Galli C, Simopoulos AP, Tremoli E (eds): Fatty Acids and Lipids: Biological Aspects.
World Rev Nutr Diet. Basel, Karger, 1994, vol 75, pp 26–29

The Role Played by β-Oxidation in Unsaturated Fatty Acid Biosynthesis[1]

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It has long been recognized that long-chain polyunsaturated fatty acids are partially β-oxidized with the subsequent esterification of chain-shortened intermediates into membrane lipids. For example, when 22:5ω6 [1] and 22:4ω6 [2] are fed to rats raised on a fat-free diet there is a large accumulation of 20:4ω6 in liver lipids. When [3-^{14}C]22:3ω6 was injected into rats fed chow it was possible to detect esterified 20:4 in microsomal and mitochondrial phospholipids suggesting that 22:3ω6 was chain shortened to 20:3ω6, at a site for β-oxidation, and then transferred to the endoplasmic reticulum where it was desaturated to yield 20:4ω6 [3]. The physiological relevance of this partial β-oxidation pathway remained undefined but the above studies were carried out before the discovery of peroxisomes. Recently we showed that 22:5ω3 was not desaturated to 22:6ω3 by a Δ 4-desaturase but that 22:5ω3 was metabolized in the endoplasmic reticulum as follows: 22:5ω3→24:5ω3→24:6ω3 [4]. The metabolism of 24:6ω3 to 22:6ω3 requires one revolution of the β-oxidation spiral followed by esterification of 22:6ω3 into membrane lipids. We have recently shown that 22:5ω6 is made via an analogous pathway thus requiring that 24:5ω6 is metabolized to 22:5ω6. It is generally accepted that peroxisomes partially β-oxidize long-chain fatty acids and that the chain-shortened compounds are transported, by some unknown process, to mitochondria where β-oxidation is completed [5]. We hypothesized that a role for peroxisomes might be to chain shorten fatty acids but that the product(s) would be transported to the endoplasmic reticulum where they would be esterified into lipids. The rationale to use [3-^{14}C]22:4ω6 as a substrate to evaluate this hypothesis stems

[1] Supported by NIH Grant DK 20387.

Table I. Influence of microsomes and 1-acyl-*sn*-glycero-3-phosphocholine on the rate of peroxisomal β-oxidation of unsaturated fatty acids

Substrate	Minutes			
	A		B	
	5	30	5	30
[1-^{14}C]18:2ω6	28.7	57.7	14.1	23.5
[1-^{14}C]20:4ω6	9.0	31.1	5.0	11.4
[1-^{14}C]22:4ω6	25.8	69.1	23.9	69.7
[3-^{14}C]22:4ω6	3.8	19.1	2.3	5.0

Values are nanomoles of acid-soluble radioactivity produced when radiolabeled acids were incubated alone with peroxisomes(A) or along with microsomes and 1-acyl-*sn*-glycerol-3-phosphocholine(B).

from the observation that when it is intravenously injected into chow-fed rats, 96% of the esterified radioactivity in liver phospholipids is 20:4ω6 [6].

Peroxisomal Fatty Acid β-Oxidation

Highly purified peroxisomes were prepared by centrifuging the light mitochondrial fraction of liver from clofibrate-treated rats as described by Das et al. [7] except that we used a 35% Nycodenz gradient. Incubations were then carried out using 100 *μM* [1-^{14}C]18:2ω6, [1-^{14}C]20:4ω6, [1-^{14}C]22:4ω6 and [3-^{14}C]22:4ω6 with 300 μg of peroxisomal protein and the cofactors used by Bartlett et al. [8] when they showed that [U-^{14}C]16:0 was catabolized to give a homologous series of chain-shortened acyl-CoAs. The results in table 1 compare the nanomoles of acid-soluble radioactivity produced when incubation mixtures contained only peroxisomes as well as when they contained 300 μg of microsomal protein and 100 *μM* 1-acyl-*sn*-glycero-3-phosphocholine. Clearly, addition of microsomes and 1-acyl-*sn*-glycero-3-phosphocholine inhibits β-oxidation of all substrates except for [1-^{14}C]22:4ω6. After 30 min the lipids from all four incubation mixtures containing microsomes and 1-acyl-*sn*-glycero-3-phosphocholine were extracted by the procedure of Folch. With [1-^{14}C]18:2, [1-^{14}C]20:4, [1-^{14}C]22:4 and [3-^{14}C]22:4 the amount of radioactivity recovered in the organic phase for the four substrates was 56, 61, 14 and 62% respectively of what was added to the incubation. The upper aqueous layer contains both unreacted long-chain acyl-CoAs and acetyl-CoA formed by β-oxidation. When the lipids in the bottom layer were separated into neutral

lipids and phospholipids, over 95% of the radioactivity was recovered in the phospholipid fraction. When the phospholipids from the incubation mixtures containing [1-^{14}C]18:2 and [1-^{14}C]20:4 were fractionated by thin-layer chromatography, 90 and 93% of the radioactivity respectively was associated with the choline phosphoglycerides. When [3-^{14}C]22:4 was the substrate, 78 and 15% of the radioactivity was esterified respectively into choline- and ethanolamine-phosphoglycerides. When the entire phospholipid fractions were interesterified and the methyl esters separated by reverse-phase HPLC, over 95% of the esterified radioactivity comigrated with 18:2ω6 and 20:4ω6 when [1-^{14}C]18:2 and [1-^{14}C]20:4 were the substrates. When [3-^{14}C]22:4 ω6 was the substrate, 75% of the esterified radioactivity was 20:4ω6 with the remainder being 22:4ω6. These data show that when microsomes and 1-acyl-*sn*-glycero-3-phosphocholine are added to peroxisomes that competing reactions take place – i.e. β-oxidation and esterification. With [1-^{14}C]18:2ω6 and [1-^{14}C]20:4ω6 the reduced rate of β-oxidation is due to esterification of the two substrates into an acceptor. The addition of microsomes and 1-acyl-*sn*-glycero-3-phosphocholine did not markedly alter the metabolism of 22:4ω6 to 20:4ω6 but once 20:4ω6 was produced it was preferentially esterified rather than β-oxidized.

Our findings raise several questions. We propose that β-oxidation of 24:6ω3 to 22:6ω3 and 24:5ω6 to 22:5ω6 are obligatory steps in providing 22:6ω3 and 22:5ω6 for membrane lipid biosynthesis. Why does partial β-oxidation of 24:5ω6 and 24:6ω3 at least in part stop when 22:5ω6 and 22:6ω3 are produced since 22:5ω6 [1] and 22:6ω3 [9] themselves are substrates for partial β-oxidation? Why does the nutritional status of the animal modify the activity of the partial β-oxidation-esterification process and why are there differences in the metabolism of ω3 and ω6 fatty acids [4, 6]? What role do mitochondria play in this partial degradative pathway? This is a particularly relevant question in light of the recent findings that mitochondria contain a fourth dehydrogenase which is specific for very-long-chain fatty acids with the resulting intermediates being channeled into a trifunctional enzyme to complete the β-oxidation spiral [10].

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