Synthesis and Regulation of Omega-3 and Omega-6 Long Chain Polyunsaturated Fatty Acids in Salmonids

26/08/2024

Student number: 6787053

A literature review by Anne Kuijper

Daily supervisor: Affif Grazette

Examiner: Alessia Longoni

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Abstract

Omega-3 (n-3) and omega-6 (n-6) long-chain polyunsaturated fatty acids (LC-PUFA) are essential components of human nutrition, necessary for cellular function and overall health. However, Western diets often lack adequate n-3 LC-PUFA, altering the recommended n-3 to n-6 ratio. Salmonids are an important dietary source of n-3 LC-PUFA, but the transition in aquaculture diet from fish oils to plant oils has decreased their n-3 content. With rising global fish consumption, this reduction highlights the need for sustainable, nutrient-rich alternatives, like cultured fish meat. This review examines LC-PUFA biosynthesis and regulation in salmonids, identify existing knowledge gaps, and explores how these insights could benefit the cultured fish industry.

LC-PUFA biosynthesis in salmonids involves a complex process of desaturation and elongation of dietary precursors, facilitated by two main enzyme families: fatty acyl desaturases (FADS) and elongases (ELOVL). Regulation of this pathway occurs primarily through transcription factors such as sterol regulatory element-binding proteins (SREBPs), liver X receptors (LXR), and peroxisome proliferator-activated receptors (PPARs). Additionally, hormonal influences, specifically insulin and estrogen, and factors like diet, developmental stage, and temperature, also influence LC-PUFA synthesis.

LC-PUFA are vital for various physiological functions in salmonids, such as immune function, neural development, and cellular function, yet gaps remain in the understanding of the regulatory mechanisms of LC-PUFA biosynthesis. Key proteins and pathways, for instance the roles of SREBP1, and PPARβ, along with the β-oxidation process, require further research. Dietary strategies in aquaculture to enhance LC-PUFA content are ongoing, and the most effective methods remain debated in literature. Future research should focus on optimizing LC-PUFA content in lab-grown fish meat, considering factors like diet and life stage of the salmonid harvested, and utilizing alternative cell types to improve the n-3 LC-PUFA biosynthesis.

Plain language summary

Long-chain polyunsaturated fatty acids, better known as omega-3 and omega-6 fatty acids, are essential nutrients for the human body. They are vital components in the outer layer of cells, known as the cell membrane, contribute to energy production, and regulate inflammation in the body. Additionally, these fatty acids have been shown to help prevent diseases. Especially omega-3 fatty acids are known for their health benefits, such as reducing the risk of heart disease. It is important to maintain a balance between omega-3 and omega-6 in our diet. However, the typical Western diet tends to be higher in omega-6 and lower in omega-3. Therefore, increasing intake of omega-3 is often recommended.

Salmon and other oily fish are excellent sources of omega-3 fatty acids. However, nowadays, more of these fish needs to be eaten to get the same health benefits. This is due to the changes in fish farming (aquaculture), which affects the nutritional quality of farmed fish. Understanding how salmon naturally produce these omega-3 and omega-6 fatty acids could assist in developing farmed or lab-grown fish meat that offer similar health benefits as wild-caught varieties.

Salmon cannot produce omega-3 and omega-6 from scratch. They must eat essential precursor fatty acids, which their bodies convert into omega-3 and omega-6 fatty acids. These conversions require specific enzymes; desaturases and elongases, which introduce double bonds and lengthen the fatty acid chains, respectively. The final step in the conversion involves shortening the already double-bonded and elongated fatty acid chains in a process called β-oxidation.

Fatty acids are transported into cells by specialized proteins that help their movement across cell membranes. Once inside the cells, fatty acids are further transported by binding proteins, ensuring they reach the right location.

In salmon, the production of omega-3 and omega-6 fatty acids is controlled by both gene-level (transcriptional) and hormonal regulation. The proteins and hormones involved create complex interplays and feedback mechanisms to keep balance in fatty acid levels. External factors such as temperature of the water, developmental stage of the salmon, and the availability of precursor fatty acids in their diet also influences the production of omega-3 and omega-6.

These external factors tie into why salmon produce omega-3 and omega-6 in the first place. They are mostly used for critical functions. Like humans, they are important for cell membranes. Furthermore, they play a crucial role in the (neural) development of salmon, cell signaling, immune system function, and other processes.

Despite significant progress in understanding how omega-3 and omega-6 are produced in salmon, several gaps in our knowledge remain. For instance, certain molecular mechanisms and the influence of diet on fatty acid production need further research.

Ultimately, the goal is to produce lab-grown fish products to provide global food security. To do this, future research should focus on enhancing the production of omega-3 and omega-6 fatty acids in specific cell types, such as fat or muscle cells, that are most desirable for fish meat.

Glossary

ACAA1	CoA acyltransferase 1
ACOX	Acyl-CoA oxidase
ALA	α-linoleic acid
ARA	Arachidonic acid
CD36/FAT	Cluster of differentiation 36/fatty acid translocase
CoA	Fatty acyl coenzyme A
CPT	Carnitine palmitoyl transferase
CVD	Cardiovascular disease
D-BP	D-bifunctional protein
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
EFSA	European Food Safety Authority
ELOVL	Elongases of very long fatty acids
EPA	Eicosapentaenoic acid
ER	Endoplasmic reticulum
ERs	Estrogen receptors
FABPpm	Plasma membrane-associated fatty acid binding protein
FADP	Fatty acid binding proteins
FADS	Fatty acyl desaturase
FATP	Fatty acid uptake or transport proteins
FO	Fish oil
GH	Growth hormone
LA	Linoleic acid
LC-PUFA	Long chain polyunsaturated fatty acids
LXR	Liver X receptor
LXRE	LXR response element
n-3	Omega 3
n-6	Omega 6
NF-Y	Nuclear transcription factor Y

PO	Plant oil
PPARs	Peroxisome proliferator-activated receptors
PPREs	Peroxisome proliferator response elements
PUFA	Polyunsaturated fatty acids
RXR	Retinoic X receptor
SCP-X	Sterol carrier protein-x
SP1	Specificity protein 1
SR-B1	Scavenger receptor class B1
SRE	Sterol regulatory elements
SREBP	Sterol regulatory element-binding protein
TAG	Triacylglycerols
VLDL	Very-low-density lipoprotein

Introduction

Omega-3 (n-3) and omega-6 (n-6) long-chain polyunsaturated fatty acids (LC-PUFA), also known as highly unsaturated fatty acids, are essential for the human body (1). LC-PUFA are crucial components of cell membranes, precursors in the synthesis of several bioactive molecules, and involved in the regulation of enzyme activity and gene expression (2). Beside these vital functions, LC-PUFA are important as source of energy for the body.

In addition, n-3 LC-PUFA, such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), have been shown to have beneficial effects on human health. For example, n-3 LC-PUFA have been proven to be effective against cardiovascular disease (CVD) due to, among other factors, lowering blood pressure, which is a known risk factor for CVD (2). Moreover, n-3 LC-PUFA seem to be beneficial against dementia, Alzheimer's disease, certain inflammatory diseases, and types of cancer (1).

Carr *et al.* (1) explains that n-3 fatty acids have anti-inflammatory effects and affect the immune system, thereby positively influencing health. In contrast, n-6 fatty acids can exert negative influence on bodily health by driving inflammatory responses (1). Due to this, the ratio between n-3 and n-6 LC-PUFA is advised to be around 1:2.5-5. In western diets the ratio of LC-PUFA is drastically tilted towards n-6 fatty acids. Thus, an increase in n-3 fatty acids is suggested (1).

In contrast, the European Food Safety Authority (2016) states that there is not enough evidence to suggest that n-6 fatty acids negatively influence bodily health (2). Moreover, the importance of n-3 and n-6 fatty acids is underlined by stating that they are crucial in “maintaining metabolic integrity” (2).

Overall, LC-PUFAs are an important component of human nutrition. The precursors of n-3 and n-6 LC-PUFA, linoleic acid (LA, 18:2n-6) and α-linoleic acid (ALA, 18:3n-3), can only be acquired through diet (1). From these PUFA, LC-PUFA like arachidonic acid (ARA 20:4n-6), EPA (20:5n-3), and DHA, (22:6n-3) can be synthesized (3). However, the conversion rate is low, and therefore it is recommended to eat direct sources of LC-PUFA, such as rapeseed oil or oily fish (1,4).

Salmonids, such as Atlantic salmon and rainbow trout, are an excellent source of LC-PUFA, particularly n-3 LC-PUFA (1,4,5). Aquaculture of salmonids plays a crucial role in meeting the rising demand for seafood in the world (4,6). To meet these demands and pursuit sustainable aquaculture, fish oils (FO) have been gradually replaced with plant oils (PO), also referred to as vegetable oils, in the diet of the farmed fish (4). However, while FO is high in LC-PUFA, which are essential fatty acids for salmonids, PO mainly contains the PUFA precursors. Salmonids can biosynthesize LC-PUFA from the PUFA LA (18:2n-6) and ALA (18:3n-3), but the conversion rate depends on genetic as well as environmental factors, such as the composition of the diet (4). As a result, the fatty acid composition of the resulting fish fillets has changed (4). Consequently, a higher intake of fish meat is necessary to acquire the sufficient of n-3 LC-PUFA in the human body (1).

As the demand for fish meat rises, meeting the nutritional demands of communities worldwide requires innovative approaches to aquaculture. A part of the solution could be to replicate the nutritionally LC-PUFA rich profile of salmonids fillets in laboratory cultivated varieties. Laboratory cultivated meat is a novel industry that aims to improve global food security. Aquaculture faces challenges related to disease management, sustainability, and food safety, while traditional fisheries contend with issues such as overfishing and climate change (7). Cultivated fish production offers a potential solution to address these concerns.

However, ensuring that cultivated fish replicates the nutrient quality, including micronutrients like LC-PUFA, of its wild counterparts is a challenging task (6). In addition, the salmonid fillet fat content is not only essential for its nutritional value, but also for the flavor and texture of the product (5).

The cells used to produce cultivated salmonid fish are derived from either aquaculture or wild-caught salmonids (7), emphasizing the importance of understanding the regulation and biosynthesis of LC-PUFA in this species.

The biosynthesis of n-3 and n-6 LC-PUFA in salmonids is well-researched, yet it remains a complex process that is not fully understood. The challenge lies in identifying and addressing the gaps in knowledge of the pathways and mechanisms involved. Moreover, current knowledge needs to be translated into practical applications.

In this review, we delve into the current state of knowledge regarding the biosynthesis and regulation of LC-PUFA in salmonids. The gaps in knowledge will be explored and in the future perspectives an effort will be made to suggest applications for the cultured fish meat industry. By doing this, we aim to contribute to the development of sustainable fish meat that not only meet the growing demand for fish but also provide a nutrient-rich alternative to aquaculture and wild-caught varieties.

An overview of the salmonid fatty acid metabolism

To elucidate the regulation and biosynthesis of LC-PUFA in salmonids, a general understanding of the salmonid lipid metabolism is necessary. The fatty acid metabolism in salmonids encompasses several key processes, including lipid uptake, transport, storage, energy utilization, and biosynthesis (8).

Multiple tissues are involved in fatty acid metabolism. In the intestine, dietary fatty acids are taken up by enterocytes and subsequently transported to various other tissues. The liver serves as the central hub for fatty acid metabolism, where the biosynthesis of LC-PUFA occurs (9). Adipose tissue is important for the storage and catabolism of fatty acids. Red and white muscle are also involved in the storage of fatty acids but play a role in the utilization of fatty acids as well (9–11). There is competition among different fatty acid pathways in salmonids, and this competition is tissue-dependent (9).

Fatty acids are primarily stored in the form of triacylglycerols (TAG), which are mainly produced in the liver, though other tissues, such as adipose tissue, can also synthesize TAG (9). TAG molecules are secreted and transported in very-low-density lipoprotein (VLDL) particles and stored in the cytoplasm of cells within lipid droplets (9,12). For energy production, fatty acids undergo β-oxidation, which breaks them down to generate energy (12). Lastly, fatty acids can be functionalized through conversion into other fatty acids and lipids, including LC-PUFA, which will be explored in greater detail in subsequent chapters. Phospholipids, produced in the endoplasmic reticulum (ER), are an example of functionalized fatty acids and are essential components of cell membranes (9).

The produced fatty acids and metabolites significantly influence the various pathways of fatty acid metabolism (8). These pathways can be modulated through transcriptional, post-transcriptional, and post-translational mechanisms, which alter the expression of proteins involved in fatty acid metabolism. These regulatory mechanisms are activated or inhibited by the direct or indirect effects of fatty acids and their corresponding metabolites. They can enter the nucleus independently or in association with ligand-activated transcription factors to directly influence gene expression or indirectly modulate it through various transcription factors (8).

Biosynthesis of LC-PUFA in salmonids

LC-PUFA are synthesized from PUFA precursors through a series of desaturation and elongation steps. *De novo* biosynthesis of LC-PUFA from saturated fatty acids is not possible in salmonids because they lack Δ12 and Δ15 desaturases (13,14). To compensate, salmonids must acquire essential fatty acids, LA (18:2n-6) and ALA (18:3n-3), through their diet. In continuation, these PUFA can be synthesized into LC-PUFA such as ARA (20:4n-6), EPA (20:5n-3), and DHA (22:6n-3) (13). In Figure 1, the chemical structure of the essential fatty acids LA, ALA, ARA, EPA, and DHA are elucidated. FA are considered PUFA when they contain ≥ 2 double bonds, in turn LC-PUFA have ≥ 20 carbons and ≥ 3 double bonds (13,15).

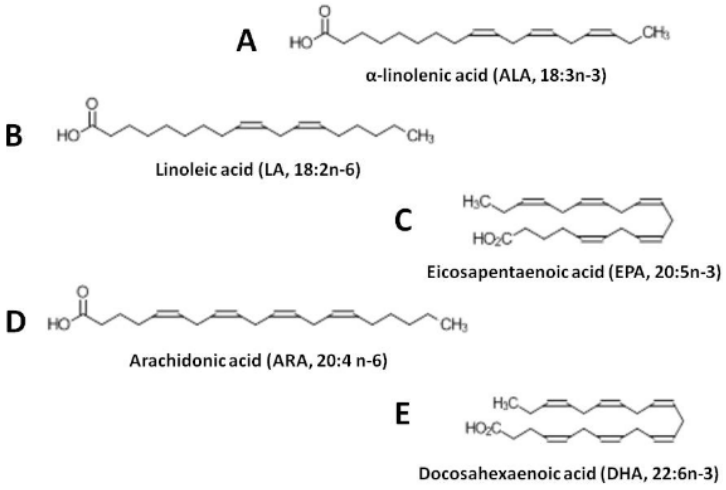

Figure 1. The chemical structure of essential fatty acids. A: α-linoleic acid, B: linoleic acid, C: eicosapentaenoic acid, D: arachidonic acid, and E: docosahexaenoic acid (15).

Fatty acyl desaturases

To biosynthesize LC-PUFA from PUFA two main enzyme families are necessary: fatty acyl desaturases (FADS) and elongases of very long fatty acids (ELOVL), which are located at the ER (Figure 4). FADS are transmembrane terminal oxygenases that catalyze the formation of additional double bonds between the carboxylic terminus and the preexisting Δ9-double bond (13,16). ELOVLs are part of the elongation reaction which takes place in four steps. First, fatty acyl coenzyme A (CoA) reacts with malonyl CoA in a condensation reaction, which is initiated by the membrane bound ELOVLs. In the remaining three steps, hydrogen atoms are added to the fabricated β-ketoacyl chain in a reduction, dehydration, and reduction reaction (13).

Salmonids possess a singular desaturase, FADS2, which is responsible for catalyzing both Δ5-desaturation and Δ6-desaturation processes (14). This in contrast to mammals which have two separate FADs. Four different *FADS2* genes have been identified, *Δ5fads2,* Δ*6fads2a*, *Δ6fads2b*, and *Δ6fads2c* (14,17–21).

The Δ5-desaturase primarily initiates the Δ5-desaturation of eicosatetraenoic acid (20:4n-3), a mid-production LC-PUFA of EPA (20:5n-3) and DHA (22:6n-3) biosynthesis. In contrast, the Δ5-desaturase minimally catalyzes the Δ5-desaturation of dihomo-gamma-linolenic acid (20:3n-6), which is an intermediate of the biosynthesis of ARA (20:4n-6) (14,15). Δ5fads2 exhibits low Δ6-desaturation activity towards the substrates, LA (18:2n-6) and γ-LA (18:3n-3) as well (15,18).

However, the Δ6-desaturation process for the intermediates tetracosatetraenoic acid (24:4n-6) and tetracosapentaenoic acid (24:5n-3) is readily initiated (14). In line, Δ6-desaturases mainly initiate Δ6-desaturation of LA (18:2n-6) and ALA (18:3n-3) (14,19). In addition, Δ6fads2a catalyzes the Δ6-desaturation of tetracosapentaenoic acid (24:5n-6) and tetracosahexaenoic acid (24:6n-3) as well (14). Δ6fads2a is also the Δ6-desaturase that demonstrates low Δ5-desaturation activity of eicosatetraenoic acid (20:4n-3) (14,17).

Interestingly, Δ5fads2, Δ6fads2b, and Δ6fads2c are able to initiate an additional Δ8-desaturation process, which converts eicosatrienoic acid (20:3n-3) and eicosadienoic acid (20:2n-6) to eicosatetraenoic acid (20:4n-3) and dihomo-gamma-linolenic acid (20:3n-6), respectively. In contrast to Δ5fads2 and Δ6fads2c, Δ6fads2b is the most efficient, with a conversion rate of 4.7% and 4% of eicosatrienoic acid (20:3n-3) and eicosadienoic acid (20:2n-6), respectively. However, overall, the Δ8-desaturation process is limited in Atlantic salmon and rainbow trout (8,22).

Elongases of very long fatty acids

Salmonids possess one elongase, ELOVL, and similarly to the desaturase, four genes have been identified that encode ELOVL; *elovl2*, *elovl4, elovl5a* and *elvol5b*. ELOVL2 and ELOVL4 elongate mainly C_{20} and C_{22} LC-PUFAs; EPA (20:5n-3), ARA (20:4n-6), docosapentaenoic acid (DPA, 22:5n-3), and adrenic acid (22:4n-6). In addition, ELOVL2 limitedly elongates stearidonic acid (18:4n-3) and γ-LA (18:3n-6) (14,21,23).

ELOVL5a and ELOVL5b both elongate C18 and C20 (LC-)PUFAs efficiently. Specifically, they elongate stearidonic acid (18:4n-3), γ-LA (18:3n-6), EPA (20:5n-3), and ARA (20:4n-6). Moreover, adrenic acid (22:4n-6) and DPA (22:5n-3) are elongated as well, but with a lower rate (14,18,21,23). Another possible ELOVL5 substrate is ALA (18:3n-3), which is converted to eicosatrienoic acid (20:3n-3). This is an alternative pathway for the synthesis of EPA (20:5n-3) and ARA (20:4n-6) in combination with the aforementioned Δ8-desaturation process in Atlantic salmon and rainbow trout (8,22). Interestingly, ELOVL5a is mostly present in intestinal tissue, while ELOVL5B is mainly found in the liver (14,24).

In **Error! Reference source not found.**, the full overview of the LC-PUFA biosynthesis pathway in salmonids is given. Overall, the n-3 pathway seems to be preferred by the enzymes related to the LC-PUFA biosynthesis over the n-6 pathway in salmonids (14,18,23). In Figure 4, the various metabolic fates of ALA (18:3n-3) are depicted, which includes the LC-PUFA biosynthesis of EPA (20:5n-3) and DHA (22:6n-3).

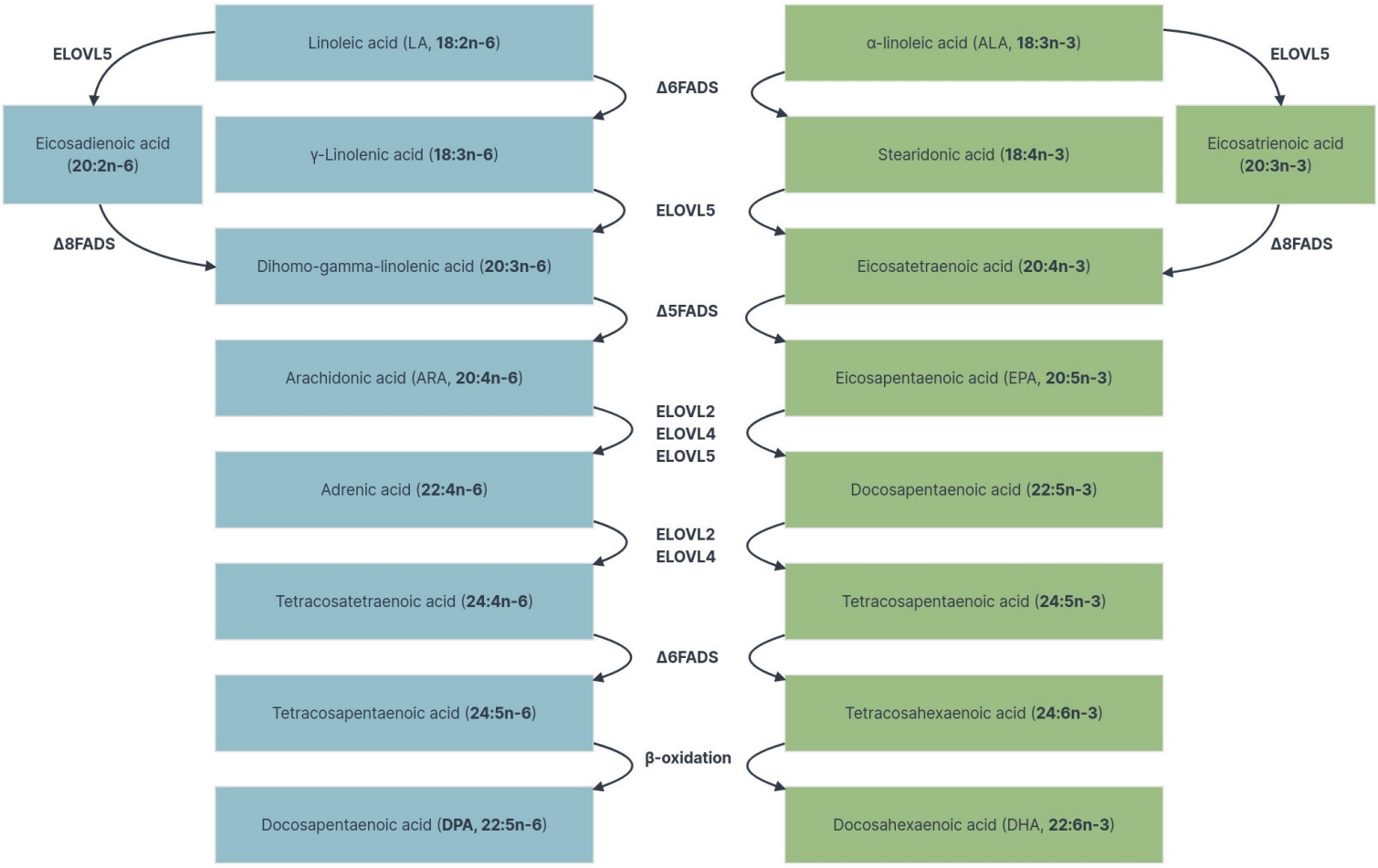

Figure 2. Biosynthesis pathway of polyunsaturated fatty acids. ELOVL and FADS refers to elongases of very long fatty acids and fatty acyl desaturases, respectively. Adapted from Schiller Vestergren (8) *and Monroig et al.* (22).

Fatty acid β-oxidation

The last conversion in both the n-3 and n-6 pathway is a β-oxidation step, which takes place in the peroxisomes (Figure 2). The process is carried out in four reactions, a dehydrogenation, hydrolysis, second dehydrogenation, and thiolysis step. As a result, the LC-PUFA chain is shortened by two C-atoms (25,26). This process can be repeated until the fatty acid chain is reduced to less than 18 carbon atoms, resulting in octanoyl-CoA. At this stage, the PUFAs can be transferred to the mitochondria, where following oxidation steps generate energy for the cell (26).

The enzymes involved in peroxisomal β-oxidation are acyl-CoA oxidase (ACOX), D-bifunctional protein (D-BP), CoA acyltransferase 1 (ACAA1), and sterol carrier protein-x (SCP-X) as shown by Lu *et al.* (26) in mammals. It is assumed that this process is also reflected in salmonids (27). In brown trout, which is part of the salmonid family, two ACOX genes have been verified, *acox1* and *acox3*. ACOX1 is also known as palmitoyl-CoA oxidase and ACOX3 as prystanoyl-CoA oxidase (27). Two isoforms of ACOX1 have been identified, ACOX1-3I and ACOX-3II. The ACOX enzymes are responsible for the rate limiting first dehydrogenation step in the β-oxidation process (27). Dehydrogenation involves the oxidation of a C-atom, wherein a hydrogen is removed, resulting in the formation of a double bond (Figure 3). According to our knowledge D-BP, ACAA1, and SCP-X have not been identified and characterized in salmonids yet.

In mammals, D-BP realizes the hydrolysis and second dehydrogenation in the peroxisomal β-oxidation. During the hydrolysis step D-BP performs as a hydratase, catalyzing the hydration of the former

created double bond. The last step is mitigated by ACAA1, a thiolase that cleaves fatty acyl-CoA from the remaining fatty acid molecule (26) (Figure 3).

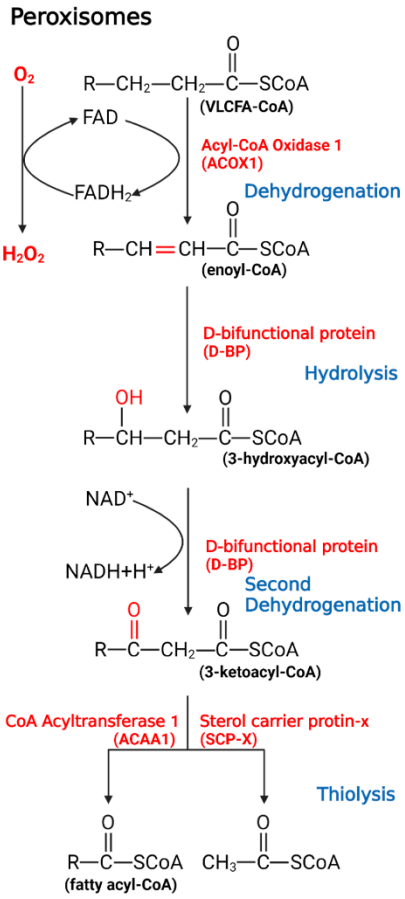

Figure 3. β-oxidation pathway in mammals. The involved proteins are depicted in red while the related processes are depicted in blue. Adapted from Lu et al. (26).

Fatty acid transportation

Zhou *et al.* (12) showed that in cultured primary hepatocytes of Atlantic salmon fatty acids are transported over the cell membrane into the cell through both active protein mediated processes and passive diffusion as well as carrier mediated transport. LC-PUFA, including EPA (20:5n-3), DHA (22:6n-3), ALA (18:3n-3), and LA (18:2n-6), were shown to be mainly reliant on membrane protein mediated processes for transport across the cell membrane. Specifically, in the order of EPA (20:5n-3) being the most dependent on protein mediated transport, followed by DHA (22:6n-3) and ALA (18:3n-3), and lastly LA (18:2n-6) being the least dependent on protein mediated transport (12).

The protein mediated transport was shown to be saturable, meaning that the rate of transport across the cell membrane cannot be increased by adding more fatty acids at a certain threshold. Furthermore, the intracellular metabolism of the hepatocytes did not seem to be the main regulator or activator of trans-membrane fatty acid transport (12).

Transmembrane transport of fatty acids

There are many proteins that play a role in the transport of fatty acids. Fatty acid uptake or transport proteins (FATP) are a necessary component in fatty acid transport and metabolism (11). FATP transport very long and long fatty acids, which include LC-PUFA (12). Torstensen *et al.* (11) states that the specific membrane-bound FATP in different tissues have not been characterized in Atlantic salmon

at that time. However, they do show that cluster of differentiation 36 (CD36), also known as fatty acid translocase, and FATP1-like protein are active in Atlantic salmon (11). CD36 is a transmembrane glycoprotein that transports LC-PUFA across the plasma membrane (8,11).

Harvey *et al.* (5) mentions a gene, *fatp2f*, related to FATP that is unmentioned by Torstensen *et al.* (11) and Zhou *et al.* (12). The use of this gene in their genomic study suggests that an additional FATP protein may be present in Atlantic salmon, namely FATP2F. In humans, six FATP (FATP1 to FATP6) have been characterized. This implies that in Atlantic salmon, FATP2f might represent a specific isoform of FATP2. The specific function of FATP2F is not mentioned by Harvey *et al.* (5).

Carnitine palmitoyl transferase 1 (CPT1) and carnitine palmitoyl transferase 2 (CPT2) are instrumental in the transport of fatty acids into the matrix of the mitochondria (8,11). CPT1 is attached to the outer membrane of the mitochondria where it facilitates the transport of fatty acids across the outer membrane into the intermembrane space. This process involves initiating the exchange of the acyl group of long-chain fatty acyl-CoA from CoA to carnitine, resulting in the formation of acyl carnitine. Subsequently, CPT2 mediates the transfer of acyl carnitine across the inner mitochondrial membrane into the matrix where mitochondrial β-oxidation takes place (8,11).

In the peroxisome, ACOX performs a similar function to the CPT proteins. There, ACOX facilitates the relocation of the activated acyl group to the inside of the peroxisome (8).

Lastly, scavenger receptor class B1 (SR-B1) functions as a cell surface high-density lipoprotein receptor and is involved in the transmembrane transport of fatty acids (8). In mammals, it facilitates the bidirectional transport of lipids between cells and high-density lipoproteins and is crucial for lipid homeostasis (28). In salmonids, SR-B1 is presumed to perform a similar function. However, in Atlantic salmon, SR-B1 is highly expressed in the intestine, particularly in the midgut. This localization suggests that SR-B1 plays a significant role in the uptake and regulation of dietary lipids, including LC-PUFA (28,29). Sundvold *et al.* (29) discovered an additional SR-B1, named SCARB1-2. SCARB1-2 was expressed at a lower level in comparison to SR-B1 (29).

Intracellular transport of fatty acids

In addition to transmembrane transport, intracellular transport is required as well. Fatty acid binding proteins (FABP) facilitate this process by acting as transfer vehicles for fatty acids within the cytoplasm. In Atlantic salmon, two FABPs have been characterized, FABP3 and FABP10, which seem to perform a similar function in both red and white muscle tissue and the liver, respectively (11). Colombo *et al.* (10) mention two variations of the FABP3 gene, *fabp3a* and *fabp3b*. Once more, in the genomic study of Harvey *et al.* (5), a specific gene encoding a FADP was mentioned, *fabp7b*, which most likely corresponds to the protein FABP7. The function of the two variants of FABP3 and FABP7 were not elucidated.

Zhou *et al.* (12) identified an additional FABP-related protein, the plasma membrane-associated fatty acid binding protein (FABPpm), which plays a role in the transport of very long and long-chain fatty acids as well. This protein potentially collaborates with CD36 in the transmembrane transport of these fatty acids (12). However, while FABPpm has been characterized in rat studies, its characterization in salmonids has not yet been accomplished to our knowledge.

The aforementioned β-oxidation related protein SCP-X, is a carrier protein that participates in the intracellular transport of lipids as well. While not mentioned by Lu *et al.* (26), SCP-X was most likely included in Figure 3 due to its role in shuttling lipids between intracellular compartments, such as the ER, peroxisomes, and mitochondria.

The regulation of LC-PUFA biosynthesis in salmonids

Transcriptional control

Sterol regulatory element-binding protein

LC-PUFA biosynthesis is regulated both at the transcriptional and hormonal level (14). One of the main transcriptional regulators is sterol regulatory element-binding protein (SREBP), whereof two genes exist, SREBP1 and SREBP2 (Figure 4) (14,30). Harvey *et al.* (5) suggests that Atlantic salmon possess three isoforms of SREBP1, *srebp1b, srebp1c, and srebp1d*, similar to those found in mammals. However, the specific regulatory functions of these isoforms were not clarified. SREBP1 has been suggested to be the most direct regulator of LC-PUFA biosynthesis as it responds to the partial knockouts of essential enzymes in the LC-PUFA biosynthesis pathway (14). SREBP1 binds to sterol regulatory elements (SRE) in the promoter region of Δ*6fads2a*, *elovl5a,* and *elovl5b* (14).

Interestingly, SREBP2 also binds to the SRE of *elovl5a* and *elovl5b*. *Elovl5b* shows higher activity when SREBP2 binds in comparison to SREBP1. In contrast, the response of *elovl5a* is similar between SREBP1 and SREBP2 (24,30).

To regulate the biosynthesis of fatty acids, SREBPs work together with other regulatory factors like specificity protein 1 (SP1), used interchangeably with stimulatory protein 1 in literature, and nuclear transcription factor Y (NF-Y) (14,30). Curiously, no SP1 binding sites were identified in the promoter region of FADS2 in rainbow trout (31). In contrast, to gain complete expression of Δ*6fads2a* in Atlantic salmon*,* the SP1 binding site in the promoter region is required (14,32). The FADS2 promotors do contain NF-Y cofactor sites and is up-regulated by SREBP1 in rainbow trout (31). NF-Y binds in close proximity to SRE sequences to NF-Y cofactor sites in the promotor sequence of *elovl5a* and *elovl5b* (30). Similarly to SP1, the cooperation of NF-Y is most likely needed to gain full activation of *elovl5* (30).

Liver X receptor

SREBPs are mainly regulated by liver X receptor (LXR), a zinc finger protein that dimerizes with retinoic X receptor (RXR) when both have bound to the LXR response element (LXRE), which is situated in the promoter region of the target genes (33). One isoform of LXR has been found in salmonids, LXRα (14). Upon ligand binding, which include oxysterols, glucose, or fatty acids, the corepressors inhibiting activation of LXR are removed. LXR is subsequently translocated into the nucleus from the cytoplasm (Figure 4). After prementioned binding to LXRE and dimerization with RXR, coactivators bind which ultimately enable transcription of the target genes (33).

LXR is a metabolic sensor for fatty acids and cholesterol metabolism (33). In the liver, LXR activates SREBP1c, which is responsible for *de novo* fatty acid biosynthesis. Furthermore, the catabolism of cholesterol is induced as well (33). It has been suggested that LXRα binds to *elovl5a* and ACOX (8,30). LXRα would therefore play a direct role in the regulation of LC-PUFA biosynthesis. In contrast to the indirect regulation via the activation of SREBP1.

Peroxisome proliferator-activated receptors

LXR is closely related to peroxisome proliferator-activated receptors (PPARs) (31). PPARs are part of the nuclear hormone receptor superfamily, bind to peroxisome proliferator response elements (PPREs), and, like LXR, are ligand activated (31,34). In salmonids three subtypes have been discovered, PPARα, PPARβ, and PPARγ (Figure 4). Additionally, PPARα can be divided into two isoforms; PPARα1 and PPARα2. Saturated fatty acids, PUFA, ALA (18:3n-3), ARA (20:4n-6), and DHA (22:6n-3) include the

ligands that have an up regulatory effect on PPARα expression, while EPA (20:5n-3) has a downregulatory effect (8). Curiously, while structurally and evolutionary very similar, PPARα2 exclusively demonstrated regulatory activity in the promoter region of FADS2, while PPARα1 did not (31). The isoform not specified, PPARα has been shown to bind to the promoter of *elovl5a* (ref 35). In contrast, *elovl5b* is more dependent on SREBP induced activation, as made evident by the lack of LXR and PPAR binding sites (30). Furthermore, PPARα also demonstrates a pivotal role in the regulation of peroxisomal beta oxidation by activating ACOX1 (27). Notably, PPARα has been denoted as the primary inducer of the β-oxidation pathway in the liver (8,14). Overall, PPARα plays a direct role in the regulation of LC-PUFA biosynthesis.

PPARβ has been studied by Leaver *et al.* (34), who identified four subtypes of PPARβ, which were divided into two families, ssPPARβ1 and ssPPARβ2, that each contained two isotypes, ssPPARβ1A, ssPPARβ1B, ssPPARβ2A, and ssPPARβ2B, respectively. The isotypes ssPPARβ1A and ssPPARβ2A were functionally characterized and showed different levels of expression and activation. Datsomor *et al.* (14) suggest that ssPPARβ1A plays an important role in the fatty acid homeostasis in the liver. ssPPARβ1A is activated by lipids such as palmitoleic acids, oleic acids, and GW501516; a mammalian ligand specific for PPARβ. As the salmonid reacts to this mammalian ligand as well, it suggests that salmonids contain the same ligand or a version thereof (34). In contrast, ssPPARβ2A does not react to these compounds. Leaver *et al.* (34) suggests that there may be undiscovered ligands that could bind to ssPPARβ2A and state that ssPPARβ2A may play a role in the suppression of other PPARs in the gill, where it is mainly expressed. Direct regulation of LC-PUFA biosynthesis through PPARβ has not been found, however PPARβ does seem to be essential for fatty acid homeostasis (14,34).

Lastly, two subtypes of PPARγ, PPARγ *short* and PPARγ *long*, have been described, which are associated with fatty acid metabolism and adipocyte differentiation, respectively (8). In addition, Datsomor *et al.* (14) described PPARγ as a "master regulator of adipogenesis". Jin *et al.* (21) speculated that PPARγ targets genes related to fatty acid synthesis and elongation. Moreover, PPARγ is involved in the transcription of CPT1 and ACOX1 and activates the transcription of CD36 and SR-B1. Thus, PPARγ has a regulatory role in both the β-oxidation pathway, as well as in the transportation of fatty acids. The genes that code for CPT1 and ACOX1 are targeted by PPARα and PPARβ as well (8,27). Likewise, the transcription of CD36 and SR-B1 is initiated by PPARα (8).

Inhibitory and antagonistic regulation

Most of the factors discussed thus far activate transcription and ultimately influence the activity of FADS and ELOVL enzymes. However, in addition to these stimulatory regulatory pathways, there are also inhibitory or antagonistic mechanisms that regulate these processes. One of these pathways includes the suppressed activity of Δ*6fads2a*, Δ*6fads2b*, Δ*6fads2c*, and SREBP1 through the inhibitory effect of EPA (20:5n-3) (14,32). This inhibitory effect is presumably mediated through similar binding sites that SREBP and NF-Y utilize (32). In addition, PUFA can act as antagonists to LXR, which inhibits the function of LXR, and consequently inhibits transcription of SREBP1c (14). Similarly, in the liver, high levels of DHA (22:6n-3) seem to lower the expression of PPARα and PPARγ, while low levels of DHA

(22:6n-3) are related to an increase of PPARα and PPARγ expression. A parallel relation exists between DHA (22:6n-3) and Δ*6fads2a*, Δ5*fads2*, and SREBP1 (14,21).

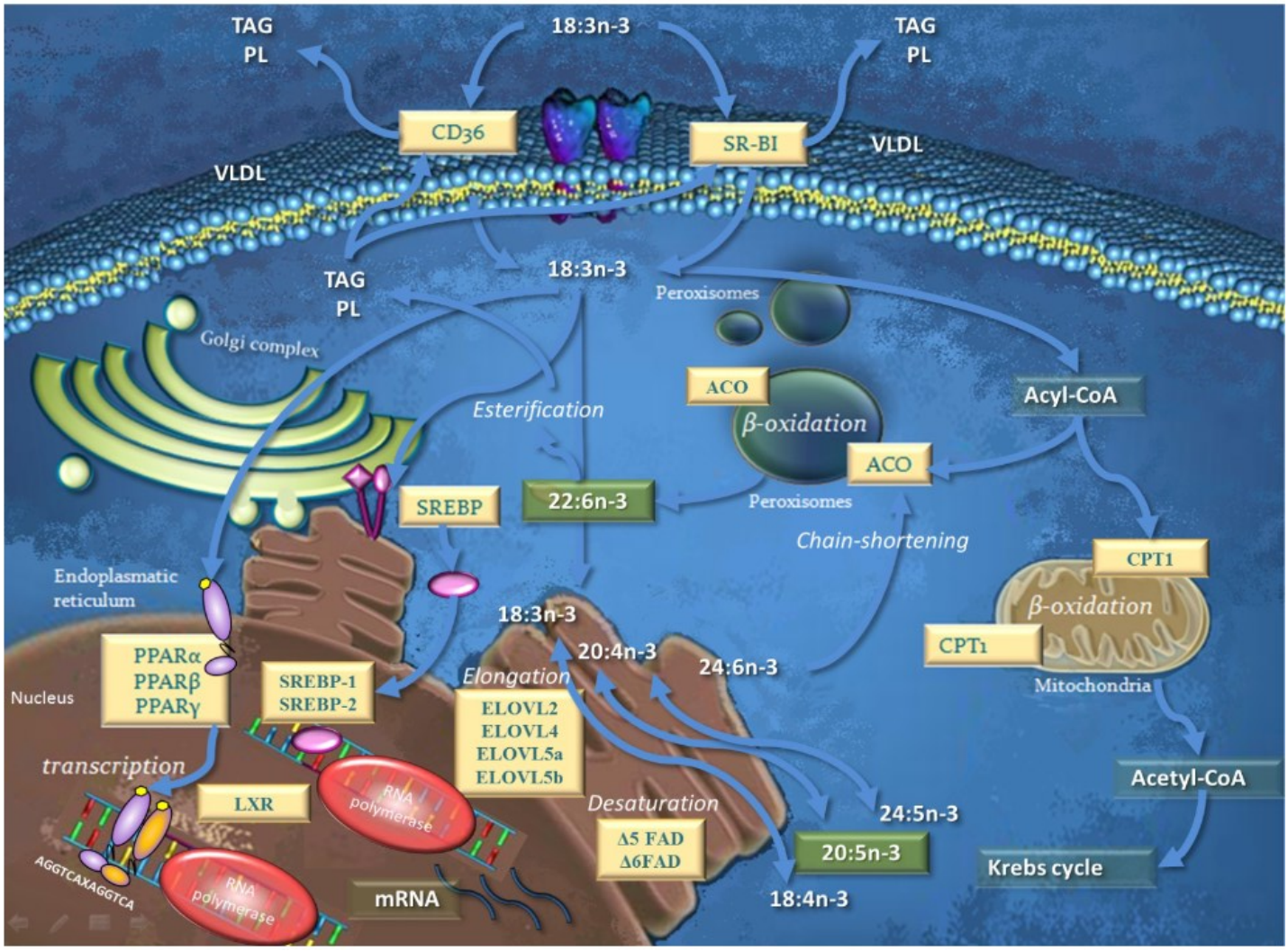

Figure 4. A visual representation of the lipid metabolism, specifically of the PUFA α-linoleic acid (ALA, 18:3n-3). The yellow boxes highlight the location of several genes and proteins discussed in the text. The associated metabolic mechanisms are indicated in italics. Abbreviations are explained in the glossary, except for ACO and PL, which refer to acyl-CoA oxidase (ACOX) and phospholipids, respectively. Reproduced from Schiller Vestergren (8).

Hormonal control

Insulin

Curiously, in Figure 5 insulin is visible as a possible regulator of LXR. Despite this, insulin is not further mentioned in the paper of Datsomor *et al.* (14). The reason why they might have included insulin in Figure 5 is due to suggested relation between insulin and growth hormone (GH) production and LXR expression by Cruz-Garcia *et al.* (33). This relation has already been proven in mammals; insulin activates LXR, which in turn activates PPARα, and stimulates the production of fatty acids. GH has the opposite effect in mammals; the expression of LXR, and therefore SREBP1c, is lowered (33).

Insulin might also influence β-oxidation, Polakof *et al.* (35) discovered that the activity of CPT1 and hydroxyacyl-CoA dehydrogenase (HOAD) increased in the liver of salmonids when infused with insulin. However, Polakof *et al.* (35) also stated that this increase was most likely not mediated by insulin but instead through high levels of LC-PUFA in the diet of the salmonids.

Kleveland *et al.* (28) discovered that insulin increases SR-B1 expression in Atlantic salmon hepatocytes as well. Although the exact mechanism underlying this interaction remains unknown, it is logical to infer a connection (28). SR-B1 is anticipated to facilitate lipid uptake in the intestine of Atlantic salmon, which is essential for the synthesis of LC-PUFA (28,29). Given that insulin stimulates fatty acid production (33), it is reasonable to expect that SR-B1 plays a role in this process and that its expression is elevated as a result.

Estrogen receptors

The β-oxidation pathway may be partly regulated through estrogen receptors (ERs) as well. The expression of ACOX1 is downregulated and the expression of ACOX3 is upregulated at a certain level of 17α-ethynylestradiol input, a synthetic estradiol derivative, which binds to ERs (27). Madureira *et al.* (27) propose that this may indicate adverse crosstalk between estrogens, specifically the ERs, and PPARα, given that ACOX1 is transcriptionally regulated through PPARα.

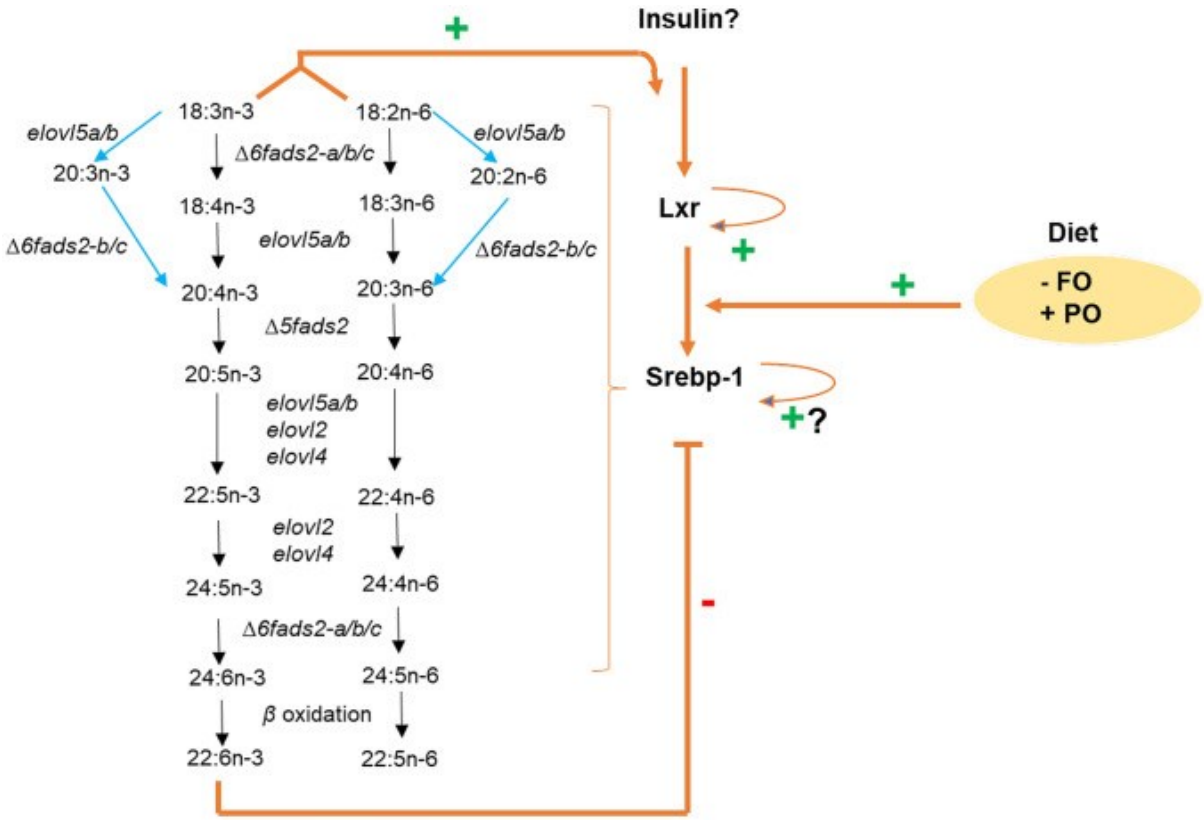

Figure 5. Biosynthesis pathway of long chain polyunsaturated fatty acids and influences thereon. The meaning of the abbreviations can be found in the glossary. Reproduced from Datsomor et al. (14).

Main factors affecting the biosynthesis of LC-PUFA in salmonids

The regulation of LC-PUFA biosynthesis is transcriptionally and hormonally controlled. However, temperature, developmental stage, and the availability of precursors in diet, LA (18:2n-6) and ALA (18:3n-3), influence the regulation of LC-PUFA biosynthesis as well.

Precursors in diet

As mentioned in the introduction, FO is gradually replaced by PO in the diet of salmonids in aquaculture. This affects the levels of PUFA and LC-PUFA in the diet and in the body of the salmonids. PO contain PUFA, such as LA (18:2n-6) and ALA (18:3n-3), while LC-PUFA, including ARA (20:4n-6), EPA (20:5n-3), and DHA (22:6n-3), are absent (24). In contrast, FO contains high levels of ARA (20:4n-6), EPA (20:5n-3), and DHA (22:6n-3) (36).

LC-PUFA are essential for healthy salmonids. While salmonids can biosynthesize a significant amount of the LC-PUFA they need, these levels are often not enough to meet de LC-PUFA levels required (1). Diet influences the LC-PUFA biosynthesis significantly. For example, Colombo *et al.* (36) showed that with high enough levels of dietary ALA (18:3n-3), Atlantic salmon can biosynthesize approximately 25% of needed LC-PUFA (4). However, salmonids need higher levels of LC-PUFA for optimal grow and health, than the basic requirement level to survive (4).

The dietary level of fatty acids seems to be directly related to the level of fatty acids in muscle tissue (36). Riera-heredia *et al.* (37) stated that salmonids fed a VO-diet changed or had more fat deposition in comparison to salmonids fed a FO-diet. The change in available fatty acids dysregulated the lipid metabolism and caused the hypertrophic adipose tissue growth (37). However, these changes are more complex *in vivo* in contrast to *in vitro* salmonid adipocytes. Yet, the lipid and fatty acid levels differ in salmonid fish between salmonids fed mainly VO-diets in comparison to FO-diets (1). It has also been suggested that the number LC-PUFA needed is relative to the total amount of lipids present in the body of the salmonids (4,38). Thus, if the amount of lipids is increased in the diet, the amount of LC-PUFA should increase as well (38).

A high content of LC-PUFA in salmonid diet, like a FO-diet, has an up regulatory effect on *pparα* and *pparβ* expression (8). PPARα is a crucial regulator in the LC-PUFA biosynthesis pathway (8,14,27,30). This suggest that LC-PUFA biosynthesis is initiated as PPARα promotes *elovl5a* and activates ACOX1 (27,30). In contrast, the function of PPARβ seems to be more indirectly related to the LC-PUFA biosynthesis pathway (14,34). Thus, an unknown interaction via PPARβ might alter the initiation of the LC-PUFA biosynthesis pathway. Additionally, the expression of *lxr*, another important player in the biosynthesis of LC-PUFA, is also increased in salmonids fed the FO-diet in comparison to salmonids fed a VO-diet (33).

In contrast, the expression of Δ5-desaturase and Δ6-desaturase genes, *srebp1*, and *pparα* were upregulated in the liver of salmonids that were fed a diet which replaced FO with VO (31). Thus, the LC-PUFA biosynthesis pathway might also be induced by high number of PUFA precursors. To further prove this point, the expression of *elovl2*, *elovl5b*, and Δ5-desaturase and Δ6-desaturase genes increased in salmonids fed the VO-diet as well (13,23,39).

However, genes that encode β-oxidation related proteins ACOX, CPT1, and CPT2 were down regulated in Atlantic salmon that were fed a VO-diet instead of a FO-diet (11). This contradiction might point to production of more ARA (20:4n-6) and EPA (20:5n-3) in comparison to β-oxidation products, DPA (22:5n-3) and DHA (22:6n-3).

Development stage

The developmental stages of salmonids are influenced by their anadromous nature. Most salmonids are born in freshwater streams and rivers and migrate to a marine environment to mature, whereafter they return to original birthplace to spawn (14). In the salmonid lifecycle, the fish undergo smoltification, which takes place during the parr stage and the smolt stage (Figure 6). The smoltification process prepares the salmonids for the transition from a freshwater to marine environment (40).

These changes are reflected in the regulation of LC-PUFA biosynthesis (14). Parr accumulate energy stores for the smoltification process (33). During this life stage, LXR expression is high and therefore the expression of SREBP1 is elevated as well. SREBP1 plays a key role in regulating the expression of genes involved in fatty acid synthesis, such as fatty acid synthase and acetyl-CoA carboxylase (33). Additionally, SREBP1 is responsible for the regulation of FADS and ELOVL enzymes (14,30,32).

During the initial start of the smoltification process insulin levels rise, which increases the LXR activation, and energy is generated and stored in the form of fatty acids (33). Later in the process, insulin levels lower and GH levels increase. Consequently, it has been hypothesized that LXR expression decreases, which encourages the output of the stored energy required for smoltification (33).

Furthermore, during juvenile development, the fry to parr stage, it has been shown that the n-3 LC-PUFA biosynthesis is especially efficient, which decreases throughout smolt and adult stages (41). Interestingly, these findings are opposed by Jin *et al.* (42), who state that in the fry and parr stage the liver is still immature as they found low activity of the lipid metabolism. They suggested that the activity increases over time as the fish matures (42).

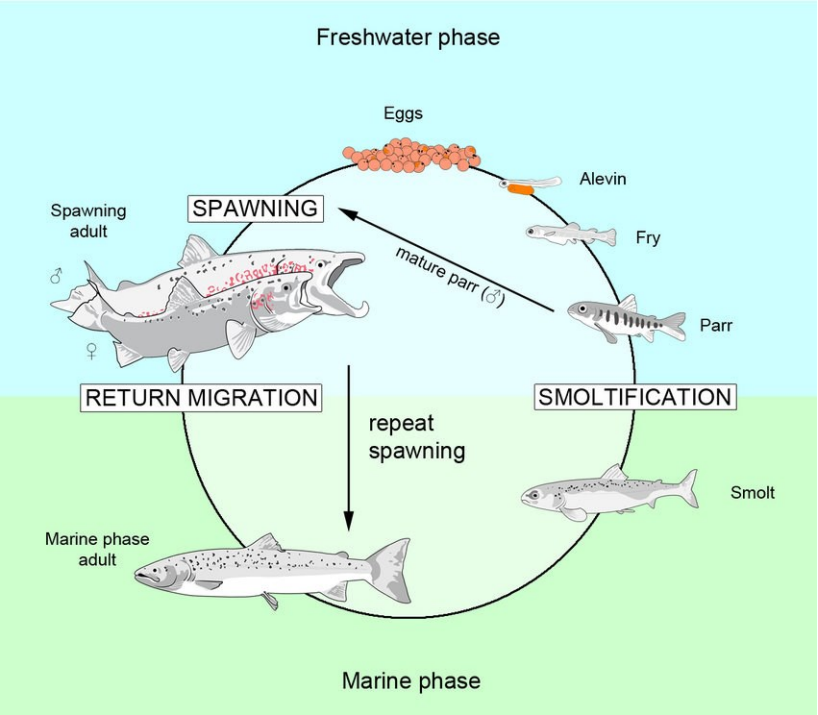

Figure 6. The life cycle of salmonids. Reproduced from Mobley et al. (40).

Temperature

Beside dietary availability of LC-PUFA precursors and the developmental stage of salmonids, the body temperature of the salmonids, and therefore the temperature of the water they reside in, has an impact on LC-PUFA biosynthesis (3). (LC-)PUFA fulfill a critical function by influencing cell membrane fluidity, flexibility, and function in salmonids.

The membrane fluidity changes according to the temperature of the salmonid. When the temperature rises, the need for LC-PUFA lowers (10). To adapt to lower temperatures, Atlantic salmon modify the composition of fatty acids in their cell membranes. The proportion of PUFAs in the cell membrane increases to maintain membrane fluidity and functionality (3). Specifically, the content of EPA (20:5n-3) and DHA (22:6n-3) increase at lower temperatures and vary inversely with oleic acid (18:1n-9) and LA (18:2n-6). Rosenlund *et al.* (3) argues that the oxidative capacity of mitochondria changes with thermal variations as well, as shown by the aforementioned inverse relation.

Interestingly, contrary to expectation, the evidence of Colombo *et al.* (10) demonstrated that a higher water temperature resulted in an increased expression of Δ*5fads*, Δ*6fads2a*, and Δ*6fads2b*. The increased enzymatic activity of the desaturases resulted in an enhanced conversion of ALA (18:3n-3) to DHA (22:6n-3). In addition, the amount of stored DHA (22:6n-3) increased in the muscle as well. Both phenomena were demonstrated when salmonids were fed a diet containing low LC-PUFA (10).

In conclusion, raising water temperatures, when kept below 23 °C, combined with a diet low in LC-PUFA, can collectively promote metabolism, growth, and increased fat storage in salmonids (10). Of note, at temperatures above 23 °C, negative effects appeared, as these temperatures can be lethal to salmonids (43).

According to the Washington State Department of Ecology (2000), elevated water temperatures reduce the amount of dissolved oxygen and increases the metabolism of salmonid fry. The heightened metabolism leads to greater food intake and, consequently, increased exposure to predators (43). Additionally, higher temperatures elevate the vulnerability of salmonids to toxins, enhance the spread of diseases, and weaken the fish, making them more susceptible to illness. Moreover, juvenile salmonids experience slower swimming speeds under these conditions, further impairing their ability to evade predators (43).

The physiological need for LC-PUFA in salmonids

LC-PUFA are not only essential fatty acids for humans but for salmonids as well, and aside from understanding the regulation and biosynthesis of LC-PUFA in salmonids, it is crucial to recognize how LC-PUFA are utilized in salmonids.

Lipids, fatty acids, and proteins are the major components in the diet of salmonids that provide energy. The β-oxidation pathway plays an important part in converting fatty acids and lipids into usable energy sources for the cell. While LC-PUFA and PUFA can be used as energy source, the β-oxidation pathway seems to prioritize monounsaturated fatty acids and saturated fatty acids (15). LC-PUFA, especially when combined with a diet low in these fatty acids, are mostly utilized in other ways.

As mentioned before, both n-3 and n-6 LC-PUFA serve as vital components in cell membranes (1,10,24). Zhou *et al.* (12) calculated the cellular distribution of lipids and found that DHA (22:6n-3) is mainly found in phospholipids, verifying DHA (22:6n-3) as key component of cell membranes. Additionally, LC-PUFA are involved in pathways revolving around cell synthesis, cellular signaling, homeostasis, ionic regulation, and pigmentation (1,4,12,24). Moreover, endocrine pathways, like steroid biosynthesis, are controlled by LC-PUFA in Atlantic salmon as well (1). Salmonid immune system functions, reproduction, and gut health, which influences salmon growth, also involve LC-PUFA (1,4,12).

Furthermore, LC-PUFA are of utmost importance for ontogenesis and the early development and functionality of neural tissue in salmonids, including the neural development of the nervous system, brains, and eyes (1,4,12). Especially n-3 LC-PUFA, EPA (20:5n-3) and DHA (22:6n-3), have been established to play key roles in this process (4). Beside the neural development, n-3 LC-PUFA are involved in metabolic regulation and functionality as chemical and effector of secondary messengers (1,4). In addition, the n-3 and n-6 LC-PUFA, ARA (20:4n-6) when converted to eicosanoids, function as stress and disease resistors (4,12). The eicosanoids synthesized from ARA (20:4n-6) are also active in lipid deposition and bone development (4).

Discussion

While considerable progress has been made in understanding the regulation and synthesis of LC-PUFA in salmonids, several gaps in knowledge remain that require further research.

For instance, various key proteins in salmonids have yet to be identified and characterized, and the functions of certain isoforms remain unexplored. This includes the regulatory roles of SREBP1 isoforms, as suggested by Harvey *et al.* (5) and the working mechanisms of SR-B1 and FABPpm (12,28,29). Moreover, several proteins related to β-oxidation, such as D-BP, ACAA1, and SCP-X, have not been fully characterized in salmonids (26).

It is assumed that the β-oxidation pathway in salmonids operates similarly to that in mammals. However, without further study this cannot be confirmed. Salmonids have demonstrated functional differences from mammals in other areas, such as possessing a single FADS where mammals possess two (14). Comparably, there may be multiple FATP and FABP proteins in salmonids that remain uncharacterized. Harvey *et al.* (5) identified genes encoding these proteins but did not specify their functions, indicating the need for further study.

In addition to characterizing proteins and their functions, there is a need for further clarification of related undiscovered ligands and the interactions between associated proteins. For instance, Leaver *et al.* (34) suggested that ssPPARβ2A might have several unidentified ligands that could bind to it. For example, the mammalian ligand GW501516 interacted with salmonid PPARβ, but a salmonid version of this ligand remains uncharacterized.

Furthermore, the role of PPARβ in the LC-PUFA biosynthesis pathway remains unclear. Datsomor *et al.* (14) hypothesized that ssPPARβ1A is essential for maintaining fatty acid homeostasis in the liver. Conversely, Leaver *et al.* (34) proposed that the function of ssPPARβ2A is to suppress other PPARs. Future research should focus on exploring these isoforms and their roles, especially in relation to the LC-PUFA biosynthesis pathway.

The regulatory influence and mechanisms by which insulin affects LC-PUFA metabolic pathways are not fully elucidated and require further study as well (14). In mammals, a connection has been established between insulin, GH production, and LXR expression (33). Similarly, in salmonids, there may be an association between insulin, CPT1, and HOAD, though this interaction has not been explicitly demonstrated (35). Lastly, the underlying mechanism by which insulin-mediated amplification of SR-B1 impacts fatty acid metabolism remains unclear (28).

Similarly, the regulatory function of ERs in the biosynthesis of LC-PUFAs needs further elucidation as well. The current hypothesis implies a potential negative interaction between PPARα and ERs, which influences the expression of ACOX1 and ACOX3, respectively (27). Therefore, ERs might function as regulators of the β-oxidation pathway.

The factors that influence the LC-PUFA biosynthesis pathway could benefit from clarification as well, particularly with regard to diet. Extensive research has examined how dietary changes, such as the transition from FO to VO, impact salmonids overall and specifically affect the n-3 LC-PUFA content in the final salmonid product. Zhang *et al.* (4) emphasizes the importance of including n-3 LC-PUFA, specifically EPA (20:5n-3) and DHA (22:6n-3), in the diet of aquacultured salmonids to ensure healthy growth and optimal product composition.

However, the strategies for enhancing n-3 LC-PUFA content in salmonids when FO is replaced with VO remain debated. Potential strategies include optimizing the lipid content in the salmonid diet,

supplementing with bioactive compounds to enhance the conversion of ALA (18:3n-3) to EPA (20:5n-3) and DHA (22:6n-3), and employing selective breeding to optimize genetic traits related to the conversion (4,8).

Future perspectives

To produce cultivated fish, specifically salmonid fillet, in a laboratory context, the optimal cell culture conditions, cell source, and type need to be determined. A key component of this process is the *in vitro* production of LC-PUFA, which are currently lacking in cultured fish products (6).

Based on the literature reviewed, Atlantic salmon hepatocytes appear to be most suitable cell type for *in vitro* LC-PUFA production, as the liver is the main organ responsible for LC-PUFA synthesis in salmonids (9). However, incorporating hepatocytes into the final salmonid product could result in a fillet that is less appealing to consumers due to the differing texture and taste of liver tissue compared to the muscle and fat typically found in fish fillets (44). Thus, increasing LC-PUFA production in alternative cell types is necessary to enhance the quality and acceptability of cultivated fish products.

Another potential cell type for *in vitro* LC-PUFA production is salmonid adipocytes, which are inherently capable of de novo lipogenesis (45). However, it is unclear whether adipocytes are also capable of synthesizing LC-PUFA. In vertebrates it has been shown that cells other than the liver are able to produce long chain fatty acids, particularly in adipose and mammary tissue (46). Myocytes are another option, having been previously studied in the context of cultivated meat products (6). Future research could explore the capacity of salmonid adipocytes and myocytes for LC-PUFA biosynthesis and strategies to optimize this process.

Mesenchymal stem cells are another interesting cell type for this type of research, as they can be differentiated into adipocytes and potentially into myocytes as well (47). If the goal were to produce just n-3 fatty acids, microorganisms like algae could be used as an alternative approach as well (48,49).

The biosynthesis of n-3 LC-PUFA is most efficient during the fry and parr stages of the Atlantic salmon life cycle (41). Therefore, extracting cells from salmon at these life stages might be optimal for laboratory LC-PUFA production. Beside life stage, the diet of the salmonid from which cells will be harvested is also an important factor. For example, Rosenlund *et al.* (3) found that the biosynthesis of n-3 LC-PUFA, specifically EPA (20:5n-3) and DHA (22:6n-3), was enhanced in hepatocytes from Atlantic salmon fed a diet low in essential fatty acids. Nonetheless, similar to what was stated in the discussion, the effects of varying levels of PUFA precursors in the diet on different cell types involved in LC-PUFA biosynthesis remain a topic of ongoing discussion in literature.

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