MINIREVIEW

The Role of n-3 Fatty Acids in Gestation and Parturition

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Preterm birth is the most common cause of low infant birth weight and infant morbidity and mortality. Evidence from human and animal studies indicates that essential fatty acids of both the n-3 and n-6 series, and their eicosanoid metabolites, play important and modifiable roles in gestational duration and parturition, and n-3 fatty acid intake during pregnancy may be inadequate. Prostaglandins (PG) of the 2-series are involved in parturition and connective tissue remodeling associated with cervical maturation and rupture of membranes. In the absence of infections, preterm birth is characterized by lower reproductive tissue PG production and decreased inducible cyclooxygenase expression. Women who deliver prematurely have increased pools of n-6 fatty acid and decreased n-3 fatty acids, despite the lower PG production. Several human pregnancy supplementation trials with n-3 fatty acids have shown a significant reduction in the incidence of premature deliver and increased birth weight associated with increased gestational duration. Supplementation with long chain n-3 fatty acids such as docosahexaenoic acid may be useful in prolonging the duration of gestation in some high-risk pregnancies. Evidence presented in this review is discussed in terms of the roles of dietary n-3 and n-6 fatty acids in gestation and parturition, mechanisms by which they may influence gestational duration and the human trials suggesting that increased dietary long-chain n-3 fatty acids decrease the incidence of premature delivery.

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Background

Birth weight and gestational age at birth are critical determinants of infant morbidity and mortality in industrialized countries. In the United States, preterm birth resulting in low birth weight (LBW) comprises 6% to 10% of infant births, which are approximately 300,000 annually (1). The immediate consequences of immaturity at birth include respiratory distress syndrome, intraventricular hemorrhage and necrotizing enterocolitis, and long-term morbidity includes cerebral palsy, impaired vision and hearing, cognitive impairment, and possible cardiovascular disease in adulthood (1–5). U.S. preterm birth costs are estimated at several billion dollars annually (5). Successful strategies to prevent significant numbers of preterm births are most likely to be effective when they are scientifically based, when they address common conditions, and when they are shown to be effective in well-controlled trials. Evidence presented by several research groups suggests that essential fatty acids and their metabolites of both of the linoleic acid (n-6) series and the linolenic acid series (n-3) play important and modifiable roles in prolonging gestation in both human and animal studies.

Essential Fatty Acid Metabolism

Linoleic acid (18:2n-6, LA) and α-linolenic acid (18:3n-3, LnA) are required in the diet. Dietary LA serves as the precursor for the n-6 series of polyunsaturated fatty acids (PUFAs) and dietary LnA is the precursor for the n-3 PUFA series. It has been suggested that man evolved on a diet with a n-6:n-3 ratio of approximately 1:1, whereas the current diet ranges from 10:1 to 25:1 (6), fueling concern that today's diet may be insufficient to meet n-3 EFA requirements, particularly for docosahexaenoic acid (DHA, an elongation and desaturation metabolite of LnA), since it has an essential function in neural and other tissues (7, 8). Long

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chain n-6 and n-3 PUFAs (LCPUFA) are synthesized from dietary LA and LnA by microsomal desaturase and elongation enzymes that metabolize both the n-6 and n-3 families of PUFAs, and the delta-6 (Δ^6) desaturase is the rate-limiting enzyme in this process (9–11). Binding affinity for Δ^6 desaturase is highest for LnA, high for LA, and lowest for oleic acid (12), and for this reason, desaturation and elongation of n-9 PUFAS is only observed when combined n-3 and n-6 EFA deficiency (EFAD) occurs. Sprecher *et al.* (13) have reevaluated the pathway for DHA synthesis and have shown that insertion of the 4-position double bond (such as occurs in DHA synthesis from LnA) occurs by an additional Δ^6 desaturation of a 24-carbon microsomal elongation product, followed by peroxisomal oxidation back to the 22-carbon fatty acid. Thus, the participation of two Δ^6 desaturase steps in DHA synthesis from LnA may introduce more potential competition from n-6 fatty acids (LA series) in the synthesis of DHA from LnA. It is well established that the Δ^6 desaturation is the rate-controlling step in both LA and LnA metabolism to LCPUFA. A common metabolic response to n-3 EFAD is a compensatory increase in n-6 FAs, particularly n-6 docosapentaenoic acid (22:5 n-6 DPA) which is also synthesized by an additional Δ^6 desaturation and peroxisomal oxidation (14–17). Humans convert dietary LnA to both eicosapentaenoic acid (EPA, n-3) and DHA, but the capacity for this conversion is limited, especially with excessive dietary LA (18), which has been estimated to provide 7% of caloric intake. Dietary sources of preformed n-3 LCFA can provide large amounts of these fatty acids and are primarily derived from certain species of fish (also fish oils or marine lipids). Thus, commonly consumed human diets in the U.S. may provide small amounts of LnA and excessive LA, which when combined with infrequent fish consumption, could lead to low n-3 LCPUFA status (18).

Essential Fatty Acid Deficiency (EFAD) in Preterm versus Full Term Infants

EFA status of the neonate has been shown to be related to gestational age (17). Marginal EFA status in preterm infants compared with full term infants has been documented (19). LnA availability may be insufficient to fully support functional development of the eye and brain of preterm infants (20). In full term newborns, n-3 FA accretion rates in brain tissue show a lag phase, suggesting that newborn desaturation of parent EFAs may be limited (20–22) and that n-3 LCPUFA supplementation may be essential for maximal neural development. In the U.S., commercial formula for both preterm and term infants provides only LA and LnA, whereas breast milk contains a range of EFAs and chain-elongation and -desaturation products, including α-linolenic acid (18:3n-6), dihomo-γ-linolenic acid (20:3n-6), arachidonic acid (AA), EPA, and DHA (23). DHA in erythrocyte membrane phospholipids in both term and preterm infants is lower in formula-fed than breast-fed infants (23, 24). Supplementation with n-3 LCPUFA increases membrane phospholipid DHA and improves visual function through retinal and occipital cortex development (20, 25). Low-level supplementation with DHA improves growth in preterm infants (26). Fetal DHA accumulation occurs primarily during the last intrauterine trimester; thus, the DHA status of preterm infants may be compromised (27). Al *et al.* (28, 29) have suggested that pregnancy is associated with maternal difficulty in coping with the high demands for DHA. In summary, two factors contribute to EFA deficiency in preterm infants: lack of EFA accretion during the last trimester and limited desaturase activity in the immature liver. During gestation the placenta selectively extracts AA and DHA in substantial amounts from the maternal circulation to enrich fetal pools of these fatty acids (2) and there appears to be little placental conversion of the parent fatty acids (LA and LnA) to AA and DHA. Placental development and size, and hence extractive capacity, appears to be primary determinant of fetal AA and DHA in cord blood and provision of these fatty acids to the developing fetus (2).

Prostaglandin Biosynthesis

Prostaglandins (PG) are eicosanoids synthesized from phospholipid derived AA by prostaglandin H synthase (PGHS or cyclooxygenase, COX). The common intermediate of PG biosynthesis, PGH_2, gives rise to a variety of prostaglandins such as prostacyclin (PGI_2), PGE_2, and $PGF_{2\alpha}$. Two forms of COX occur in tissues. COX-1 in the constitutive enzyme, and COX-2 in the inducible form. Sadovsky (30) has recently shown that COX-2 expression increases in amnion, chorion, decidua, and myometrium in women in labor, and that this COX-2 expression is paralleled by increased PGE_2 content of these tissues in laboring women. However, the increase in amnion COX-2 expression in women in labor delivering preterm was lower compared with women in labor delivering at full term (30). Membrane phospholipid AA gives rise to the 2-series PGs, whereas n-3 fatty acids, particularly EPA, which is the n-3 analogue of AA, when present in sufficient amounts in phospholipids, can give rise to the 3-series PGs and depress the synthesis of 2-series PGs by competition at the level of PGHS, or by incorporation into membrane phospholipid precursor pools at the expense of AA (31, 32). The amount of dietary long-chain n-3 fatty acids, provided as a fish oil (MaxEPA) supplement, in human studies necessary for suppression of 2-series PGs accompanied by small increases in 3-series PGs is quite large: approximately 9 g of EPA per day plus 6.0 g of DHA per day (31, 32). This amount of EPA plus DHA provides nearly 7% of the calories in a 2000 calorie per day diet. Since the major effect of this large dose of fish oil, a mixture of EPA and DHA, is to suppress 2-series PG production with a small increase in 3-series PG, this suggests that the EPA component of the supplement acts as a competitive inhibitor of AA, either at the level of PGHS or by incorporation into tissue phospholipids at the expense of AA. Thus, these changes in PG production may

be wholly, or mainly, attributable to the EPA component. However, human supplementation studies with 1.6 g of DHA (containing no EPA) for 6 weeks have shown that 9% of the DHA is retroconverted to EPA, and that this DHA supplement significantly decreased plasma and platelet phospholipid AA content (33). In addition, there is evidence that DHA supplements containing no EPA influence both phospholipid AA concentrations and PG production. In neonatal pigs, 0.7 g of DHA per 100 g of total fatty acids (a modest level of DHA supplementation) significantly increased lung phospholipid DHA by 3- to 5-fold with only a 10% increase in lung phospholipid EPA and a 10% decrease in lung phospholipid AA (34). Lung TXB_2 and 6-keto-$PGF_{1\alpha}$ were also suppressed by this modest DHA supplement (34). Recently, a study in mice has also indicated that supplementation with DHA, using DHA ethyl ester at a level of approximately 8% of calories, significantly depressed basal small intestine PGE_2 and 6-keto-$PGF_{1\alpha}$ concentrations by approximately 50% (35). Interestingly, this suppression of basal PG concentrations was equal to that observed in mice supplemented with EPA (35). Thus, supplements providing only DHA can reduce PG production. Possible mechanisms for this effect include competition at the level of PGHS, retroconversion to EPA, and decreased phospholipid AA.

Leukotrienes and Lipoxygenase Products in Pregnancy

Leukotrienes (LT) are noncyclized AA metabolites containing three conjugated double bonds and LT synthesis is dependent on 5-lipoxygenase activity. The role of LTs in inflammation, vascular permeability, and chemotaxis is well recognized. Recently, a growing body of evidence suggests that AA 5-lipoxygenase products, particularly 5- hydroxyeicosatetraenoic acid (5-HETE) and LTC_4, are important regulators of the onset of labor (36, 37). 5-HETE can induce uterine contractions, and 5-HETE concentrations in amniotic fluid increase 2- to 3-fold 1 week prior to labor in human pregnancy (36). Rhesus monkeys treated with indomethacin, a cyclooxygenase inhibitor that reduces PG production, are able to deliver without increases in amniotic fluid $PGF_{2\alpha}$ concentrations, but with increased amniotic fluid 5-HETE and LTC_4 concentrations (36). These data suggest that the 5-lipoxygenase products of AA, particularly LTC_4 and 5-HETE, are important in the parturition process and they challenge the primacy of PGs as the sole mediators of labor (36, 37). Large doses of preformed n-3 LCPUFA (EPA/DHA) have been demonstrated to decrease human neutrophil and monocyte 4-series LTs (38). Again, it is important to recognize that as was the case for 2-series PG suppression, the dose of preformed n-3 LCPUFA provided as a fish oil (MaxEPA) supplement necessary for suppression of 4-series LTs was large: 5.5 g of n-3 LCPUFA per day providing 3.2 g of EPA and 2.2 g of DHA per day (32, 38). These suppressive effects may be due to both EPA and DHA (see Prostaglandin Biosynthesis section).

Prostaglandins in Gestation and Parturition

Numerous studies suggest that eicosanoids regulate gestational length and parturition (39–45) and initiation of labor, although the exact mechanism of action remains unclear. AA concentrations are elevated in the amniotic fluid of women during labor, and intra-amniotic injections of AA stimulate labor. Levels of PGE_2, $PGF_{2\alpha}$, LTC_4, and LTB_4 are elevated in the maternal circulation prior to the onset of spontaneous labor (39). Exogenous administration of either PGE_2 or $PGF_{2\alpha}$ induces cervical ripening, uterine contractions, and emptying in both full term and preterm labor, and inhibitors of cyclooxygenase inhibit labor (39, 42). PGE_2 production increases 2- to 5-fold in laboring women compared with nonlaboring women (30). Both primary PGs, PGE_2 and $PGF_{2\alpha}$, and the major metabolite of $PGF_{2\alpha}$ (15-keto-13,14-dihydo-$PGF_{2\alpha}$), are increased in the amniotic fluid during labor, and prostaglandin metabolites increase in the peripheral circulation during labor, indicating that PG synthesis increases during parturition at term (46–48).

Thromboxane (TX) and PGI_2 may exert effects on myometrial contractility, although these effects are not as well established. (49). Tissue sample homogenates (amnion, chorion, placental arteries, placenta, and myometrium) extracted both before and after labor, all demonstrated capability for conversion of labeled AA into one or more PGs (50). COX has been localized in the amnion epithelium and the cytoplasm of fibroblast-like cells, in the subepithelial connective tissue, and in the villous and chorionic cytotrophoblast, villus syncytotrophoblasts, and decidualized stroma (51, 52). PGE_2 and $PGF_{2\alpha}$ have been identified in all gestational tissues. The PGI_2 metabolite, 6-keto-$PGF_{1\alpha}$, has been found in the myometrium, placental arteries, and only sporadically in the amnion and chorion (50). TX has been found in the placenta, placental arteries, and myometrium, but this may represent contribution by blood platelets.

Recent studies have shown that reproductive tissue PG production is decreased in women with idiopathic preterm delivery. We have found a decrease in PG production in women delivering preterm (<37 weeks gestation) compared with women delivering at term (≥37 weeks gestation) (53). For amnion and placenta, both PGE_2 and $PGF_{2\alpha}$ were significantly reduced in women delivering preterm (53). The circulating plasma $PGF_{2\alpha}$ metabolite (PGFM; 13,14-dihydro-15-keto- $PGF_{2\alpha}$) was not different between women delivering preterm and at term. Plasma PGFM concentrations peak 1.5 hr after rupture of membranes, followed by a decrease within 4 hr of rupture (54). Since samples of plasma for PGFM analysis were obtained following completion of labor, this may have resulted in an inability to detect differences. Sadovsky (30) has recently shown that PGE_2 increased 2- to 5-fold in amnion, chorion, decidua, and myometrium in laboring women compared with nonlaboring women. However, women who delivered preterm, whether laboring or nonlaboring, showed a markedly lower PGE_2 content in these tissues compared with women deliv-

ering at term (30). It is well recognized that infections can cause premature labor and that this is associated with massive increases in PG production (see below in Eicosanoids and Infections section). In both our and Sadovsky's study care was taken to exclude women with evidence of intrauterine or systemic infections (30, 53). As regards mechanistic explanations for the lower PG production in idiopathic preterm delivery, two interpretations have been suggested. It has been proposed that the reduced PG production is due to depletion of AA due to chronic unidentified infections or contractions (53, 55). However, high concentrations of tissue AA have been reported in women delivering preterm, which makes this interpretation unlikely (56). Sadovsky (30) has suggested that the lower amniotic PG production and COX-2 expression in women in preterm labor compared with women at term, but before labor, may indicate that the preterm uterus is more responsive to PG stimulation than the term uterus. The mechanism underlying this postulated increased preterm uterine responsiveness may reflect the expression of PG receptors, the function of these PG receptors in the preterm uterus, and metabolism of PGs to inactive products (30).

Prostaglandins may play a role in maintaining maternal-fetal blood flow. Fish oil administration has been shown to reduce blood pressure in men with essential hypertension by changes in TX/PGI ratios (57). However, the dose of fish oil supplement in this short duration human study was large, at 9 g of EPA plus 6 g of DHA per day (57) and both the EPA and DHA components may have contributed to the changes in the TX/PGI ratio (see Prostaglandin Biosynthesis section). Increases in uterine and placental vascular tone favoring increased blood flow could contribute to the increased uterine growth and birth weight observed in n-3 supplementation and diet intake studies (2, 58). Umbilical artery endothelia in LBW infants have been shown to have reduced PGI_2 synthesis and increased n-6 DPA content, an indicator of n-3 deficiency (2, 58). Wang *et al.* (59) have shown increased placental peroxides and reduced PGI_2 in women with preeclampsia. These observations suggest reduced fetal blood delivery with reduced growth and n-3 provision to the fetus.

Cervical Changes and Prostaglandins

The nonpregnant cervix is a firm, rigid structure compared with the edematous compliant cervix at delivery. Cervical ripening involves changes in the arrangement and concentration of the collagen fibers and a reduction in the tensile strength of the tissue (60). Collagen bundles surrounded by proteoglycans are degraded at delivery, and compositional studies show a 70% decrease in collagen concentrations at term (61). Prostaglandins are involved in many aspects of connective tissue synthesis and breakdown. Both LnA and AA, PGE_2 precursors, decrease collagen synthesis (62) and PGE_2 has been shown to decrease procollagen mRNA production in human fibroblasts (63). Conversely, n-3 fatty acids, particularly EPA, increase collagen synthesis in ligament fibroblasts, most likely by inhibiting PGE_2 production (64). Part of the PG-mediated effects on collagen synthesis may be attributed to a PG-mediated interleukin-(IL) 6 pathway (64). The enzymes that destroy the proteins of the extracellular matrix, the metalloproteinases (MMP), are also influenced by cytokines and eicosanoids. Eicosanoids derived from AA, particularly PGE_2 and LTD_4, induce MMP production (65, 66). These eicosanoid influences on MMP production may also be mediated by changes in cytokines such as IL-1, IL-6, and tissue necrosis factor- (TNF) α, since n-3 LCPUFA decreases 2-series PG and 4-series LT production in association with decreased cytokines. Tissue inhibitors of MMPs (TIMPs) control the activity of MMPs and the conversion of pro-MMPs to their active forms. Eicosanoids and n-3 LCPUFA may also influence TIMP production and connective tissue remodeling (63). Thus, dietary fatty acids of the n-3 and n-6 series may influence the connective tissue matrix, both synthesis and breakdown, which may prepare the cervix for delivery. A recent study of plasma concentrations of MMP-9 (92-kD MMP-9 gelatinase) has shown a 3-fold increase at spontaneous labor, but plasma levels of MMP-9 do not appear to predict spontaneous preterm birth (67). The human amnion is a significant source of TIMPs, which may be important in maintenance of the structural integrity and the prevention of rupture of the fetal membrances (68). Systemic or local PGE_2 administration induce biochemical changes in the unripened cervix, similar to those seen in spontaneously ripened cervix, without a concomitant increase in myometrial activity (69). Delineation of the separate roles of PGE_2 and $PGF_{2\alpha}$ in cervical ripening or uterine contractions has proven to be difficult, but it is clear that they are both involved in the control of parturition at term. Several different studies have suggested that PGE_2 predominates in early labor (cervical ripening), whereas $PGF_{2\alpha}$ dominates during late labor, possibly by affecting uterine contractions (70–73).

PGs have been implicated in myometrial intracellular calcium release, which may stimulate contraction (74, 75), and in inducing gap junctions between myometrial cells, which is crucial for the efficient and rapid spread of cell action potentials and synchronized generation of contractions in the entire myometrium.

Eicosanoids and Infections during Gestation

Chronic and acute infections, including urogenital infections and chorioamnionitis, cause premature labor (55, 76–78). The massive increases in PG production play a role in infection-associated premature delivery; however, in the absence of infections, PG production during labor is lower in preterm women than those delivering at term (30, 53). Uterine infections, both chronic and acute, are associated with localized release of free AA due to phospholipase A_2 and C production by the infective organisms, which leads to increased AA substrate availability and massively increased PG production.

Unexamined Roles for n-3 Fatty Acids in Gestational Length

Long-chain fatty acids of the n-3 series exert effects on cardiac muscle and are antiarrythmic (79). These effects occur at n-3 fatty acid intakes that are much lower than those needed to alter eicosanoid metabolism. The postulated mechanisms for these effects, which have been used clinically to reduce arrythmias associated with heart disease, include direct effects of these fatty acids on Ca^{2+} channels and effects on cell signal transduction pathways involved with contraction of the myocardium (79). It is possible that n-3 fatty acids have similar roles in myometrial contraction, influencing gestational length.

Dietary n-3 Fatty Acid Supplementation Studies

Human Studies. Human studies involving supplementation of n-3 fatty acids and gestational length have been conducted in Denmark (80–82). An estimated 104 g of increased birth weight in Faroese women (high n-3 intake) was observed, which could be attributed to the 4-day increase in gestational length, with an additional 90-g increase due to more rapid fetal growth due to n-3 provision, or increased fetal blood delivery (see above) (80). In a similar study, Danish women showed a significant positive increase in gestation of 5.7 days, associated with a 20% increase in erythrocyte n-3/n-6 ratio (81). In this study, erythrocyte fatty acid profiles were obtained within 2 days of delivery, reflecting recent dietary lipid intake. In a recent randomized, controlled fish-oil supplementation trial by Olsen *et al.* (82), gestation was significantly prolonged by approximately 4 days when pregnant women consumed a fish oil supplement (Pikasol, four 1-g capsules per day) providing 920 mg of DHA and 1.3 g of EPA per day (total n-3 fatty acid intake of 2.7 g per day). Supplementation was commenced at the 30th week of pregnancy. A case control study conducted in Seattle, WA found that women with preeclampsia were 7.6-times more likely to have low levels of n-3 fatty acids compared with control women without preeclampsia, and a 15% increase in n-3:n-6 fatty acid ratio was associated with a 46% reduction in risk for preeclampsia (83). A recent randomized placebo-controlled pilot trial using DHA-enriched eggs, providing a mean DHA intake of 205 mg of DHA per day without any EPA, demonstrated fewer LBW and preterm infants, larger placentae, and less gestational diabetes in women receiving the DHA-enriched eggs (84).

A recent study (85) has focused on fish oil administration in high-risk pregnancies and comprised both prophylactic and therapeutic trials in pregnant women. In the prophylactic trials, women with high-risk pregnancies were randomly assigned to receive either a fish oil supplement, providing 920 mg of DHA and 1.3 g of EPA per day, or olive oil commencing at 20 weeks of gestation. High-risk pregnancies were classified as an uncomplicated pregnancy in women who, in a previous pregnancy, had experienced either preterm delivery (<37 weeks of gestation), intrauterine growth retardation (<5th percentile), or pregnancy-induced hypertension (diastolic blood pressure >100 mmHg), and an additional group of women with current twin pregnancies. These four prophylactic trials were mutually exclusive in that the previous preterm delivery group did not contain any women who, in a previous pregnancy, had either intrauterine growth retardation or pregnancy-induced hypertension. Similarly, the previous intrauterine growth retardation group did not contain women who had pregnancy-induced hypertension in a previous study. Fish oil, at the prophylactic dose, significantly reduced the recurrence risk of preterm delivery from 33% to 21%. However, this dose of fish oil had no effect on recurrence risk of intrauterine growth retardation or recurrence risk of pregnancy-induced hypertension. In the twin pregnancy group, the prophylactic fish oil dose had no effect upon preterm delivery, risk of intrauterine growth retardation, or on the risk of development of pregnancy-induced hypertension. In the therapeutic fish oil trial, women were identified with an existing pregnancy complication, either signs or symptoms of preeclampsia, with or without intrauterine growth retardation in the current pregnancy, or suspected intrauterine growth retardation (<10th percentile by ultrasonography) in the current pregnancy. The women were supplemented with the therapeutic fish oil dose, 2.1 g of DHA and 2.9 g of EPA per day, beginning at 33 weeks of gestation. The therapeutic fish oil dose had no effect on the mean duration of pregnancy in the preeclampsia group, or on the mean birth weight adjusted for gestational age in the suspected intrauterine growth retardation group (85).

Not all n-3 supplementation trials in human pregnancy have shown an effect on gestational length. A case-control nested cohort study in Danish women of dietary marine n-3 intakes, estimated by food frequency questionnaire 0.5 to 3.5 years after delivery, showed no relationship with pregnancy duration (86). A self-administered questionnaire and interview at the 30th week of gestation to assess n-3 intakes in Danish women found no association with gestational length (87). However, both of these studies were retrospective, estimated n-3 intakes by food frequency questionnaires and did not employ controlled experimental designs.

A recent prospective study (53, 56) examined the fatty acid status and PG production in women who deliver prematurely. In this study, 37 preterm (gestational age $\leq$37 weeks) and 34 control mother baby dyads (gestational age >37 weeks) were studied. No significant differences were found between maternal age and sociodemographic characteristics. Gestational age at delivery and infant weight were significantly decreased by 16% and 38%, respectively, in the preterm group compared with control (term) deliveries. Fatty acid analyses of samples from women who delivered at $\geq$37 weeks of gestation (controls) were conducted both at 34 weeks of gestation and at delivery in order to control for gestational duration effects on maternal pools.

Maternal plasma and erythrocyte AA was significantly

higher in preterm than in controls sampled at comparable gestational age (34 weeks) and at delivery. For erythrocytes, AA preterm samples showed a remarkable 278% increase compared with controls at delivery, and a 149% increase compared with controls at 34 weeks. Plasma total lipids AA showed a 42% and 18% increase, respectively, compared with controls at delivery and at 34 weeks. The AA enrichment in both erythrocytes and plasma total lipids is quite striking, and our interpretation is that this reflects the diet consumed by these women; that is, a diet high in LA (n-6) fatty acids and low in n-3 fatty acids. The higher LA in preterm erythrocytes, 190% and 47% increase, respectively, compared with controls at delivery and at 34 weeks, supports this interpretation

No significant differences were seen in the sum of n-3 in maternal plasma total lipids between preterms and controls at 34 weeks and controls at delivery. However, the sum of n-3 in plasma phospholipids at delivery were 34% lower in preterms than in controls at delivery. In erythrocytes, the sum of n-3 was significantly, 22%, lower in preterms than in controls at delivery, but not at 34 weeks.

Differences in maternal pools of DHA and EPA were observed, but not in all tissues sampled. This is not surprising since levels (percent of total fatty acids) were very low for DHA and EPA and ranged from 0.41% to 3.1%—much lower than for LA. In all but one case there was a trend towards lower EPA and DHA in maternal pools in preterm samples compared with controls at delivery and/or controls at 34 weeks. However, this decrease in preterm was only significant for DHA in plasma phospholipids (32%), DHA in plasma total lipids (29%), and EPA in erythrocytes (129%).

Preterm maternal erythrocyte membrane ω-6 DPA was 950% higher than controls at delivery, 641% higher than controls at 34 weeks, and 119% increased in preterm amnion at delivery. These large increases in preterm maternal n-6 DPA strongly support the interpretation of a diet high in n-6 fatty acids and low in n-3 fatty acids. In summary, the maternal fatty acid data indicate that women who deliver prematurely have increased pools of total n-6 fatty acids, LA, AA, and n-6 DPA, and decreased pools of total n-3 fatty acids, EPA, and DHA compared with women delivering at term. It is well recognized that pregnancy places high demands on the essential fatty acid status of the mother, especially for DHA (28). Pregnancy may impose maternal difficulty in meeting the DHA demands of the fetus, especially in the last trimester of pregnancy, such that maternal DHA status (the ratio of DHA to 22:5n-6, n-6 DPA) may be compromised (28). These fatty acid data are in broad agreement with this fetal demand interpretation, but these data strongly suggest that there are differences in the n-3 and n-6 fatty acid status of women who deliver prematurely compared with women delivering at term. These differences are still quite striking even when comparisons are made at 34 weeks of gestation in both women who subsequently delivered prematurely and at term. Thus, the results cannot be interpreted as due to pregnancy duration differences on essential fatty acid demands. These results, although associative and not causative, suggest that these n-3 and n-6 fatty acid status differences may be due to diet or possible differences in maternal essential fatty acid metabolism. It is difficult to invoke differences in fetal delivery of these fatty acid as a cause of the changes in maternal essential fatty acid status since these differences were evident at 34 weeks of gestation and since preterm infants were 38% smaller than term infants.

Amnion and placenta PGE_2 and $PGF_{2\alpha}$ concentrations were all significantly decreased in preterm samples, and maternal plasma PGFM showed a nonsignificant 30% reduction in preterm samples at delivery compared with controls at delivery (53). These findings, and the similar results reported by Lopez Bernal *et al.* (88) and by Sadovsky (30), emphasize that in the absence of infection, preterm delivery is associated with decreased PG production, most probably due to reduced COX-2 expression (30).

Animal Studies. Animals with n-6 fatty acid deficiency and those fed high levels of n-3 fatty acids have depressed PG synthesis and increased lengths of gestation (89). Dietary fish oils rich in n-3 LCPUFA (20:5n-3 and 22:6n-3) increase gestational length in laboratory animals (90–92). In studies with timed-mated rats intubated with 480 mg/d fish oil (containing 200 mg of DHA) that had been surgically delivered on day 20 of gestation, fish oil selectively depressed PG synthesis in amnion and uterine homogenates, and nonsignificantly depressed PG synthesis in placenta. Treatment with fish oil significantly increased incorporation of DHA and EPA into placenta and uterus, but not at the expense of AA (78). It is also clear, however, that excessive amounts of n-3 LCPUFAs may be associated with prolonged and difficult labor and excessive bleeding times in animals fed oils high in EPA and DHA (89, 92), but not in human studies (82, 85). In a recent study in pregnant sheep, intravenous infusion for 2 days of an emulsion of fish oil in late gestation (Day 123 of a 147-day ovid gestation) prevented, delayed and, in some cases, reversed Day 125 betamethasone-induced premature delivery (93). Although this study used a high dose of EPA and DHA, it is important to note that EPA/DHA infusion was performed for only 2 days, and premature delivery was induced by infusion of the potent synthetic glucocorticoid (93). Perturbations of maternal EFA status and membrane phospholipid pools, as precursors for eicosanoids, may represent a reasonable pathophysiological mechanism contributing to preterm births.

Summary

The evidence linking n-3 fatty acid intakes and changes in maternal n-3 fatty acid status with alterations in gestational length is strong. The mechanism for this effect is not clear, but may involve eicosanoid-mediated changes in myometrial contractions and connective tissue remodeling,

changes in eicosanoid receptors, or membrane effects of these fatty acids such as alterations in signal transduction pathways or modulation of ion-mediated contractions. If diet or n-3 LCPUFA supplementation can increase gestational length, as some studies suggest, then we should consider how the dietary fatty acid profile has changed over the last few years, and how this could be changed such that gestational duration is increased. Even a small increase in gestational length can have a profound influence on infant mortality and morbidity. A recent study has reported that mild preterm birth (birth at 34 to 36 weeks of gestation) is associated with a high relative risk of infant death (94). The typical U.S. diet is characterized by high dietary intakes of n-6 fatty acids (LA alone provides 7% of caloric intake) and low n-3 fatty acid intakes (95). Raper *et al.* (96), using USDA Economic Research Service data, has documented a 23% increase in the n-6/n-3 ratio of U.S. diets from 8.4:1 in 1935 to 1939 to 10.3:1 in 1985. Kris-Etherton *et al.* (18) have estimated that the dietary n-6/n-3 ratio in the U.S. has decreased from 12.4:1 in 1985 to 10.4:1 in 1994, primarily due to a 5.5-fold increase in canola oil consumption, but have suggested that this ratio is still very much higher than the recommended ratio of 2.3:1 (18). These authors have also emphasized the impracticality of increasing LnA intakes as a means of increasing conversion to n-3 LCPUFAs, in view of the high LA intake in the U.S. Daily intakes of DHA in the diets of vegans, ovo-lacto vegetarians, and omnivores are 0, 33 (mainly from eggs), and 78 mg, respectively, with the vast majority of omnivores' intake derived from fish consumption (97). Achieving the proposed recommended intake of 0.65 g of EPA plus DHA per day (18, 98) would require a 4-fold increase in fish consumption, an unlikely dietary change. Alternative approaches such as n-3 or DHA-enriched foods have been recommended for meeting the U.S. proposed n-3 dietary recommendations (18). Sattar *et al.* (99) have emphasized the importance of maternal supplementation trials during pregnancy being conducted with DHA-, rather than with EPA-containing products, since high EPA depresses neonatal growth by reducing neonatal AA status (100–102). This effect has also been observed in neonatal pigs provided with DHA supplements (34), where an approximate 10% reduction in lung phospholipid AA concentration occurred. However, the possibility that EPA supplementation on its own may have beneficial effects cannot be excluded on the basis of existing data.

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