy. In rats rendered diabetic with streptozotocin, the attenuated levels of C32:6$n-3$ esterified in phosphatidylcholine, is paralleled by reductions in the expression of *Elovl2* and *Elovl4* [158].

Two inherited forms of macular degeneration, Stargardt-like macular dystrophy (STGD3) and autosomal dominant macular dystrophy (adMD), are caused by a deletion of five base-pairs in the *Elovl4* gene that results in a frame-shift generating a premature stop codon, as a consequence of which the 51 amino acids at the *C*-terminus of this protein, including the signal for its retention in the ER, are missing [159–162]. Subsequently, several other mutations and deletions in the *Elvol4* gene have been shown to be associated with the pathogenesis dominant macular dystrophies in humans [163,164]. Mice heterozygous for ablation of *Elovl4* do not demonstrate any early signs of macular degeneration. However, STGD3 patients heterozygous for the five base-pair deletion described above exhibit all of the major features of the retinal pathology [165]. This latter observation suggests that a dominant negative effect, rather than haploinsufficiency, is the primary mechanism underlying juvenile retinal degeneration in these patients. This conclusion is supported by the finding that the mutant form of ELOVL4 aggregates with the wild-type protein, resulting in mislocalization of the complex to the cytosol rather than the ER, giving a null phenotype.

The recent application of both cell cultures and transgenic mice to examine the role of FADS1 and 2, and ELOVL5, 2 and 4 in the metabolism of $n-6$ and $n-3$ PUFAs has advanced our knowledge in this area considerably. These enzymes are involved in the same biochemical pathways and share certain aspects of transcriptional regulation.

At the same time, little is presently known concerning the possible significance of certain newly identified genes that may encode PUFA desaturases. Although a very recent study establishes for the first time that several forms of the FADS3 protein are expressed differentially in various tissues of the rat [55], the functions of these various forms remains unknown. The occurrence of different forms of FADS3 is consistent with the alternative splicing described in baboon [54]. Interestingly, expression of FADS3 has recently been shown to be up-regulated markedly in mice lacking FADS2 [132]. Furthermore, two other putative genes belonging to the FADS family have been reported (FADS6 GeneID: 328035 and RIKEN GeneID: 228151) but the functions of their products remain to be established.

Although it is now clear that FADS1, FADS2, ELOVL2 and ELOVL5 are involved in the metabolism of essential $n-6$ and $n-3$ fatty acids, it remains unclear which enzymes are involved in the synthesis of "non-essential" PUFAs, i.e., those of the $n-7$ and $n-9$ series. The early work by Mead and collaborators [166] indicates that this synthesis proceeds through the same pathways involving the same enzymes as in the case of the $n-6$ and $n-3$ fatty acids. However, the activities of FADS1, FADS2, ELOVL2 and ELOVL5 with $n-7$ and $n-9$ fatty acids as substrates remain to be examined.

Finally, the Mead acid (C20:3$n-9$) is known to be synthesized in animals fed a diet free of fat or deficient in essential fatty acids [167] and, indeed, this fatty acid is used as an indicator of PUFA deficiency. When induced experimentally in rodents, PUFA deficiency results in various syndromes, including those described initially by Burr and Burr [168,169]. However, while it is clear that essential fatty acid deficiency exerts a potent impact on health and disease, it remains unclear which PUFAs and/or its derivatives are required for various biological functions and, thus, which fatty acid is actually essential [170]. Further work with the transgenic mouse strains currently available, together with the development of new strains in which the various desaturases and elongases involved in PUFA biosynthesis have been knocked out will help us to understand the biological significance of PUFAs in greater detail. The present review does not deal with investigations on transgenic mice that overexpress desaturases and/or elongases. For example, *Caenorabditis elegans* fat-1 gene encoding a desaturase that converts $n-6$ to $n-3$ fatty acids has been introduced into mice to create a model for investigating the biological effects of elevated $n-3$ fatty acids without dietary alterations [171].

4. Concluding remarks

Purification of membrane-bound elongases and desaturases to homogeneity has proven to be a daunting task and, consequently, biochemical characterization of these enzymes and of the pathways in which they participate is still rather limited. Interactions between desaturases and elongases, as well as their relationships to proteins responsible for the incorporation of fatty acids into complex lipids and the intracellular transport and partial degradation of fatty acids (e.g., in the peroxisome) require further elucidation. Cloning of the relevant genes in mammals has improved our understanding of the functions of many of the known elongases and desaturases. Moreover, both gain-of-function investigations in cell cultures and appropriate knockout strains of mice have allowed us to begin sketching a map over the contributions of elongases and desaturases to VLCFA metabolism. However, the substrate specificities and affinities of certain elongases and desaturases must be examined in more detail.

The alterations in phenotype and cellular fatty acid profiles observed in mice that lack a particular elongase or desaturase gene clearly illustrate the significance of these enzymes in maintaining fatty acid homeostasis and, thereby, proper cellular functions and metabolic control. In particular, observations on the most recent transgenic strains of mice with a tissue-specific deficiency in SCD-1 emphasize both the relevance and potency of this approach. In addition, investigations on transgenic mice lacking an elongase and/or desaturase together with dietary manipulations provide invaluable information concerning how fatty acid composition influences cellular processes. Moreover, the significant levels of expression of most elongases and desaturases in the liver are also indicative of their central roles in maintaining global lipid homeostasis.

In addition, modern lipidomic technologies that allow the quantitation of minor species of lipid will complement the use of tissue-specific knockouts of elongases and desaturases in mice to reveal new novel physiological functions for fatty acids and their metabolites.

Acknowledgement

The authors wish to thank Dr. Joe DePierre for critical reading of the manuscript. Both laboratories are supported by a joint grant from INRA and FORMAS.

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