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Differences in the Regulation of Biosynthesis of 20- versus 22-Carbon Polyunsaturated Fatty Acids

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Recent studies, as summarized in this brief review, show that different factors regulate the biosynthesis of 20- versus 22-carbon (n-6) and (n-3) polyunsaturated fatty acids. It is generally accepted that dietary linoleic and linolenic acids are metabolized to arachidonic acid and 20:5(n-3) respectively in the endoplasmic reticulum as follows:

9,12–18:2 → 6,9,12–18:3 → 8,11,14–20:3 → 5,8,11,14–20:4
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 incorporation of (n-3) versus (n-6) fatty acids into membrane lipids is regulated by a number of different processes within the cell. It is generally accepted that a common 6-desaturase uses both linoleate and linolenate as substrates. Desaturation of linoleate and linolenate are generally considered to be the rate limiting steps in the biosynthesis of arachidonate and 20:5(n-3) respectively (1). The addition of fatty acids to the diet, beyond the rate limiting 6-desaturation step, would thus appear to be a way to increase the amount of arachidonate or 20:5(n-3) that is produced and potentially made available for esterification into membrane lipids.

In order to evaluate this hypothesis, we carried out a number of feeding studies with deuterium labeled (n-6) fatty acids using the following general protocol. Male weanling Sprague-Dawley rats were maintained on a modified AIN diet that contained 3.3% fat the composition of which was 2.1% oleate, 1.0% linoleate and 0.2% linolenate. After 4 weeks on this diet, the animals were changed to one in which all of the linoleate was replaced by 17,17,18,18-d_4 linoleate. After 4 days the animals were sacrificed and it was observed that 34 mol % of arachidonate esterifed in liver phospholipids was derived from the deuterium labeled linoleic acid. Another group of animals was maintained on the AIN diet that contained 1.9% oleate, 1% linoleate, 0.2% linolenate and in addition 0.2% ethyl 18:3(n-6). Again, all of the linoleate was replaced by d_4-linoleate but now only 27% of the esterified 20:4(n-6) was derived from the labeled precursor. The same above protocol was now repeated except that 17,17,18,18-d_4-18:3(n-6) replaced the unlabeled 18:3 (n-6). Now 25% of the arachidonate esterifed in liver phospholipids was derived from the d_4-18:3(n-6). The absolute amount of arachidonate in liver phospholipids was the same when animals were fed on diets with and without 18:3(n-6). By adding the amount of deuterium labeled arachidonate esterified in phospholipid from the latter two groups; i.e. 27% plus 25% it is apparent that 52 mol % of the esterified arachidonate was derived from 18:2(n-6) and 18:3(n-6). This value of 52% corresponds to only 34% when linoleate was the only dietary (n-6) fatty acid.

Addition of 18:3(n-6) to the diet resulted in the synthesis of more esterified 20:4(n-6) without altering the mass amount of 20:4(n-6) in liver phospholipid. The implication of this finding is that dietary supplements of 18:3(n-6) may possibly increase the amount of 20:4(n-6) in the free fatty acid pool. Secondly, dietary 18:3(n-6) may down regulate the activity of the 6-desaturase that metabolizes 18:2(n-6) to 18:3(n-6), however, this remains to be determined. If 18:3(n-6) does down regulate 6-desaturase activity the effect is not unique for this (n-6) fatty acid since the addition of deuterium-labeled 20:3(n-6) or 20:4(n-6) to the diet also depressed the amount of d_4-18:2(n-6) that was metabolized to 20:4 (n-6) and esterified into liver phospholipids.

When animals are maintained on a chow diet, the liver phospholipids contain linoleate and arachidonate but only low levels of 18:3(n-6) and 20:3(n-6). Liver lipids of rats fed chow contain low levels of all 18- and 20-carbon (n-3) fatty acids. These compositional differences have never been explained by specificities for any single process. Rates of reactions for desaturation and chain elongation are similar for analogous substrates from the two families of fatty acids (2), as are rates of acylation using 1-acyl-*sn*-glycero-3-phosphocholine as

an acceptor (3). Several other processes, which have not as yet been examined in detail, may play a role in regulating membrane lipid fatty acid composition. In general, diets contain much higher levels of linoleate than linolenate. Higher intracellular levels of linoleate than linolenate may in part explain why membrane lipids contain large amounts of esterified linoleate but little 18:3(n-3). Alternatively, or in addition, there may be differences as to how linoleate and linolenate partition themselves between anabolic and catabolic pathways. If linolenate was a better substrate for β-oxidation than was linoleate, the possibility exists that it could be selectively channeled into the β-oxidation pathway rather than being used for polyunsaturated fatty acid biosynthesis. Peroxisomes β-oxidize 18:3(n-3) somewhat more rapidly than they do linoleate (4). However, it remains to be determined how linoleate and linolenate distribute themselves between mitochondria and peroxisomes for β-oxidation.

Once 18:3(n-6) and 18:4(n-3) are produced, are they channeled to longer chain fatty acids in such a way that they and their chain elongation products, i.e. 20:3(n-6) and 20:4(n-3) in essence are not being made available for acylation into membrane lipids in vivo? When [1-^{14}C]18:3(n-6) and [1-^{14}C]18:4(n-3) were incubated with hepatocytes these two acids were initially esterified into membrane phospholipids. However, during the 2 h incubation both acids were released from membrane phospholipids for subsequent metabolism to longer chain (n-6) and (n-3) acids (5). We suggested that hepatocytes might contain a phospholipase A_2 which was specific for certain phospholipid molecular species to rapidly release 18:3(n-6) and 18:4(n-3) after they were esterified. However, other studies have shown that fatty acids are released directly from rat microsomal lipids by transfer to CoA via an ATP-independent mechanism (6). If this pathway was highly fatty acid specific then acids such as 18:3(n-6) and 18:4(n-3) might initially be incorporated into membrane phospholipids. They could then rapidly be released to CoA to yield the acyl-CoA which is the true substrate for malonyl-CoA dependent chain elongation (7). Further studies are obviously required to evaluate the validity of this hypothesis.

Once arachidonate and 20:5(n-3) are produced then a complex type of intracellular communication must play a role in determining what regulates their metabolism to 22:5(n-6) and 22:6(n-3) respectively. We recently showed that 22:5(n-3) was the precursor of 22:6(n-3) but the pathway was independent of a 4-desaturase and proceeded as follows: 22:5(n-3) → 24:5(n-3) → 24:6 (n-3) → 22:6(n-3) (8). An analogous pathway operates for metabolizing 22:4(n-6) to 22:5(n-6). In these two reaction sequences the first two reactions take place in the endoplasmic reticulum while the partial β-oxidation process, i.e. 24:5(n-6) → 22:5(n-6) and 24:6(n-3) → 22:6(n-3) probably takes place in peroxisomes (9, 10). The Figure shows revised pathways of (n-6) and (n-3) polyunsaturated fatty acid biosynthesis. In this Figure the solid arrows depict reactions taking place in the endoplasmic reticulum while the dashed arrows show

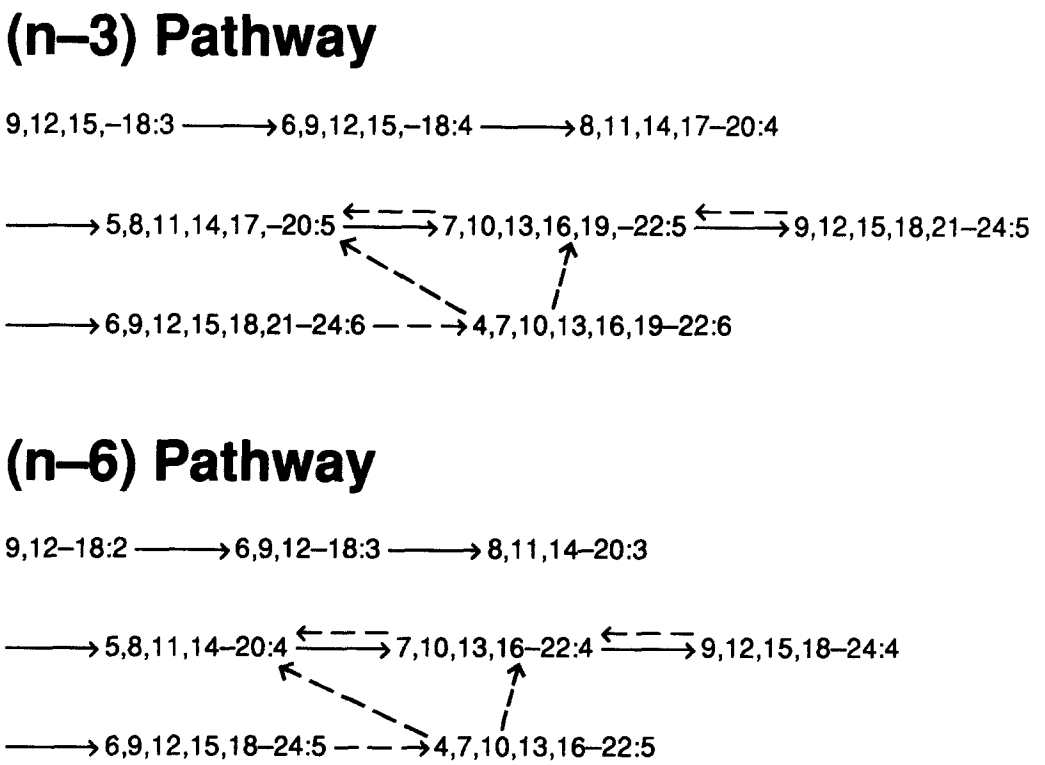

Figure Revised pathways of (n-3) and (n-6) polyunsaturated fatty acid biosynthesis. The solid arrows denote reactions taking place in the endoplasmic reticulum while the dashed arrows show acids that are substrates for partial β-oxidation.

partial β-oxidation reactions that most likely take place in peroxisomes. These revised pathways of polyunsaturated fatty acid biosynthesis raise three new, as yet, unanswered questions. What regulates the intracellular movement of fatty acids between the endoplasmic reticulum and a site for partial β-oxidation? Secondly, both 24:4(n-6) and 24:5(n-3) are substrates for desaturation at position 6. Does the endoplasmic reticulum contain chain length specific 6-desaturases? In a series of competitive substrate studies we were not able to obtain any evidence for multiple forms of a 6-desaturase (11). However, there is clearly a need to purify this enzyme or enzymes to conclusively answer the above question. Thirdly, does the endoplasmic reticulum contain multiple chain elongating enzymes? Evidence has accumulated to suggest that different condensing enzymes use saturated versus unsaturated primers. Once β-ketoacyl-CoAs are formed presumably common enzymes complete the chain elongation process (12). According to the revised pathways of (n-3) and (n-6) PUFA biosynthesis six different acids must be chain elongated as linoleate and linolenate are metabolized respectively to 22:5(n-6) and 22:6(n-3). It would seem unlikely that a single condensing enzyme would recognize six different substrates. In any case, the regulation of the chain elongation process and the number of enzymes required may well be different in liver versus in many extrahepatic tissues where membrane lipid contain small amounts of unsaturated acids with 4, 5, or 6 double bonds with up to 36 carbon atoms (13).

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