

LIPIDS AND SPERMATOGENESIS: A VERY LONG STORY

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Our story of lipids containing PUFA with very long chains (VLCPUFA) started three decades ago with the finding, in the membranes of retinal rod outer segments, of phosphatidylcholine species that contained 22:6n-3 at *sn*-2 and a series of fatty acids that were elongated versions (up to C36) of common C20 and C22 PUFA (20:4n-6, 20:5n-3, 22:5n-6, and 22:6n-3) at *sn*-1. Other authors found similar VLCPUFA, amide-bound to sphingosine in sphingomyelin (SM), in mammalian testis and spermatozoa. Shortly after, SM species with nonhydroxy and 2-hydroxy VLCPUFA (n-V and h-V, respectively) were identified in rat testis. In retina and seminiferous tubules (ST), [$1-^{14}\text{C}$]-24:5n-3 and -24:4n-6 were actively desaturated (to 24:6n-3 and 24:5n-6) and all C24 PUFA were rapidly elongated to n-V. In rat ST, (n-6) C20-C22 PUFA-rich glycerophospholipids (GPL), and (n-6) C28-C34 (n-V and h-V)-rich SM and ceramides (Cer) belonged to spermatogenic cells. Situations that *in vivo* led to apoptosis of germ cells but spared Sertoli cells (SC) led to decreased amounts of membrane GPL and SM, as well as cholesterol, and increases of (n-V-rich) cholesterol esters (CE) and triglycerides (TG), which concurred with a marked buildup of lipid droplets located to SC. Both neutral lipids accumulated PUFA and n-V (including n-V with uneven carbon chains). The content of n-V and h-V in Cer and SM was then studied in rat testis with postnatal development and germ cell differentiation. Spermatocytes had n-V sphingolipids (SL), spermatids acquired h-V, and sperm SL contained both in their heads. The TG subclasses (including those with ether bonds) with PUFA and n-V increased in germ cells as they differentiated from spermatocytes to spermatids. Along with lipid (and non-lipid) disposable materials, the TG of mature spermatids merged into lipid droplets that collected in residual bodies. These particles were phagocytized, and their contents recycled, by SC. The expression (mRNA, protein) of two diglyceride acyl transferases, five fatty acid binding proteins, and a member of the perilipin family of lipid droplet-associated proteins followed a similar trend with germ cell differentiation. Then, we followed the expression of selected elongases (Elovl), with a focus on Elovl4 (responsible for the synthesis of n-V), and a fatty acid 2-hydroxylase, Fa2h (for that of h-V). Along with *Elovl5* and *Elovl2*, *Elovl4* was actively transcribed in the adult testis. *Elovl4* mRNA was high in immature testes and SC, though the protein was absent. The Elovl4 protein was a germ cell product. Spermatocytes displayed the highest Elovl4 protein levels and enzymatic activity. Fa2h mRNA and protein was expressed mostly in round spermatids, and the protein concentrated in late spermatids. The expression of Elovl4 and Fa2h in germ cells thus correlated with the relative abundance of n-V and h-V in their SL. Most of the *de novo* biosynthetic activity of membrane lipids including SL took place during meiosis. Eventually, SM species with n-V (bull, ram) or with n-V and h-V (rat, mouse) collected on sperm heads. The biophysical properties of these SMs markedly differed from those of “conventional” SMs with saturated fatty acids. Conditional deletion of *Elovl4* in rods leads to lack of n-V and results in harmed rod function and longevity. Although a similar deletion remains to be done in germ cells, interferences with the biosynthesis of testicular VLCPUFA result in infertility.

El resumen debe ser en inglés y contener: introducción, objetivos, métodos, resultados y conclusiones del trabajo.

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