Hepatic Phospholipid Molecular Species in the Guinea Pig. Adaptations to Pregnancy

Graham C. Burdge* and Anthony D. Postle
Child Health, University of Southampton, Southampton S09 4XY, United Kingdom

Incorporation of polyunsaturated fatty acids (PUFA), particularly 22:6n-3, into fetal brain at specific gestational ages is critical for development of normal brain function. We have studied adaptations to maternal liver phospholipid molecular species compositions that may be related to the supply of PUFA to fetal brain. The increase of 22:6n-3 in brain phosphatidylethanolamine (PE) was maximal at day 25 to day 35 of gestation, consistent with early prenatal development of guinea pig brain. At the same gestational ages, there was a transient increase in maternal liver concentration of 16:0/22:6 phosphatidylcholine (PC), which preceded the progressive increase in total PC concentration toward term (day 68). This effect was specific for the sn-1 16:0 species, as there was no significant increase in 18:0/22:6 PC concentration. These results are consistent with a specific role for 16:0/22:6 PC in the directed supply of 22:6n-3 from maternal liver to the fetus. Concentrations of all PE species in maternal liver decreased at day 25 and day 35 of gestation. The gradual accumulation of 22:6n-3 in fetal liver throughout gestation did not correlate with the pattern of acquisition of 22:6n-3 into fetal brain PE. Maternal plasma PC and cholesterol concentrations decreased dramatically by day 25 of gestation, and remained low until term. This hypolipidemia of pregnancy in the guinea pig may be due to increased lipid-mediated turnover of plasma lipoproteins and contrasts strongly with the well-characterized hyperlipidemia in human and rat gestation.


Optimal fetal growth requires an adequate supply of essential polyunsaturated fatty acids (PUFA) at defined periods in gestation. Failure to acquire sufficient and appropriate PUFA at such critical periods in fetal development may have irreversible and harmful consequences for postnatal growth and function. The major PUFA arachidonate (20:4n-6) and docosahexaenoate (22:6n-3) must be derived from the essential fatty acids linoleate (18:2n-6) and a-linolenate (18:3n-3) in the maternal diet. Studies in preterm human infants fed formula either lacking or supplemented with marine oils (1,2) have shown that the capacity of the fetus and neonate to desaturate and chain elongate 18:2n-6 and 18:3n-3 is inadequate to meet the demands of rapid growth in development. The conclusion from these and many other studies is that the bulk of the long-chain PUFA must be synthesized by the mother and supplied to the fetus and neonate either by placental transport or through the milk. Further evidence to support this view was provided by analysis of phospholipid fatty acid composition in cerebral cortex obtained from victims of Sudden Infant Death Syndrome, who had been born at term (3). Brain phosphatidylethanolamine (PE) 22:6n-3 was deficient in infants who had been fed formula not supplemented with preformed 22:6n-3 as compared with breast-fed infants. The mechanisms regulating the maternal synthesis of 20:4n-6 and 22:6n-3 destined for supply to the fetus are still not understood. Indeed, despite knowledge of the maternal hyperlipidemia of human pregnancy for almost 150 years (4), it is still not clear which lipid or lipoprotein subfraction in the circulation is the principal carrier of PUFA to the placenta.

A significant proportion of the 22:6n-3 delivered to the fetus is destined for incorporation into neuronal membranes of the developing brain, preferentially as PE (5). The period of 22:6n-3 accumulation in brain PE is associated with the process of neurite extension and axonal formation and precedes the brain growth spurt characterized by the incorporation of saturated fatty acids into brain myelin (6). The timing of maximal 22:6n-3 incorporation into brain PE and brain development differs considerably between animal species (7). Consequently, for any given species, any adaptations of maternal lipid metabolism proposed to be involved in the directed supply of 22:6n-3 must correlate temporally with its accumulation in fetal brain.

In human development, 22:6n-3 incorporation into fetal brain is initiated early in gestation, at about 16 wk (8,9), and continues into early postnatal life. This process in the rat is restricted to the postnatal period (10). Analyses of the ontogeny of the accumulation of 22:6n-3 in fetal guinea pig brain phospholipids have not previously been reported. However, neuronal differentiation is essentially prenatal in the guinea pig (11), and the timing of neuritogenesis at about day 40 of gestation term (term = 68 d) (12) suggests that brain 22:6n-3 accumulation should also be substantially prenatal.

In maternal rat liver (13) and in maternal human plasma (14), the period of 22:6n-3 accumulation by perinatal brain is preceded by an increased concentration of the phosphatidylcholine (PC) molecular species, 16:0/22:6 PC. This suggested that 16:0/22:6 might be a preferential carrier for transport of 22:6n-3 from the maternal liver either to fat stores destined for use during lactation or to the placenta for supply to the developing fetus.

In the present study we have characterized the molecular species compositions of PC and PE in fetal and maternal liver and in maternal plasma throughout guinea pig gestation, and have compared the adaptations with the ontogeny of PUFA accumulation in fetal brain.

*To whom correspondence should be addressed at Child Health, Level G, Centre Block, Southampton General Hospital, Tremona Rd., Southampton S09 4XY, United Kingdom.

Abbreviations: DPH, 1,6-diphenyl-1,3,5-hexatriene; FA, fatty acid(s); GC, gas chromatography; PC, phosphatidylcholine; PE, phosphatidylethanolamine; TFE, trifluoroethanol; UV, ultraviolet.
MATERIALS AND METHODS

Materials. HPLC grade methanol and trifluoroethanol (TFE) were obtained from Rathburn Ltd. (Walkerburn, Scotland). Chloroform, choline chloride and 1,6-diphenyl-1,3,5-hexatriene (DPH) were purchased from Merck Ltd. (Poole, Dorset, United Kingdom). All other reagents and lipid standards were from Sigma (Poole, Dorset, United Kingdom).

Animal procedures. Outbred adult female Dunkin-Hartley guinea pigs were fed maintenance diet (FD1; Special Diet Services, Witham, Essex, United Kingdom) ad libitum before mating and throughout pregnancy. The diet contained 3.4% fat by weight, and its fatty acid (FA) content, expressed relative to total esterified fatty acids, was 22.2% saturated FA, 30.9% monounsaturated FA, 22.7% linoleic acid (18:2n-6), 16.3% α-linolenic acid (18:3n-3) and 7.9% PUFA, mainly 20:4n-6. At gestational ages 25, 35, 55 and 68 (term) days, fetal guinea pigs were delivered by Caesarian section (15). Maternal livers, fetal livers and fetal brains were collected, immediately frozen in liquid nitrogen and stored at −20°C. Obtaining fetal tissues at earlier gestational ages was not technically feasible.

HPLC analysis of phospholipid molecular species. Liver or brain tissue, approximately 100 mg, was homogenized in 0.8 mL of 0.9% (wt/vol) NaCl with an Ultra-Turrax homogenizer (Janke & Kunkel, Staufen, Germany). One hundred nmoles each of 14:0/14:0 PC in TFE and 14:0/14:0 PE in chloroform were added to liver homogenates as internal standards. Lipids were extracted with chloroform/methanol (16); PC and PE were purified using aminopropyl BondElut cartridges (Jones Chromatography, Hengoed, Mid Glamorgan, United Kingdom) (15,17). Intact liver PC and PE molecular species were resolved by reverse-phase HPLC on a 25 cm × 4.6 mm APEX II ODS column (Jones Chromatography) (15,18) maintained at 50°C using a mobile phase of methanol/water (925:75, vol/vol) containing 40 mM choline chloride. These isocratic conditions were chosen as a compromise between optimal resolution of phospholipid species containing PUFAs, and the ability to resolve 16:0/16:0 and 16:0/18:1 species effectively. Although adding acetonitrile up to 20% (by vol) improved the resolution of PUFAtaining species, this modification resulted in co-elution of 16:0/18:1 and 18:0/22:6 species, and consequently was not used. The elevated temperature of 50°C was essential to control the elution order of phospholipid species (18). The mass of eluted PC and PE molecular species was determined by fluorescence detection following post-column derivatization with DPH (18). Ultraviolet (UV) absorbance at 205 nm, recorded simultaneously with the fluorescence signal, was routinely used to confirm the identity of molecular species. For all chromatographic peaks of PC and PE, the principal component species, determined by gas chromatography (GC), is quoted. Calculation of the ratio of UV absorbance to fluorescence responses for each eluted peak at each gestational age confirmed that the gestational changes observed in phospholipid composition were substantially due to the major species indicated.

Analysis of brain phospholipid fatty acids by GC. Brain PC and PE fractions eluted from BondElut cartridges were dried under nitrogen and dissolved in 20 μL of chloroform/methanol (2:1, vol/vol). Twenty nmoles of 17:0/17:0 PC was added as internal standard. Fatty acid methyl esters, prepared by transmethylation of phospholipids with sodium methoxide, were resolved by GC using a fused silica capillary column (30 m × 0.25 mm, DB225; Jones Chromatography).

Statistical analysis. Statistical analysis was carried out using the Mann–Whitney U-test.

RESULTS

Fatty acid composition of fetal guinea pig brain PC and PE. The fatty acid composition of the major phospholipid classes PC and PE in fetal guinea pig brain were analyzed at intervals throughout gestation to define the timescale for the acquisition of the PUFAs 22:6n-3 and 20:4n-6. The concentration (mole %) of the principal FAs in fetal brain PC, 16:0 and 18:1n-9, remained essentially constant between day 25 and day 68 (term) (Fig. 1). The corresponding values for 20:4n-6 and 22:6n-3 both increased significantly in early gestation, reaching maxima at day 55. These FAs, however, remained minor components of fetal brain PC throughout gestation, and neither contributed more than 6% to total PC. In contrast, the gestational changes in fetal brain PE were considerably more marked. Both 20:4n-6 and 22:6n-3 were major components of brain PE at term (Fig. 2); the concentration (mole %) of 22:6n-3 in brain PE increased dramatically between gestational ages of day 25 and day 35, and then continued to increase steadily toward term. The accumulation of 20:4n-6 in brain PE occurred slightly earlier in gestation. At day 25, the earliest time...