Metabolism of highly unsaturated n-3 and n-6 fatty acids

Howard Sprecher *

Department of Molecular and Cellular Biochemistry, The Ohio State University, 337 Hamilton Hall, 1645 Neil Avenue, Columbus, OH 43210, USA

Received 7 March 2000; received in revised form 17 May 2000; accepted 24 May 2000

Keywords: Metabolism; Unsaturated fatty acids; Fatty acids

1. Introduction

It is generally accepted that dietary linoleate and linolenate are metabolized, respectively, to 5,8,11,14-20:4 and 5,8,11,14,17-20:5 via the use of position-specific Δ6 and Δ5 desaturases and a malonyl-CoA-dependent chain elongation step as follows:

- the n-6 pathway starting from dietary linoleate, 9,12-18:2 → 6,9,12-18:3 → 8,11,14-20:3 → 5,8,11,14-20:4;
- the n-3 pathway starting from dietary linolenate, 9,12,15-18:3 → 6,9,12,15-18:4 → 8,11,14,17-20:4 → 5,8,11,14,17-20:5.

The first objective of this review is to summarize some of the more important contributions that have been made to establish the validity of these pathways.

For many years, it was assumed, but not proven, that the synthesis of 22-carbon acids with their first double bond at position 4 requires an acyl-CoA-dependent Δ4 desaturase. It has now been established that the synthesis of these compounds requires a degradative step and proceeds as follows:

- the n-6 pathway, 5,8,11,14-20:4 → 7,10,13,16-22:4 → 9,12,15,18-24:4 → 6,9,12,15,18-24:5 → 4,7,10,13,16-22:5;

The second objective of this review is to summarize evidence that led to the elucidation of these pathways and controls that are operative for regulating the synthesis of polyunsaturated fatty acids (PUFA) with their first double bond at position 4.

The revised pathways for the biosynthesis of PUFA require the participation of both microsomes and peroxisomes as they relate to the synthesis of 22-carbon acids with their first double bond at position 4. The last objective of the review is to summarize results of studies to determine what regulates biosynthesis of 20-versus 22-carbon n-3 and n-6 PUFA.

2. Synthesis of 20-carbon PUFA

The metabolic pathways of PUFA biosynthesis were elucidated primarily in the laboratories of Klenk [1] and Mead [2] more than 40 years ago. Both groups of investigators developed techniques to synthesize labeled PUFA which were administered to animals. Tissue lipid PUFA were isolated and
degraded. The labeling profiles of these compounds were used to propose pathways for PUFA biosynthesis. These studies also established that there was no direct interconversion between metabolites in the two families of PUFA. It was also shown that 5,8,11–20:3, a fatty acid that accumulates in lipids only when there is a deficiency of dietary essential fatty acids, is derived from oleate [2].

Three different experimental approaches contributed to the generally accepted, but unproven, hypothesis that PUFA biosynthesis requires position-specific Δ6 and Δ5 desaturases. First, when microsomes were incubated with PUFA, with their first double bond at different positions, the composition of the labeled products was consistent with separate desaturases [3–5]. For example, there was a decrease of the labeled products was consistent with separate double bond at different positions, the composition of the labeled products was consistent with separate desaturases [3–5]. For example, there was a decrease in the desaturation of [1-14C]9,12–18:2 when increasing amounts of 9,12,15–18:3 were added to the incubation [3]. Results of this type implied that microsomes contained a position-specific Δ6 desaturase that desaturated both 9,12–18:2 and 9,12,15–18:3. Secondly, the fatty acid composition of tissue phospholipids, when various PUFA from the n-6 and n-3 families were fed to animals, was generally consistent with the presence of position-specific desaturases [6,7]. Thirdly, a number of cell lines were able to introduce a double bond at position 5, but not at position 6, when appropriate PUFA were used as substrates [8,9]. It was not until recently that Δ6 [10–12] and Δ5 [13–17] desaturases were cloned from a number of sources and expressed in a number of different types of cells. These studies established that a single gene product desaturates both 9,12–18:2 and 9,12,15–18:3 but that fatty acids with their first double bond at position 8 are not desaturated. In a similar way the 5 desaturase accepts fatty acids with their first double bond at position 8 but not at position 9. Interestingly the gene product of the Δ5 desaturase from C. elegans, when expressed in Saccharomyces cerevisiae, desaturated 8,11,14–20:3 as well as 11,14–20:2 and 11,14,17–20:3 at position 5 [14].

When the pathways of PUFA biosynthesis were being elucidated the labeling profiles of metabolites suggested that 9,12–18:2 and 9,12,15–18:3 might initially be chain elongated, respectively, to 11,14–20:2 and 11,14,17–20:3 followed by desaturation at position 8 [1,18,19]. In these studies, labeled 11,14–20:2 and 11,14,17–20:3 were used as substrates. Total tissue lipid fatty acids were degraded. The presence of a labeled 5-carbon dicarboxylic acid was taken as evidence that it was derived from 5,8,11,14–20:4 or 5,8,11,14,17–20:5. When a variety of PUFA, with their first double bond at position 11, were incubated with rat liver microsomes only the Δ5 desaturated product was detected [20,21]. In these studies, individual fatty acids were isolated and degraded [20,21] and it was established that the double bonds were in the 5,11,–12 positions. Subsequent studies with rat [22] and human testes [23] showed that 11,14–20:2 was metabolized primarily to 5,11,14–20:3 although a small amount of label was detected in 5,8,11,14–20:4 suggesting that 11,14–20:2 was sequentially desaturated at positions 8 and 5 to yield 5,8,11,14–20:4. In their paper [22], the authors note that the tissue-specific conversion of 11,14–20:2 at position 8 might be carried out by a Δ9 desaturase, which would imply a lack of absolute specificity for one of the Δ9 desaturases [24]. This hypothesis has never been evaluated. Wallis and Browse [25] recently reported the isolation of a Δ8 desaturase from Euglena gracilis. It remains to be determined whether animals express a similar enzyme and its role in the biosynthesis of PUFA via a pathway that does not require desaturation of fatty acids at position 6.

The first evidence for the presence of a microsomal chain elongation pathway was obtained in studies carried out by Abraham et al. [26] and Matthes et al. [27]. These investigators showed that the addition of microsomes to a particle-free supernatant stimulated the synthesis of fatty acids. In 1965, Nugteren [28] published his classical paper showing that the malonyl-CoA-dependent microsomal pathway for chain-elongating myristic acid proceeds through the following four reactions.

1. Myristoyl-CoA+Malonyl-CoA→β-ketopalmitoyl-CoA+CO2+CoASH
2. β-Ketopalmitoyl-CoA+NADPH+H+→β-hydroxypalmitoyl-CoA+NADP+
3. β-Hydroxypalmitoyl-CoA→2-trans-hexadecenoyl-CoA
4. 2-trans-Hexadecenoyl-CoA+NADPH+H+→palmitoyl-CoA+NADP

It is possible to assay three of the reactions inde-
pendently. Reaction 1 is assayed by incubating an acyl-CoA with malonyl-CoA. Reaction 2 cannot be assayed independently since upon addition of a β-ketoacyl-CoA and NADPH the fully chain elongated product is produced. Reaction 3 proceeds without addition of co-factors while the final reaction is assayed by incubating 2-trans-acyl-CoAs with NADPH. The substrates and products of all reactions are acyl-CoAs [29]. In each of the four reactions, the acyl-CoA is used directly by the enzyme without transfer to some other acceptor. In liver, the overall rate of chain elongation is similar to that of β-ketoacyl-CoA synthesis and much slower than subsequent reactions [30,31]. Results obtained in a number of studies are consistent with the presence of multiple condensing enzymes with the resulting intermediates being channeled into a common set of enzymes to complete the chain elongation process [30–34]. However, since these enzymes have not been purified several other possibilities cannot be excluded. It is possible that all four reactions are carried out by a multifunctional enzyme. This is unlikely since the activity of the condensing enzyme, using palmitoyl-CoA and 6,9,12–18:3-CoA as substrates, was influenced differently by the dietary history of the animal. Reaction rates subsequent to β-ketoacyl-CoA synthesis, using appropriate unsaturated and saturated 3-hydroxyacyl-CoAs and 2-trans-2-enoyl-CoAs, were insensitive to dietary change [30]. It is also possible that a number of condensing enzymes channel their respective β-ketoacyl-CoAs into a tri-functional enzyme, with three catalytic activities, to complete the chain elongation process. In any case the microsomal chain elongation system, unlike the position-specific desaturases, accepts a wide variety of saturated and unsaturated fatty acids as primers for chain elongation [30,31,34].

Liver phospholipids do not contain significant amounts of saturated or unsaturated fatty acids with more than 22-carbon atoms. The phospholipids from a number of tissues, including brain and retina, contain small amounts of very long chain PUFA with up to 40-carbon atoms [35,36]. A number of investigators have shown that microsomes from a number of tissues, including brain, have the capacity to chain elongate both saturated and unsaturated fatty acids (reviewed in [34]) but there is a lack of information on the synthesis of the very long chain PUFA. It remains to be determined whether there are tissue-specific differences as they relate to the expression of enzymes that chain elongate fatty acids of different chain lengths.

3. Synthesis of 22-carbon PUFA with their first double bond at position 4

For years it was assumed that 4,7,10,13,16–22:5 and 4,7,10,13,16,19–22:6 were synthesized, respectively, from 7,10,13,16–22:4 and 7,10,13,16,19–22:5 by a microsomal acyl-CoA-dependent Δ4 desaturase. The first paper questioning this hypothesis was published in 1973 when Ayala et al. [37] reported that testes and liver microsomes did not desaturate 7,10,13,16–22:4. In essence, we repeated this experiment and observed that liver microsomes did not desaturate 7,10,13,16,19–22:5. When malonyl-CoA and NADPH were included in the incubation the substrate was chain elongated to 9,12,15,18,21–24:5 which was then desaturated to yield 6,9,12,15,18,21–24:6. The findings suggested that if 6,9,12,15,18,21–24:6 underwent one cycle of β-oxidation, it would yield 4,7,10,13,16,19–22:6, which could be used as a substrate for phospholipid biosynthesis [38]. It has long been recognized that long chain PUFA are partially degraded with the subsequent esterification of chain-shortened products. For example, when 4,7,10,13,16–22:5 [39] and 7,10,13,16–22:4 [40] were fed to rats raised from weanling, on a diet devoid of fat, there was an increase in the level of stearified 5,8,11,14–20:4 in liver lipids versus in controls.

To determine the pathway for the biosynthesis of 4,7,10,13,16,19–22:6 a number of labeled n-3 PUFA were incubated with hepatocytes and the radioactive metabolites, as esterified in phospholipids, were defined [38]. When [1-14C]7,10,13,16,19–22:5 was the substrate it was esterified and metabolized to 4,7,10,13,16,19–22:6. In addition, the esterification of small amounts of labeled 24-carbon acids was consistent with the hypothesis that they are metabolic intermediates in the synthesis of 4,7,10,13,16,19–22:6. When 3-14C-labeled 9,12,15,18,21–24:5 and 6,9,12,15,18,21–24:6 were used as substrates only small amounts were directly esterified. They were both primarily metabolized to yield esterified
4,7,10,13,16,19–22:6 which requires that 6,9,12,15,18,21–24:6 undergoes one cycle of β-oxidation.

Results with microsomes and hepatocytes demonstrated that 4,7,10,13,16,19–22:6 synthesis was not a process confined to the endoplasmic reticulum, but it also required the participation of degradative enzymes. This site for degradation could be either peroxisomes or mitochondria. When 3,14C-labeled 7,10,13,16,19–22:6 and 7,10,13,16,19–22:5 were incubated with fibroblasts, they were esterified and also partially β-oxidized to yield, respectively, esterified 5,8,11,14–20:4 and 5,8,11,14–17:0. When fibroblasts from patients with Zellweger’s disease, who lack peroxisomes, were incubated under identical conditions, the substrates, and not the chain-shortened products, were esterified [41]. When 3,14C-labeled 9,12,15,18,21–24:5 and 6,9,12,15,18,21–24:6 were incubated with fibroblasts, both acids were metabolized to yield esterified 4,7,10,13,16,19–22:6. When [3,14C]9,12,15,18,21–24:5 was incubated with fibroblasts from patients with Zellweger’s disease it was metabolized to 6,9,12,15,18,21–24:6 but no 4,7,10,13,16,19–22:6 was made. When [3,14C]6,9,12,15,18,21–24:6 was incubated with fibroblasts from patients with Zellweger’s disease it also was not converted to 4,7,10,13,16,19–22:6. In their important study, Moore et al. [42] also observed that control fibroblasts metabolized [1,14C]9,12,15–18:3 to 4,7,10,13,16,19–22:6 but 6,9,12,15,18,21–24:6 was the end metabolite when fibroblasts from Zellweger’s patients were used. These studies established that microsomal desaturation and chain elongation reactions were not affected in fibroblasts from patients with Zellweger’s disease. Conversely, these cells were unable to partially degrade fatty acids, which is required for converting 6,9,12,15,18,21–24:6 to 4,7,10,13,16,19–22:6. When [3,14C]7,10,13,16,19–22:5 was incubated with retinal pigment epithelium the specific activities of the metabolites were consistent with what would be expected if 24-carbon acids were the precursor of 4,7,10,13,16,19–22:6 [43]. Both retinal endothelial cells and cerebral endothelial cells metabolized [3,14C]7,10,13,16,19–22:5 to 4,7,10,13,16,19–22:6 via 24-carbon acids [44]. The above studies all document that 24-carbon acids are the precursors of 22-carbon acids with their first double bond at position 4 and their synthesis requires the participation of peroxisomes.

One other remote possibility, that has not been ruled out, exists for the synthesis of 4,7,10,13,16,19–22:6. Pugh and Kates [45] reported that 1-acyl-2-[14C]eicosatrienoyl-sn-glycero-3-phosphorylcholine was desaturated to 1-acyl-2-[14C]arachidonoyl-sn-glycero-3-phosphorylcholine by rat liver microsomes. Phospholipids containing esterified 7,10,13,16,19–22:5 have not been used as substrates to determine whether they are directly desaturated by microsomes. As noted above fibroblasts from Zellweger’s patients maintain their ability to desaturate and chain elongate fatty acids. If esterified 7,10,13,16,19–22:5 was a substrate for desaturation at position 4, it would be expected that labeled 4,7,10,13,16,19–22:6 would have been synthesized from phospholipids containing esterified 7,10,13,16,19–22:5 by fibroblasts from patients with Zellweger’s disease. No such conversion was observed [42].

Results analogous to those described above were obtained for the synthesis of 4,7,10,13,16–22:5 from the appropriate 24-carbon n-6 PUFA [46,47]. It remains an interesting question why microsomes desaturate fatty acids at positions 9, 6, and 5, but not at position 4. However, the participation of enzymes in more than one intracellular compartment, as it relates to lipid synthesis, is not confined to the synthesis of 22-carbon acids with their first double bond at position 4. For example, the de novo synthesis of phospholipids containing the 1-O-alkenyl bond at the sn-1 position requires the participation of both microsomes and peroxisomes [48].

4. Control of the biosynthesis of PUFA with their first double bond at position 4

The synthesis and esterification of 22-carbon acids, with their first double bond at position 4, is a more complex process than is the synthesis of 20-carbon acids with their first double bond at position 5. Enzymes in both peroxisomes and microsomes are used. In addition, fatty acids move between these two subcellular compartments. The diagram in Fig. 1 depicts a simplified overview of some of these processes. When 24-carbon PUFA are produced from precursors in hepatocytes, or used directly as substrates, only trace amounts are esterified [38,46]. When 24-carbon acyl-CoAs are incubated with liver micro-
somes and 1-acyl-sn-glycero-3-phosphocholine rates of esterification were slightly above background using a spectral assay method [47,49]. When 24-carbon acids were incubated with liver microsomes their rates of chain elongation to 26-carbon acids were low [38]. The possibility exists in liver that small amounts of 24-carbon acids may be used as substrates for esterification or chain elongation, but most likely they primarily move to another intracellular site for metabolism. The substrate for the conversion of 22-carbon to 24-carbon fatty acids by microsomes has not been determined. However, based on other studies [29,50], it is assumed that acyl-CoAs are the substrates and products of all microsomal desaturation and chain elongation reactions. As shown in Fig. 1 it is not known whether fatty acids move to another cellular compartment as acyl-CoAs or whether acyl-CoAs are hydrolyzed to free fatty acids in the cytosol by acyl-CoA hydrolases [51,52]. Microsomes, peroxisomes and mitochondria all contain a long chain acyl-CoA synthase but only peroxisomes and microsomes have a very long chain acyl-CoA synthase [53,54]. If 24-carbon fatty acids were targeted primarily to mitochondria, and used as substrates for β-oxidation, the degradative process most likely would go to completion without accumulation of chain-shortened metabolites [55,56].

Studies were carried out to determine whether liver mitochondria degrade 24-carbon PUFA. Rates of acyl-CoA synthesis, acylcarnitine synthesis and β-oxidation of the acyl-CoA derivatives of linoleic acid and palmitic acid were compared with those of 9,12,15,18-24:4 and 6,9,12,15,18-24:5. In these studies, 24-carbon n-6 acids rather than the corresponding 24-carbon n-3 acids were used. The possibility exists that 24-carbon n-3 acids might be processed differently. This is unlikely since in all ways the corresponding 24-carbon n-3 and n-6 were metabolized in a similar manner [38,46,47,49]. Rates of activation of palmitic and linoleic acids by liver mitochondria

---

Fig. 1. The intracellular movement of PUFA and their metabolism in the cell. The diagram shows that when PUFA are made in the endoplasmic reticulum, they may be used directly as substrates for phospholipid biosynthesis. The substrate and products of PUFA biosynthesis are the acyl-CoA derivatives. Acyl-CoAs may move directly to another subcellular compartment or be hydrolyzed to free acids by cytosolic acyl-CoA hydrolases. Metabolism by mitochondria requires that acyl-CoAs are converted to acylcarnitines for transport into mitochondria, where β-oxidation most likely results in their complete degradation. Conversely, when 6,9,12,15,18,21-24:6 is degraded by peroxisomes the diagram shows that 4,7,10,13,16,19–22:6 preferentially moves back to the endoplasmic reticulum where it is used for membrane lipid synthesis, rather than serving as a substrate for continued peroxisomal β-oxidation.
were between 50 and 60 nmol/min/mg of protein, while the corresponding values for 9,12,15,18-24:4 and 6,9,12,15,18-24:5 were only 1–2 nmol/min/mg of protein. Rates of acylcarnitine synthesis, using the CoA esters of palmitic and linoleic acids, were between 10 and 15 nmol/min/mg of protein while those for the 24-carbon acyl-CoAs were between 1 and 2 nmol/min/mg of protein. Between 35 and 40 ng atoms of oxygen/min/mg/protein were consumed when the CoA esters of palmitic and linoleic acid were incubated with liver mitochondria while less than 2 ng atoms of oxygen/min/mg of protein was consumed for the 24-carbon acyl-CoAs [57]. Collectively the results show that 24-carbon acids are poor substrates for mitochondrial \( \beta \)-oxidation. When they are synthesized in the endoplasmic reticulum they most likely move to peroxisomes for subsequent metabolism.

Several types of experiments were carried out to define what differences exist in the metabolism of 9,12,15,18-24:5 versus 6,9,12,15,18-24:6 [49]. Both fatty acids were converted to their acyl-CoAs by peroxisomes, albeit at slower rates than were 20- and 22-carbon \( n \)-3 fatty acids. Since 24-carbon acids were poor substrates for activation by mitochondria their activation by peroxisomes, i.e. 16–20 nmol/min/mg of peroxisomal protein, suggests that the very long chain activating enzyme was used. Acid-soluble radioactivity, as a measure of \( \beta \)-oxidation, was quantified when \( ^{3,14}C \)-labeled 9,12,15,18,21–24:5 and 6,9,12,15,18,21–24:6 were incubated with ATP, Mg\(^{2+}\), NADPH and NAD. The amount of acid-soluble radioactivity was compared with what was produced when microsomes and 1-acyl-sn-glycero-3-phosphocholine were included in the incubation. Under the later incubation conditions, a chain-shortened metabolite could either serve as a substrate for continued peroxisomal degradation or move to microsomes to esterify 1-acyl-sn-glycero-3-phosphocholine. Peroxisomes are not able to esterify the acceptor [58]. When peroxisomes were incubated with 100 nmol of \( ^{3,14}C \)9,12,15,18,21–24:5 about 35 nmol of acid-soluble radioactivity was produced after 30 min with the accumulation of 7,10,13,16,19–22:5 as the only radioactive metabolite. There was no significant decrease in the production of acid-soluble radioactivity when microsomes and 1-acyl-sn-glycero-3-phosphocholine were added to the incubation. Under these conditions, 3 and 6 nmol, respectively, of 9,12,15,18,21–24:5 and 7,10,13,16,19–22:5 were esterified. When \( ^{3,14}C \)9,12,15,18,21–24:5 is degraded to 5,8,11,14,17–20:5 no double bonds are removed and two cycles of \( \beta \)-oxidation requires only the enzymes of saturated fatty acid degradation. When \( ^{1,14}C \)7,10,13,16,19–22:5 is produced it is preferentially used as a substrate for continued degradation, rather than to move to microsomes for esterification.

When \( ^{3,14}C \)6,9,12,15,18,21–24:6 was incubated under identical conditions, the addition of microsomes and 1-acyl-sn-glycero-3-phosphocholine decreased the production of acid-soluble radioactivity from about 6 to 3 nmol after 30 min [49] and 6 and 41 nmol, respectively, of 6,9,12,15,18,21–24:6 and 4,7,10,13,16,19–22:6 were esterified. The degradation of 6,9,12,15,18,21–24:6 to 4,7,10,13,16,19–22:6 also uses only the enzymes of saturated fatty acid \( \beta \)-oxidation. The continued degradation of 4,7,10,13,16,19–22:6 results in the synthesis of 2-trans-4,7,10,13,16,19–22:7 which is a substrate for NADPH-dependent 2,4-dienoyl-CoA reductase. When peroxisomes were incubated with \( ^{3,14}C \)6, 9,12,15,12,15–24:6 there was a time-dependent increase in the accumulation of 4,7,10,13,16,19–22:6, 2-trans-4,7,10,13,16,19–22:7 and 3,5,7,10,13,16,19–22:7. The later two intermediates did not accumulate when incubations contained microsomes and 1-acyl-sn-glycero-3-phosphocholine. The results led us to hypothesize that when 4,7,10,13,16,19–22:6 was made in peroxisomes it preferentially moved out of peroxisomes to microsomes for esterification, rather than serving as a substrate for continued degradation. In this regard, the reaction catalyzed by NADPH-dependent 2,4-dienoyl-CoA reductase may be considered as a slow or perhaps rate-limiting step. When 2-trans-4,7,10,13,16,19–22:7 is produced it may be metabolized in two ways. It is further degraded since some acid-soluble radioactivity was produced even when incubations contained microsomes and 1-acyl-sn-glycero-3-phosphocholine. Alternatively, it may be metabolized in a very unique way by isomerization of the 2-trans-4-double bonds to the 3,5-positions giving rise to 3,5,7,10,13,16,19–22:7, a reaction that is catalyzed by \( \Delta^{3,5,7,12-4,6}-\)trienoyl-CoA isomerase. Liang et al. [59] recently reported that \( \Delta^{3,5,7,12,4,6}\)-dienoyl-CoA isomerase, as purified from pig heart, was a component part of \( \Delta^{3,5,7,12,4,6}\).
dienoyl-CoA isomerase. Peroxisomes contain Δ1,5,Δ2,4-dienoyl-CoA isomerase [60,61], but it has not been established that this enzyme has trienoyl-CoA isomerase activity. Based on the results of Liang et al. [59], 3,5,7,10,13,16,19-22:7 it would be converted to 2,4,6,10,13,16,19-22:7 by trienoyl-CoA isomerase. Reduction of this conjugated triene by NADPH-dependent 2,4-dienoyl-CoA reductase would yield 3,6,10,13,16,19-22:6, which upon isomerization with Δ1,Δ2,-enoyl-CoA isomerase [62], yields 2-trans-6,10,13,16,19-22:6. Completion of the β-oxidation spiral, by loss of acetyl CoA, would give 4,8,11,14-17-20:5 in which one of the double bonds is not in the skipped pattern of unsaturation. Two points should be emphasized. The β-oxidation of 4,7,10,13,16,19-22:6 must proceed through 2-trans-4,7,10,13,16,19-22:7, but it is not known whether in vivo this intermediate is isomerized to the compound containing double bonds in the 3,5,7-positions. Secondly, further studies are required to determine how a compound of this type is metabolized.

The finding that 22-carbon n-3 acids and n-6 acids [47,49] with their first double bond at position 4, are poor substrates for peroxisomal degradation, raises the question as to whether this control is specific for only 22-carbon PUFA. When 6,9,12-18:3 was incubated with peroxisomes the acyl-CoAs of the following acids accumulated: 4,7,10-16:3, 2-trans-4,7,10-16:4, 5,8-14:2, 4-10:1, 2-trans-4-10:2 and 6:0 [63]. Peroxisomes metabolized 12(S)-hydroxy-5,8,10,14-eicosatetraenoic to 8-hydroxy-4,6,10-hexadecatrienoic acid [64]. Fibroblasts from controls, but not those from patients with Zellwegers disease, metabolized arachidonic acid to 5,8-14:2 [65] and 4,7,10-16:3 [66] and both metabolites were released into the medium. In a similar way 6,9,12,15-18:4, 4,7,10,13-16:4 and 5,8,11-14:3 accumulated when 5,8,11,14,15,17-20:5 was the substrate. The 16-carbon metabolite, with its first double bond at position 4 [67], was the major product. Studies, using different substrates, all suggest that the peroxisomal removal of the double bond at position 4 is a slow reaction.

5. Revised pathways for the biosynthesis of PUFA

The diagram in Fig. 2 depicts revised pathways for the biosynthesis of n-3 and n-6 PUFA. These pathways raise several new questions about the regulation of PUFA biosynthesis. Both 9,12-18:2 and 9,12,15-18:3 are metabolized via identical reaction sequences. The pathways show that there is recycling of 22- and 24-carbon PUFA. These pathways show that fatty acids are partially degraded without the loss of any double bonds. These conversions use only the enzymes of saturated fatty acid degradation. The pathways also show that fatty acids are partially degraded without the loss of any double bonds. These conversions use only the enzymes of saturated fatty acid degradation. The pathways also show that fatty acids are partially degraded without the loss of any double bonds. These conversions use only the enzymes of saturated fatty acid degradation. The pathways also show that fatty acids are partially degraded without the loss of any double bonds.
the endoplasmic reticulum its reduction by 2-trans-enoyl-CoA reductase, the last reaction in microsomal chain elongation, would also yield 7,10,13,16,19-22:5.

The pathways in Fig. 2 imply that the biosynthesis of both 5,8,11,14-20:4 and 5,8,11,14,17-20:5 are processes confined to the endoplasmic reticulum and that no recycling occurs whereby these two 20-carbon acids can be made by a partial degradation-resynthesis cycle. This may not necessarily be totally correct. When 6,9,12–18:3 was incubated with peroxisomes and microsomes along with NAD, NADPH and malonyl-CoA a number of acyl-CoAs accumulated including 6:0-CoA, 5,8–14:2-CoA 7,10–16:2-CoA, and most importantly, 9,12–18:2-CoA [70]. The accumulation of small amounts of 6:0-CoA shows that some of the substrate was degraded with the removal of all double bonds. When the CoAs of 5,8–14:2 and 7,10–16:2 are produced, they may be further degraded by peroxisomes, or move to microsomes where they are chain elongated to 9,12–18:2. When 5,8–14:2, 7,10–16:2 [71] and 4,7,10–16:3 [72] were fed to rats, raised on a diet devoid of essential fatty acids, they were metabolized to yield esterified 5,8,11,14–20:4. Hansen et al. [73] found that 5,8,11,14–20:4 was metabolized to 9,12–18:2 in rats raised on a diet devoid of fat. These studies show that when the dietary content of essential fatty acids is restricted, there is recycling of acids containing less than 22-carbon atoms. It is not known how important this recycling process is when adequate amounts of linoleate and linolenate are included in the diet.

The synthesis and esterification of 20-carbon PUFA, with their first double bond at position 5, are processes that are both confined primarily to the endoplasmic reticulum. It has never been established why phospholipids accumulate specific 18- and 20-carbon acids. For example, liver phospholipids accumulate both 9,12–18:2 and 5,8,11,14–20:4, but only trace amounts of 6,9,12–18:3 and 8,11,14–20:3 and very low levels of any 18- or 20-carbon n-3 PUFA. Reaction rates for individual steps in PUFA biosynthesis [74] are much slower than for the metabolism of the analogous n-6 acids [77,78]. When [3-14C]9,12,15,18,21–24:5 was incubated with peroxisomes, microsomes and 1-acyl-sn-glycero-3-phosphocholine 3 and 6 nmol, respectively, of 9,12,15,18,21–24:5 and 7,10,13,16–22:5 were esterified [49]. Using identical conditions, 3 and 9 nmol, respectively, of 9,12,15,18–24:4 and 7,10,13,16–22:4 were esterified when the 24-carbon n-6 acid was the substrate [47]. When [3-14C]6,9,12,15,18,21–24:6 and [3-14C]6,9,12,15,18–24:5 were used as substrates less than 6 nmol of either substrate was esterified but 41 and 32 nmol, respectively, of 4,7,10,13,16,19–22:6 and 4,7,10,13,16–22:5 were incorporated into the acceptor [47,49]. Individual reaction rates for the conversion of 20-carbon acids, with their first double bond at position 5, to 22-carbon acids with their first double bond at position 4, are thus also of little value to predict why phospholipids accumulate specific 22-carbon PUFA.

It is also possible that there may be competitive interactions between 18- and 20-carbon metabolites...
with longer chain PUFA for the enzymes of PUFA biosynthesis. The revised pathways of PUFA biosynthesis, in Fig. 2, show that both 18- and 24-carbon PUFA are desaturated at position 6. Desaturation of 9,12-18:2 and 9,12,15-18:3, at position 6, is generally recognized as the rate-limiting step in PUFA biosynthesis [74]. As noted previously, it is now established that a single position-specific Δ6 desaturase accepts both 9,12-18:2 and 9,12,15-18:3. Competitive substrate studies, where mixtures of 18- and 24-carbon acids were incubated with microsomes, showed that there were competitive interactions between fatty acids for desaturation at position 6 [77]. A possible interpretation was that a single Δ6 desaturase accepts both 18- and 24-carbon acids. By using trans-9,12-18:2 as a Δ6 desaturase inhibitor, Marzo et al. [79] reported that the synthesis of 4,7,10,13,16,19-22:6 from [1-14C]9,12,15-18:3, in Y-79 cells, was depressed to a greater degree than was observed when the substrate was [1-14C]5,8,11,14,17-20:5. These results are consistent with the presence of separate chain-length-specific Δ6 desaturases. Studies have not yet been carried out to determine whether the recently cloned Δ6 desaturases accept both 18- and 24-carbon substrates. If a single enzyme accepts both 18- and 24-carbon acids, the control of this enzyme will be of considerable interest since, in vivo, four different acids would be desaturated at position 6. The revised pathways also show that three acids in each metabolic pathway are chain elongated, and two of these chain elongation reactions take place in sequence. The results of competitive substrate studies were most consistent with the presence of chain length specific condensing enzymes [78]. It must be stressed that until the enzymes of malonyl-CoA-dependent chain elongation are purified, the results of competitive substrate studies must be interpreted with a great degree of caution.

A more subtle type of metabolic control may be operative in vivo which cannot be detected when subcellular particles are incubated with a radioactive substrate. The diagram in Fig. 2 shows that 22-carbon acids with their first double bond at position 7, may be used as precursors for the synthesis of 22-carbon acids with their first double bond at position 4. In addition, when these acids are incubated with a number of different cells, they are also partially degraded to yield 20-carbon acids with their first double bond at position 5, which are esterified [42,44]. In vivo studies suggest that 7,10,13,16-22:4 and 7,10,13,16,19-22:5 may be important central metabolites that are metabolized somewhat differently. When [3-14C]7,10,13,16-22:4 was injected into the tail veins of rats, 96 and 4%, respectively, of the esterified radioactivity in liver phospholipids was 5,8,11-14-20:4 and 7,10,13,16-22:4, but no labeled 4,7,10,13,16-22:5 was detected. Conversely, when [3-14C]7,10,13,16,19-22:5 was the substrate 20, 56 and 24%, respectively, of the esterified radioactivity was 5,8,11,14,17-20:5, 7,10,13,16,19-22:5 and 4,7,10,13,16,19-22:6 [80]. The results suggest that differences exist for the metabolism of these central intermediates that cannot be determined by ex vivo studies using subcellular particles. The labeling patterns are generally consistent with liver phospholipid compositional data since larger amounts of 4,7,10,13,16,19-22:6 than 4,7,10,13,16-22:5 are esterified.

Rat testes are atypical since their phospholipids contain large amounts of esterified 4,7,10,13,16-22:5, but only low levels of the corresponding n-3 acids [81]. Rat testes convert linoleate to 24-carbon n-6 fatty acids [82,83]. We compared the metabolism of 3-14C-labeled n-6 acids with the corresponding n-3 analogs. It was our expectation that labeled 7,10,13,16-22:4 would readily be metabolized to yield esterified 4,7,10,13,16-22:5. Surprisingly, 6 h after the intratesticular injection of [3-14C]7,10,13,16-22:4, the percentage of esterified radioactivity in phospholipids for 5,8,11,14-20:4, 7,10,13,16-22:4 and 4,7,10,13,16,19-22:5 was, respectively, 42, 55, and only 3%. When [3-14C]7,10,13,16,19-22:5 was injected 29, 13 and 47% of the radioactivity was esterified, respectively, in 5,8,11,14,17-20:5, 7,10,13,16, 19-22:5 and 4,7,10,13,16,19-22:6. In contrast to the 22-carbon acids, there were no differences as to how corresponding pairs of 24-carbon n-3 and n-6 fatty acids were processed [84]. Even in a tissue where phospholipids preferentially accumulate 4,7,10,13,16-22:5, versus 4,7,10,13,16,19-22:6, there appears to be preferential partial β-oxidation of 7,10,13, 16-22:4 to 5,8,11,14-20:4 versus metabolism to 4,7,10,13,16-22:5. Once the biosynthesis of PUFA proceeded beyond the point of 22-carbon acids, with their first double bond at position 7, there were no differences in the metabolism of metabolic...
analogs of n-3 and n-6 24-carbon acids. However, it must be emphasized that it was not possible to accurately define specific activities of substrate pools. The possibility exists that differences in pool sizes might alter the interpretation of data particularly as it relates to the synthesis of 4,7,10,13,16-22:5.

There is a unique problem in determining how 24-carbon PUFA with their first double bond at position 9 are metabolized. As shown in Fig. 2, they are metabolized, via 24-carbon acids with their first double bond at position 6, to 22-carbon acids with their first double bond at position 4. They also may be degraded, but after two cycles of β-oxidation, the labeled carbon is lost so it is not possible to follow their continued metabolism. When [3-14C]7,10,13,16-22:4 was incubated with microsomes, peroxisomes and 1-acyl-sn-glycero-3-phosphocholine, the arachidonate that was produced was preferentially esterified rather than being degraded [85]. When 3,14C-labeled 9,12,15,18-24:4 and 9,12,15,18-21-24:5 were incubated with peroxisomes, microsomes and 1-acyl-sn-glycero-3-phosphocholine the amount of acid-soluble radioactivity produced was similar to what was generated in the presence of only peroxisomes [47,49]. When [3-14C]9,12,15,18-24:4 was incubated with peroxisomes, microsomes and 1-acyl-sn-glycero-3-phosphocholine 19.2 nmol of acid-soluble radioactivity was generated after 30 min. When [23,23,24,24-2H4]9,12,15,18-24:4 was the substrate 15 nmol of deuterium-labeled 5,8,11,14-20:4 was esterified into the acceptor. In these ex vivo experiments, the substrate was preferentially metabolized, via two cycles of β-oxidation, to give esterified 5,8,11,14-20:4 [47]. Collectively, the above results show that when a PUFA is produced, by partial degradation, with its first double bond at position 5, it is preferentially esterified rather than serving as a substrate for esterification.

Rather than serving as a substrate for esterification. However, it is now established that a second pathway exists in both peroxisomes and mitochondria for degrading fatty acids with their first double bond at position 5 (reviewed in [55]). The classical pathway of fatty acid degradation requires that 3,6,9,12-18:4 is produced from 5,8,11,14-20:4 after one cycle of β-oxidation. In the second pathway, as depicted in Fig. 3, the double bond at position 5 is removed during the first degradative cycle. The pathway requires that the product generated by fatty acid oxidase is isomerized by Δ1,Δ3-enoyl-CoA isomerase to a compound with double bonds in the 3,5-position. The conjugated 3,5 double bonds are then isomerized to the 2,4 isomer by Δ1,Δ3,5-enoyl-CoA isomerase. The next step in the degradative spiral then requires the putative control enzyme, NADPH-dependent 2,4-dienoyl-CoA reductase. The relative contribution of the two pathways for degrading fatty acids with their first double bond at position 5 has not been established. Caruso et al. [86] reported that 5,8,11,14-20:4, labeled with deuterium at the double bonds, was metabolized by THP-1 cells to a number

![Fig. 3. The pathway for the removal of the double bond at position 5 during the first cycle of peroxisomal β-oxidation. The substrate reacts with fatty acid oxidase to insert a double bond in the 2-trans position (reaction 1). The 2-trans-double bond is isomerized to the 3 position by Δ1,Δ3-enoyl-CoA isomerase (reaction 2). This 3,5-conjugated diene is then isomerized to the 2,4-conjugated diene by Δ1,Δ3,5-dienoyl-CoA isomerase (reaction 3). Reduction with NADPH-dependent 2,4-dienoyl-CoA reductase (reaction 4) followed by isomerization with Δ1,Δ3,5-enoyl-CoA isomerase (reaction 5) completes the pathway whereby the 5-cis double bond is moved to the 2-trans position for completion of the degradative cycle. Alternatively, the last metabolite may possibly be reduced in peroxisomes or move to the endoplasmic reticulum for reduction by 2-trans-enoyl-CoA reductase which is the last step in malonyl-CoA-dependent chain elongation.](image)
of labeled fatty acids including 8,11,14-20:3. The findings imply that the last metabolite in Fig. 3 was reduced. In mitochondria, two different approaches have been used to determine the contribution of the two pathways [87,88]. Both groups of investigators agree that both pathways are operative, but the relative contribution of the two pathways has not been resolved.

6. Conclusions

Recent progress in the cloning and expression of Δ6 and Δ5 desaturases has conclusively established that position-specific desaturases are required to synthesize 20-carbon acids with their first double bond at position 5. It remains to be determined if there are 18- and 24-carbon chain-length-specific Δ6 desaturases. There has been relatively little recent progress in determining how many enzymes are required to chain elongate PUFA. The number of proteins expressed in tissue-specific ways is also an unanswered question. It has long been recognized that PUFA are partially degraded with the esterification of chain-shortened metabolites. The finding that both peroxisomes and microsomes are required to synthesize fatty acids with their first double bond at position 4 establishes a specific role for this partial degradative process. In general, the movement of PUFA between peroxisomes and the endoplasmic reticulum results in a partial degradation–resynthesis cycle which may be ongoing for PUFA of all chain lengths. In this regard, PUFA biosynthesis may be a more complex process than previously recognized. PUFA are not simply synthesized and totally degraded. Partial degradation–resynthesis cycles may play an important role in regulating intracellular PUFA composition particularly for 20-, 22-, and 24-carbon PUFA. Not only are enzymes in the endoplasmic reticulum and peroxisomes required, but, moreover, there must be controlled movement of PUFA between these two subcellular particles by processes that are not understood.

Acknowledgements

The authors studies cited in this review were partially supported by NIH Grants DK20387 and DK48744.

References


