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REVIEW

Maternal PUFAs, Placental Epigenetics, and Their Relevance to Fetal Growth and Brain Development

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Abstract

Dietary polyunsaturated fatty acids (PUFAs), especially omega-3 (n-3) and n-6 long-chain (LC) PUFAs, are indispensable for the fetus' brain supplied by the placenta. Despite being highly unsaturated, n-3 LCPUFA-docosahexaenoic acid (DHA) plays a protective role as an antioxidant in the brain. Deficiency of DHA during fetal development may cause irreversible damages in neurodevelopment programming. Dietary PUFAs can impact placental structure and functions by regulating early placentation processes, such as angiogenesis. They promote remodeling of uteroplacental architecture to facilitate increased blood flow and surface area for nutrient exchange. The placenta's fatty acid transfer depends on the uteroplacental vascular development, ensuring adequate maternal circulatory fatty acids transport to fulfill the fetus' rapid growth and development requirements. Maternal n-3 PUFA deficiency predominantly leads to placental epigenetic changes than other fetal developing organs. A global shift in DNA methylation possibly transmits epigenetic instability in developing fetuses due to n-3 PUFA deficiency. Thus, an optimal level of maternal omega-3 (n-3) PUFAs may protect the placenta's structural and functional integrity and allow fetal growth by controlling the aberrant placental epigenetic changes. This narrative review summarizes the recent advances and underpins the roles of maternal PUFAs on the structure and functions of the placenta and their relevance to fetal growth and brain development.

Keywords PUFA · Epigenetics · Placenta · Angiogenesis · Brain · DNA methylation

Abbreviations

DHA	Docosahexaenoic acid, 22:6n-3
EPA	Eicosapentaenoic acid, 20:5n-3
ARA	Arachidonic acid, 20:4n-6
ALA	α-Linolenic acids, 18:3n-3
LA	Linoleic acid, 18:2n-6
LCPUFAs	Long-chain polyunsaturated fatty acids
TFA	Trans fatty acid
LPL	Lipoprotein lipase
MFSD2A	Major facilitator superfamily domain-containing 2A
ANGPTL-4	Angiopoietin-like4
VEGF	Vascular endothelial growth factor
MMPs	Matrix metalloproteinases
VCAM	Vascular cell adhesion molecule
IUGR	Intrauterine growth restriction
DNAm	DNA methylation
GpX	Glutathione peroxidase

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Introduction

Dietary fatty acids, particularly long-chain polyunsaturated fatty acids (PUFAs) supplemented to the mother, influence the production of eicosanoids. The latter act as signaling molecules to control the fatty acid metabolic function involving growth and development. Both total fats and the ratio of n-6 and n-3 fatty acids influence eicosanoid, docosanoids balance [1], placental cytokine, and pro-resolving lipid mediators [2]. Again, maternal n-6, n-3, and *trans* fatty acid (TFA) levels during early gestation influence the birth weight at term [3, 4]. Higher maternal n-6 fatty acids are correlated with lower birth weight in a population where n-3 fatty acid intake is adequate [5] or deficient [6]. The differential fatty acid composition results in altered eicosanoid production (an elevated thromboxane A2 to prostaglandin I2

ratio) and affects the placental blood vessel's vasodilation in hypertensive pro-thrombotic pregnancy.

The dietary PUFAs are predominantly enriched with essential fatty acids such as alpha-linolenic acid, 18:3n-3 (ALA), and linoleic acid, 18:2n-6 (LA). The longer chain PUFAs are usually present in smaller quantities in the diet, such as docosahexaenoic acid, 22:6n-3 (DHA), and eicosapentaenoic acid, 20:5n-3 (EPA); those play diverse physiological roles, including conception, placentation, and parturition. The inadequate reservoirs of fetal long-chain PUFAs (LCPUFAs) could arise from an insufficient supply of maternal LCPUFAs or inefficient fetal lipid metabolism. The insufficient supply of LCPUFAs results from a lower maternal reservoir of LCPUFAs and inefficient transplacental transfer of LCPUFAs. The preeclamptic women measured a lower amount of n-3 LCPUFAs (EPA) and a higher ratio of n-6 to n-3 LCPUFAs (ARA to DHA) than normotensive subject indicating a contribution of ARA-derived eicosanoids in promoting pro-thrombotic pregnancy [7].

The placenta has the potential to program the risks of adult diseases by modulating its metabolism [8]. Maternal lipid metabolism determines new-born's fetal adiposity and growth index via placental function. The placenta plays adaptive roles in trafficking maternal lipids during pathological states such as intrauterine growth restriction (IUGR) and gestational diabetes mellitus (GDM). In IUGR, impaired placental transfer of lipophilic LCPUFAs and fat-soluble vitamins promotes metabolic dysfunction and decreased birth weight. In GDM, the interplay of the ANGPTL4-LPL axis is proposed for higher fetal fat accumulation. ANGPTL4 secretion in the placenta, liver, and adipose irreversibly suppresses LPL activity. As a result, LPL involves in the lipolysis of fat in the form of triglycerides and promotes fatty acid transfer. The maternal plasma ANGPTL4 is decreased in GDM, which enhances placental LPL activity that results in a greater transfer of maternal fatty acids and leads to fetal fat accumulation [9]. Thus, the impact of maternal fatty acid levels can determine the clinical outcome of the offspring by changing placental phenotypes.

Placental vascular defects and dysfunctions may be underlying causes of fetal growth restriction [10]. Placental angiogenesis helps establish placental vascularization and thereby promotes the growth and development of the fetus. Conversely, inadequate angiogenesis and reduced trophoblast invasion lead to impaired placental development. Placental diseases, preeclampsia (PE), resulted from a maladaptive placental and circulatory balance of pro-and anti-angiogenic growth factors [11–16]. The uterine spiral arterioles undergo extensive remodeling and continue till the second trimester of pregnancy. It lowers the resistance of blood and increases nutrient flow across the placenta and utero-placental perfusion. All these establish an appropriate utero-placental architecture for optimal fetus development. Altered placental angiogenesis may directly or indirectly involve several pathological pregnancies, including PE, PTB (pre-term birth) [17], GDM [18], and IUGR [19].

Recent evidence suggests that PUFAs promote the secretion and expression of angiogenic growth factors in the trophoblast and protect diet-induced epigenetic changes in the placenta [20–27]. The deficiency of n-3 fatty acids reduced the placental transfer of fatty acids in PE- and GDM-associated fetuses [28]. The n-3 fatty acids' requirement throughout life is essential, but their availability in maternofetal circulation remained obligatory for the organ growth and development of the fetus. Although adequate n-3 fatty acids are recommended during pregnancy [29, 30], little is known about the impact of n-3 PUFA deficiency on utero-placental changes in angiogenesis and epigenetics and its implications on fetus growth and development. The present article aimed to explore the potential roles of maternal PUFAs on placental functions and their implication to fetal growth and brain development.

A literature search was performed on the PubMed database using keywords such as placenta, PUFA, epigenetics, brain development, and pregnancy. Several clinical and mechanistic preclinical studies were included to develop this narrative review. This review highlights the present understandings of the regulators of vasculogenesis, angiogenesis, and epigenetics during pregnancy and the novel roles of PUFAs in modulating these functions in the feto-placental units.

Impact of PUFA Deficiency During Pregnancy

Maternal PUFAs on Fetal Adiposity and Birth Weight

While adequate intake of n-3 LCPUFAs is recommended during pregnancy, its deficiency is rampant among pregnant women in developing nations [31, 32]. The n-6 PUFAs and TFA are predominantly higher among PUFA intake with a small amount of ALA, EPA, or DHA as total n-3 PUFAs in the diet. Pregnancy initiates with inadequate or insufficient n-3 PUFAs in these women. TFA produced from natural biohydrogenation of the rumen and industrial hydrogenation of vegetable oils show different effects on trophoblast functions and pregnancy outcomes. Low birth weight was associated with higher elaidic acid (18:1 *trans*) in the cholesterol ester fraction of umbilical cord blood-derived from premature infants [33].

On the other hand, c9,t11-CLA stimulated ANGPTL4 and other pro-angiogenic factors and DHA uptake in trophoblast cells [34]. TFA (18:1t) in maternal plasma phospholipids during early pregnancy showed a significant inverse relation with birth weight [35]. Dietary TFA ingestion decreased the

activities of delta-6-desaturase in pregnant rats [36]. The radiolabelled [^{14}C] ARA and [^{14}C] EPA uptake was significantly decreased in early placental trophoblast cells after incubating with elaidic (ELA, 18:1t) acid [34]. A large cohort ($n = 12,373$) based on 12-week gestational fatty acid composition and food intake suggests that adverse maternal n-3, n-6, and trans fatty acid profiles influence the babies' birth weight at term. Lower individual n-3 fatty acids and the precursor of ARA but higher dietary TFA (18:1n-9t) and other n-6 fatty acids were positively correlated to lower birth weight [3]. A higher mid-pregnancy n-3:n-6 PUFA ratio was associated with gestational duration and birth weight [37]. While the higher plasma ARA and total n-6 fatty acids in late pregnancy delivered low birth weight babies [4], the higher levels of n-6 fatty acids and ARA in early pregnancy lead to reduced birth weight [3, 38]. Similar to that trend, maternal intake of n-6 fatty acids (predominantly LA-based) was inversely related to birth weight in India [6]. A higher maternal n-6 PUFA was positively correlated with a higher n-6 PUFA and birthweight for the 3-month infants [39]. Keeping the ratio of the n-6 and n-3 fatty acids lower could have multiple advantages. The desaturation and elongation of n-6 and n-3 fatty acids involve similar sets of enzymes that competes by the substrates (n-6 and n-3 fatty acids), resulting in the lower conversion of n-3 fatty acids in the presence of high n-6 fatty acids [40]. The n-3 LCPUFAs improve receptor activity, membrane fluidity, and vasodilation [41]. The n-3 LCPUFAs can improve fetal growth by enhancing placental blood flow and lowering the viscosity of the blood through the optimal production of n-3 PUFA metabolites [42]. Thus, a decrease in birth weight could be attributed to the reduced beneficial effects of n-3 fatty acids or the higher presence of n-6 fatty acids in pregnancy. However, the association of maternal n-3 PUFA and birth weight remained inconsistent. The disparity could arise due to ethnicity, background diet (omega-3 fatty acid intake), chronic malnutrition, chronic vitamin deficiency, gene mutations, and others.

In addition to diet, a higher body mass index (BMI) during pregnancy is negatively related to circulating n-3 LCPUFAs in their offspring [43]. The placental uptake of unsaturated fatty acids is lower in obese women [44]. Due to increased placental fatty acid uptake from maternal circulation, obese women accelerate lipid esterification and decrease fatty acid oxidation and thus promote fetal adiposity [45]. Intake of n-3 PUFAs during pregnancy helps maintain optimal lipid metabolism of the placenta by balancing between placental fatty acid esterification and accumulation pathways. N-3 PUFAs favor lipid oxidation, lower triglyceride (TG) synthesis in the liver, downregulate the expression of lipid metabolism and its clearance, improve fatty acid utilization, and decrease tissue lipid storage. The n-3 PUFA deficiency over four generations induces fetal adiposity without any changes in food intake [46]. During n-3 PUFA deficiency, excess production of ARA metabolites such as PGE2 PGF2 prevents the conversion of white adipose into brown adipose [47], deregulating hypothalamic-leptin signaling and energy expenditure homeostasis in mice [48].

Current evidence suggests that higher maternal n-6 to n-3 PUFAs negatively correlate with the leanness of the offspring. Moreover, an excess of n-6 PUFAs inversely regulates the incorporation of n-3 LCPUFAs in the fetal tissues and alters fetal adiposity.

Maternal PUFAs on Fetal Brain Development

The fetal brain requires that PUFAs are supplied from the mother via placenta. The n-3 long-chain fatty acid, such as DHA, is the brain's major structural component critical for membrane receptor function, membrane fluidity, and neuronal signaling. The higher intake of n-3 LCPUFAs during pregnancy and lactation promotes early brain development by activating PPAR-γ [49]. As the brain cannot synthesize its own DHA, therefore, deficiency of DHA or its precursor during brain growth and development in utero possibly affects the programming of brain maturation, plasticity, and compromise its function in adult life. The n-6 LCPUFA, such as ARA, is quantitatively predominant in the brain next to DHA. Therefore, the supply of ARA needs to be continuous in the growing brain. The lipoproteins and lysophospholipids are known to contribute to brain ARA levels. ARA is released in the brain from synaptic membrane phospholipid by neurotransmitter-activated phospholipases, PLA2. Usually, calcium-dependent cytosolic PLA2 selectively releases ARA, while calcium-independent cytosolic PLA2 releases DHA at the sn-2 position of glycerol moiety of phospholipids. Dietary n-3 PUFA deficiency increases the expression of ARA-selective calcium-dependent cytosolic cPLA2 secretory sPLA2 while reducing the DHA regulatory phospholipase A2 (PLA2), calcium-independent iPLA2, in the rat frontal cortex [50]. Such adaptation increases the DHA half-life in the brain by downregulating iPLA2 activities with concomitant increased production of ARA-derived metabolites, docosapentaenoic acid, 22:5n-6 (DPA), during n-3 PUFA deprivation [51]. The higher levels of DPA are a clinical biomarker for n-3 PUFA deficiency [52].

PUFA-enriched diet alters the expression of genes involved in synaptic plasticity and learning in rats [53]. In rodents, the n-3 PUFA deficiency showed reduced expression of glutamate receptors and heightened TNF-α in the central nervous tissue independent of their effects on membrane composition [53]. Over multiple generations, the deficiency of n-3 fatty acids stimulates the stress response and anxiety in rats due to the impaired expression of neuropeptide Y-1 and glucocorticoid receptors in the pre-frontal brain hippocampus [54]. The n-3 PUFA deficiency during

brain maturation in rats lowers the plasticity and alters the brain function in adulthood [55]. The excess n-6 PUFAs leads to increased levels of ARA and its eicosanoid derivatives such as prostaglandin, leukotriene, and thromboxane, eventually promoting a proinflammatory state in the brain that disrupts the balance of anti- (n-3) and proinflammatory (n-6) eicosanoids via GPRs [56].The n-3-deficient (0.02% energy from ALA) or adequate (1.3% energy from ALA) diet showed lower concentration of DHA and docosapentaenoic acid, 22:5 n-6, and reduced telencephalon structure in the hippocampus of n-3 PUFA-depleted mice [57].The n-3 PUFA deficiency affects region-specific brain development in rats. The cerebral frontal cortex is mainly affected during the deficiency since n-3 fatty acids are concentrated in the region. The n-3 PUFA deficiency affected learning ability, motor activity, and monoamine transmission in the rat brain [58]. The maternal DHA deficiency influences gender-specific offspring's brain development due to the differential efficiency of endogenous DHA-converting enzymes in males and females. The maternal DHA deficiency negatively affects behavior during the stress response, anxiety in the offspring [59], and brain reward activities to consume calorie-dense foods [60]. Furthermore, it induces maternal stress and causes hypomyelination in the neonatal brain, which may predispose offspring to develop anxiety-related disorders later [59–61]. The n-3 PUFA deficiency in mice leads to the reward-processing deficit by selective motivational impairments [62]. Recent data suggest that dietary deficiency of omega-3 fatty acids promote microglia-oriented phagocytosis of synaptosomes in the developing rodent hippocampus [63].

A proportion of n-3 PUFA deficiency findings on brain functionalities are assimilated from animal studies, which must be confirmed in a well-designed clinical study. Moreover, endogenous conversion of n-3 PUFAs is more efficient in animals than humans should be considered while extrapolating these studies.

Protective Roles of DHA in Fetal Brain Development by Improving Signaling, Plasticity, and Antioxidant Capacity

The brain is enriched with fatty acids containing 18 or more carbon atoms as long-chain PUFA, in which DHA and ARA are predominant. In contrast to ARA (2–5%), DHA levels in the neuronal membrane are relatively higher (15–50% of total fatty acids). DHA is esterified at the sn-2 glycerophospholipids, particularly phosphatidylethanolamine of fatty acids in neuronal cells. The biophysical properties of DHA made it exclusive in the brain to other long-chain fatty acids. The presence of six double bonds in the fatty acyl axis forms a kink conformation in DHA, which cannot fit orderly during lipid packaging and causes a change in the membrane's fluidity. Several double bonds ensure lower melting points of DHA, which enables keeping the brain matrix liquid at low temperatures. Neuronal membrane fluidity is critically required for the synaptic membrane receptors, neurotransmitters in communicating and transmitting signals among the nerve cells [64]. DHA contributes to multiple neurodevelopmental functions, including synaptogenesis [65], neuritogenesis [66], neuronal differentiation [67], neurite outgrowth [68], and production of neuroprotective metabolites [69].

The DHA was first documented as a signaling mediator with its rapid presence in the free fatty acid pool at the site of brain injury [70]. The bioactive docosanoid neuroprotectin D1 (NPD1) is produced from free DHA through the action of 15-LOX-1 [71]. The DHA-derived bioactive showed an inverse relation with the number of reference memory errors (RME) in the rat brain, while EPA-derived bioactive did not affect [72]. The DHA-derived bioactive NPD1 affects NFkβ expression and controls pro-inflammatory cytokine production [73]. PPARγ activation confers DHA's anti-inflammatory action as it has the fatty acid binding pocket for PUFA, including DHA [74, 75]. The brain hippocampus's dentate gyrus constantly produces new neurons from the progenitor cells during neurogenesis. Neurogenesis is involved in learning and memory functions. The neuronal stem cells from the rat embryo showed additional morphological mature neurons in the DHA group than in the control [76]. The DHA-induced spatial memory is attributed to the DHA-stimulated neurogenesis in the hippocampus. DHA stimulates neural stem cells' differentiation by favoring activator type transcription factors such as neurogenin, mash1, arrests cell cycle at G0 phase, and suppresses the repressor type transcription factors Mes-1 [67]. Thus, DHA can dictate the differentiation terms of the brain cells' maturation and transform new neurons into maturity. These collectively form synapses to enhance synaptic connectivity in vivo, thereby adding a domain for further learning and memory functions.

The mechanism by which increased neuronal DHA affects synaptic function is unknown. Synaptic plasticity promotes improved connectivity between neurons and thus influences the capacity in learning and memory via long-term potentiation (LTP). LTP-controlled memory functions are modulated by NMDA receptors (NMDAR) and its subunits NR2A and NR2B. The disruption in the hippocampal NMDAR subunits, namely NR2A and NR2B, is associated with LTP impairment and memory functions [77, 78]. Dietary DHA may induce LTP via restoring neurotransmitters release [79, 80]. In rats, the memory impairment was directly correlated with gene expression of presynaptic membrane-associated mediators such as PSD-95, BDNF, and TrkB in the hippocampus. The hippocampus and cerebral cortex are the

major structural hotspots for facilitating memory functions. DHA-fed rats showed that increased expression of GluR2, NR2B, TrkB, and BDNF levels in the brain could mediate increased memory in rats [55, 81]. Thus, DHA directly or indirectly stimulates the gene expression of various synaptic membrane-associated mediators that may affect learning and memory functions.

The brain accounts for approximately 2% of total body weight but consumes 20% of the total oxygen demand of the body. Thus, neuronal cells can die within 3 min upon oxygen non-availability [82]. Structurally, lipids constitute 30–50% of total cerebral dry weight, in which 70% are phospholipids and 30–40% of phospholipids are enriched with DHA [83]. Despite the pro-oxidant nature of brain parenchyma, neuronal cells can protect its autoxidation by various mechanisms, including antioxidant properties of brain phospholipid DHA. DHA has a specific role as an antioxidant in the brain. As majorities of the PUFAs are incorporated in the brain's phospholipid fraction, their peroxidation can severely affect the membrane integrity, fluidity, neurotransmission, signal transduction, and nerve impulse [84]. Usually, these cells are equipped with antioxidant shields that offer protection against the damages caused by lipid peroxidation. However, lipid peroxidation can be a vicious cycle as reaction products can trigger additional lipid peroxides.

Despite being a highly unsaturated fatty acid, DHA can play as antioxidant in a pro-oxidative brain. The antioxidant defense of DHA in brain cells is facilitated by detoxifying enzymes such as thioredoxin/peroxiredoxin and glutathione/glutaredoxin systems, heme oxygenase, and oxidoreductive quinone pathways. Glutathione peroxidases (GPx4), a selenoprotein enzyme, can detoxify both free and membrane-bound hydroperoxides in membrane phospholipids. The DHA regulates the GPx4 gene expression in hippocampal cells and increases the total activities of GPx4 upon DHA exposure but not with ARA stimulation. The DHA deficiency stimulated the expression of all GPx isoforms, with the cytosolic isoform exhibiting immense stimulation [85, 86]. Thus, increased GPx4 expression is a compensatory response by stimulating GPx4 biosynthesis and thereby protecting membrane phospholipids' oxidative damage due to DHA deficiency. The cytosolic GPx4 is expressed predominantly compared to mitochondrial and nuclear isoforms. DHA has recently been reported to activate nuclear factor erythroid-related factor 2 (NRF2), a master regulator in the transcriptional activation of the brain. The n-3 LCPUFAs seems to play a stimulatory role in activating the NRF2 antioxidant pathway [87]. The antioxidant response favors in promoting neuronal differentiation and neurite outgrowth. Compared to wild type, the *fat-1* mouse brain significantly enriched the transcripts levels with neurite outgrowth and neuronal development associated with the *Nrf2*-transcriptional pathway. Notably, the *fat-1* mice increase the endogenous n-3 fatty acids and decrease n-6 fatty acids, thereby maintaining a healthy n-6/n-3 fatty acid ratio. Furthermore, DHA-treated primary cortical neurons exhibited a dose-dependent increase in *Nrf2*-targeted gene expression [88]. During n-3 PUFA deficiency in rats, liver n-3 PUFA pools are primarily replaced by n-6 PUFAs. The deficiency of n-3 PUFAs resulted in altered dopamine transmission in the brain suspect a disrupted neurogenesis pathway [89]. Compared to deficient n-3 PUFAs, the sufficient mice had significantly lower ARA levels, higher DHA levels, and dihomo-γ-linolenic acid (20:3n-6) in the brain [90]. Dietary n-3 PUFAs plays multiple roles in brain maturity and functions by modulating neural maturation, sensorimotor integration, spatial memory, and motor coordination in mice brains. The mechanism of DHA-mediated signaling and protection in the brain seems complex and much to be established [91]. Based on available evidence, a protective antioxidative role of brain phospholipid DHA is proposed (Fig. 1).

Placental Angiogenesis and Uterine Vasculature: Effects of PUFAs

The successful placentation involves coordinated vascular development and adaptation on both sides of the maternal–fetal interface. The uterine vascularity helps in establishing early implantation. The process follows the expansion and placental vasculature development to facilitate nutrient exchange and oxygen delivery to the fetus. The placental angiogenesis is augmented by a squeal of these events in close interaction with decidua for a successful pregnancy. Preclinical data suggest that the expression and secretion of angiogenic factors are critical determinants for placental growth and development [92, 93]. Placental angiogenesis and vascular development are tightly controlled by a local balance between pro- and anti-angiogenic growth factors. Thus, alteration in the expression and secretion of these growth factors can contribute to the efficiencies of invasion and angiogenesis processes mediated by cytotrophoblast. The extravillous trophoblasts (EVTs) express the junctional and trophoblast-endothelial adhesion molecules such as VE-cadherin, PECAM-1, and VCAM1. VE-cadherin involves trophoblast-endothelial cell interaction in decidual spiral arteries. Increased expression of these adhesion molecules is involved in the trans-endothelial migration of placental trophoblast cells. The matrix metalloproteinases (MMPs), another key mediator of placentation, are involved in degrading the extracellular matrix components (ECM). The MMPs cause the basement-membrane breakdown, mediate vascular remodeling, and maintain vascular integrity. Thus, regulating the factors that control placental angiogenesis could be

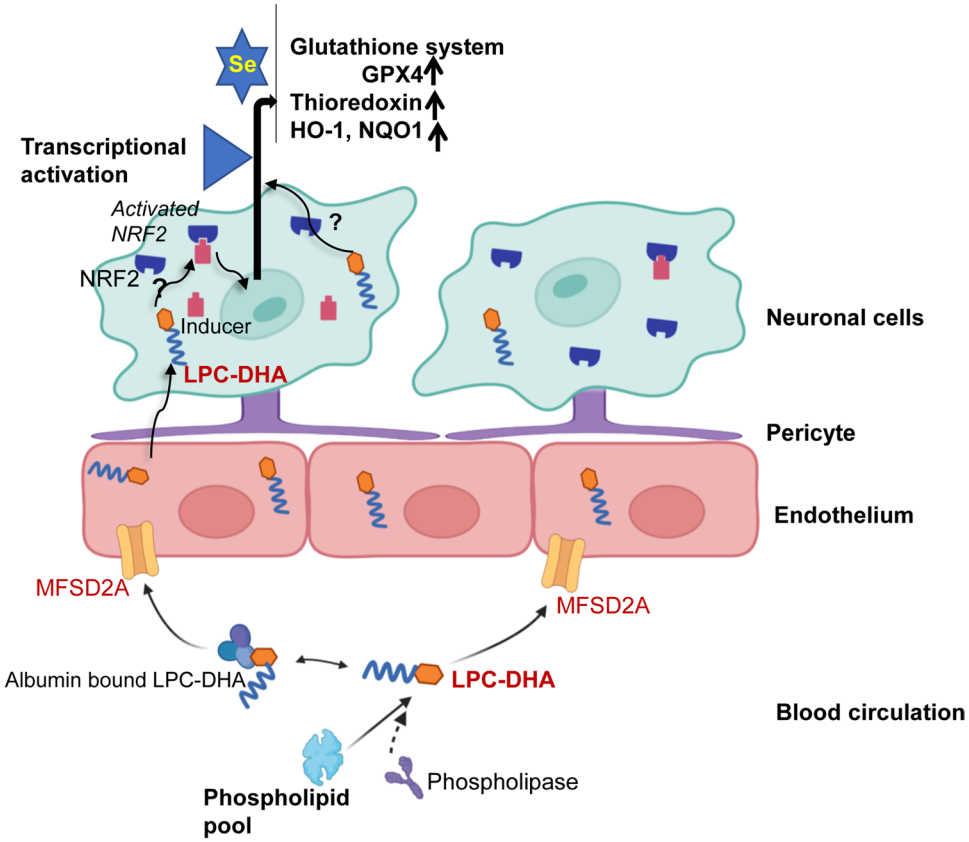

Fig. 1 Protective antioxidative roles of DHA in brain development. The blood–brain transport of LPC-DHA is promoted by specific transporter MFSD2A. In addition, DHA seems to activate nuclear factor erythroid-related factor 2 (Nrf2), a master regulator in the transcriptional activation of the brain by stimulating selenium (Se) containing glutathione peroxidase systems. The transport of other fatty acids is not shown

an appropriate intervention for ensuring successful outcomes of the pregnancies [94, 95].

The structural and functional development of the placenta is indispensable for maintaining a healthy pregnancy and fetal growth and development. The blood flow of the placental vessel network is one of the key determinants of the physiologically functional placenta. The formation of placental vasculature involves two interlinked events as vasculogenesis and angiogenesis. During vasculogenesis, the blood vessel is formed de novo from angioblast or endothelial progenitor cells. In contrast, the formation of new blood vessels occurs from the pre-existing one by angiogenesis. The sequel of placental angiogenesis follows vasculogenesis that comprises branching angiogenesis during the first trimester and non-branching angiogenesis in the third trimester [96]. Placental angiogenesis is a highly regulated and intricate process that involves migration, proliferation, and tube formation of the vascular endothelial cells [97]. Abnormal vasculature in the placenta reflects in several pathological pregnancies, including preeclampsia, recurrent spontaneous miscarriage, pre-term, IUGR, and fetal death. In the basal plate of the placenta, fetal cytotrophoblast invaded the uterus and remodeled the resident vasculature to ensure minimum maternal immune rejection. The optimal gene expression at the decidua-placental site provides coordinated cell-to-cell interaction for a successful pregnancy. Gene expression at the maternal–fetal interface is gestational-specific and tightly controlled in human pregnancy. While there was a slight change in gene expression at 14–24 weeks, many genes are differentially expressed at 37–40 weeks. The key differentially expressed genes are involved in transcription, differentiation, angiogenesis, extracellular matrix reorganization, and lipid metabolisms [98]. In addition to genetics, dietary and lifestyle factors also contribute to suboptimal placental angiogenesis by modulating epigenetics induced gene expression [99].

The placenta has a complex vascular network for fetal blood supply that requires extensive angiogenesis. The normal placentation is supported by various angiogenesis growth factors such as VEGFA, ANGPTL4, FGF, and PlGF. Angiogenesis is an energy-intensive process. The energy metabolism of angiogenesis is regulated by peroxisome proliferator-activated receptor (PPAR)-γ and its coactivator-1α (PGC-1α) by mitochondrial function. PGC-1α independently upregulates the function of VEGFA and other angiogenic growth factors, thereby helping in forming new blood vessels by stimulating angiogenesis [100]. The uterine vasculature is transformed by remodeling during early gestation to facilitate adequate maternal blood flow and nutrient delivery for the growing fetus. The arterial remodeling lowers the total uterine vascular resistance in the mouse by 47% [93]. These changes expand the uteroplacental blood volume and

ensure adequate blood flow from the placenta to the fetus. In humans and animals, uterine arterial dysfunction leads to preeclampsia [101]. A maternal high-fat diet induces ectopic lipid deposition in the placenta during pregnancy, leading to lipotoxicity and chronic inflammation [102]. Furthermore, it induces the placenta to adjust its metabolic response and adapts a structural change in placental thickness by altering angiogenesis. The placental labyrinth thickness was reduced, gene expression of insulin-like growth factor 2 (IGF2), and its receptor were elevated in the fetuses of HFD dams [103]. High fat-induced maternal obesity alters the metabolome early placental transcriptome and decreases placenta vascularity in the mouse. The sphingolipid metabolism was markedly affected due to altered gene expression related to sphingolipid processing. Upregulation of sphingolipid pathways inhibits angiogenesis and causes endothelial dysfunction. The transcriptome levels associated with angiogenesis and vascular development pathways were also disrupted. These changes in the metabolism and gene expression were thought to contribute phenotypic changes in the placenta [104].

The n-3 PUFA deficiency resembles HFD-induced impaired placental phenotypes where its development and angiogenesis are disrupted in mice pregnancy. The n-3 PUFA deficiency affected the decidual vascular development of the feto-placental unit and showed that maternal fatty acid status modulates placental vascularity [26]. The uterine spiral artery of the n-3 PUFA-deficient dam had a thicker and narrower lumen than those of the n-3-sufficient. The reduced lumen thickness indicates suboptimal remodeling where smooth muscle cells are still present due to incomplete uterine spiral arterial remodeling (uSAR). This adapted morphology may promote vasoconstriction of the spiral artery and regulate the materno-fetal blood flow. In addition to morphological and structural alterations at the utero-placental interface, the expression of VCAM1 protein was decreased in the placenta during n-3 PUFA deficiency [26]. Integrin, such as VCAM1, is an adhesion and invasion growth factor and plays a crucial role in cell-to-cell interactions and uSAR, which are required for an effective early fetal development. Decreased VCAM1 expression in spontaneous abortions suggests its essential role in uteroplacental adequacy [105]. The critical window of gD10-11 corresponds to the expression of VCAM1 on the formation of the spiral septum in rats [106]. VCAM1 knockouts-mice could not fuse chorions and severely affect placentation.

The trophoblast invasion is controlled by angiogenic factors in vivo and by remodeling of extracellular matrix proteins like MMPs [107, 108]. Proteolytic enzymes like MMPs are expressed in a controlled manner by both trophoblast and decidua during ECM remodeling. MMP-9 and MMP-2 are the most predominant MMPs produced by the cytotrophoblast in first trimester, and their involvement in the success of extravillous trophoblast functions, such as migration and invasion, has been well documented [109, 110]. MMP9 is a key mediator associated with cellular remodeling associated with trophoblast invasion and angiogenesis of the placenta [111]. The prostaglandin (PG) stimulates MMP expression and activities during labor [112]. The PG and MMP have an interactive function during parturition. The ex vivo culture of amnion, chorion, and decidua collected from fetal membranes of delivered women showed differential production of MMPs and their inhibitors in response to PGF2 stimulation in these lineages. Unlike amnion and chorion, decidual MMPs to TIMP ratios were significantly increased in response to PG stimulation [113]. The n-3 LCPUFA, DHA, regulates PG production by competing with ARA to incorporate into the phospholipid pool or suppressing COX-2 expression. DHA diet (0.7en%) during conception and pregnancy significantly reduced rats' uterus and placental ARA content.

Placental MMP-2 and MMP-9 (gelatinase) production were suppressed in response to this diet [114]. These gelatinases are actively involved in fetal membrane rupture due to their roles in degrading various types of collagen (I, IV, and V). The ratio of MMP9 to TIMP1 is an index for the tensile strength of the fetal membranes [115]. The production of MMP2 is relatively stable before and after labor in the fetal membrane, while MMP9 activities are surged with the onset of the labor and decreased after delivery [116]. The placental MMP2 and MMP9 expression in rats were significantly reduced with an n-3 LCPUFA rich diet [114]. The MMP9 to TIMP1 ratio in the placenta increased consistently over two generations in mice fed with an n-3 PUFA-deficient diet [26]. Reducing 2-series PGs by preformed n-3 LCPUFA could reduce the MMP activity associated with labor, particularly with premature rupture of membranes and delayed pre-term delivery. The placental collagenase activity and the increased expression of MMP9, KDR, and VEGF during n-3 PUFA deficiency may contribute to hypervascularization of arterial lumen due to high collagen deposition [26, 114]. All these can promote the thickening of the uterine arterial membrane due to increased vascular density and thickness of the blood vessels. The increased vascular density and higher VEGF, KDR, and MMP9 expression were noted in a model of endometriosis [117].

Thus, a maternal n-3 fatty acid-deficient state may influence uterine membrane organization, density, and placental vascularity by dysregulating proteins' expression in these pathways.

Does Maternal PUFA Deficiency Affect the Transport and Metabolism of Lipids?

Dietary conversion of PUFAs expressed as an energy percent of lipid composition can lead to a comparable outcome between humans and rodents by extrapolating the model

system [118]. The deficiency of n-3 PUFA during pregnancy affects the endogenous conversion of LCPUFAs from their precursor fatty acids. The endogenous biosynthesis of LCPUFAs such as ARA and DHA from their precursors is critically important as these are predominant n-3 and n-6 LCPUFAs for the offspring. The conversion of ARA from LA was increased by ~ twofolds in the plasma and placenta of n-3 PUFA-deficient mice. In comparison, the transformation of ALA to DHA was increased by ~ 9–16-folds in the plasma and placenta of n-3-sufficient mice [26]. Due to the differential conversion to synthesize LCPUFAs from their precursors, the ARA and DHA content in the placenta differed between n-3-deficient and sufficient mice. It may influence the capacity of placental transfer of fatty acids to the offspring. Data suggest that the ALA/LA ratio in the maternal diet can influence rodent placental fatty acid composition [119]. Low placental transfer of LA is likely to promote DHA accretion in fetal development [120]. During n-3 PUFA deficiency, placental delivery of DHA was decreased while DPA was increased, whereas the latter one was undetected in n-3-adequate mice [26]. Elevated n-6 DPA in circulation indicates a biomarker for n-3 PUFA deficiency in women delivered pre-term [52]. The lower presence of DHA in n-3-depleted placenta may utilize n-6 DPA to meet the increased demand of LCPUFAs in the fetus. DPA relatively increased in n-3 depleted mice; DHA may be spared for the placental transfer to the fetus.

Delta desaturases, the rate-limiting enzymes in LCPUFA biosynthesis, can signify fatty acid redistribution during n-3 PUFA deficiency. FADS1 and FADS2 genes encode delta-5 (D5D) and delta-6 desaturase (D6D) enzymes. Data suggest that the D6D enzyme activity can alter fatty acid profiles [121]. The n-3 fatty acid deficiency selectively upregulates the expression of FADS2, not FADS1, in mice livers [122, 123]. The FADS2 mRNA expression, but not FADS1, was upregulated in n-3 PUFA-deficient placenta [26]. The lower placental n-3 LCPUFA and reciprocal elevation of n-6 LCPUFA were correlated with increased FADS2 expression in the n-3 deficient placenta. Due to greater DHA demand of the n-3 PUFA-deficient fetus, the placenta possibly overexpressed the FADS2 gene as a part of compensatory response to desaturate available fatty acid substrates.

The dietary n-3 PUFA deficiency significantly affected the placental expression of fatty acid metabolic mediators in successive two generations in mice [26]. The n-3-depleted mice placenta upregulated the expression of intracellular fatty acid carrier and binding proteins such as FABP4, FABP3, and ADRP. Due to its adaptive capacity, the placenta probably responded by stimulating the expression of lipid metabolic and transporting machinery when the availability of n-3 PUFAs became lower [20, 124]. A maternal diet influences the expression of fatty acid transporters in the mid-gestation mice placenta [125]. Moreover, n-3-deficient or higher n-6:n-3 fatty acid fed mice increased the FATP4 and FATP2 mRNA expression in placentae [126].

The LPL immunofluorescence was found positive in fetal membrane cells. Invasive cytotrophoblasts upregulate cytoplasmic LPL expression at term with minimal expression in maternal cells, possibly to compensate demand for the prostaglandin synthesis in labor. The plasma pre-heparin LPL mass was decreased and inversely correlated with TG levels [127]. Pre-heparin LPL mass decreases when TG catabolism is impaired, such as hypertriglyceridemia. Since LPL decreases serum TG, thus, pre-heparin LPL reflects working LPL activity in pregnant women. Estrogen lowers the LPL expression in adipose tissue as observed during late pregnancy. Since estrogen increases as the pregnancy advances, it also inhibits hepatic LPL activity. Thus, LPL activity and its expression are tightly controlled with the stages of gestation and maternal hormone.

The LCPUFAs, in particular EPA and DHA, have independent effects on lipogenesis in humans [128]. DHA intake showed a reduction in TG concertation, which was correlated with increased LPL activity and enhanced lipogenesis in humans. LPL is an endothelial enzyme that catalyzes the TG carriers by VLDL and chylomicron to facilitate their uptake in recipient target tissues. Placental LPL hydrolyzes TG from post-hepatic LDL, VLDL, but not TG with chylomicron [129]. Placental LPL activity is attenuated in pathogenic pregnancy of IUGR and diabetes oppositely. A transcription factor, sterol regulatory-element binding proteins (SREBPs), plays an important role in maintaining lipid homeostasis of the human placenta. SREBPs are transcription factors that regulate the gene expression involved in lipid synthesis. The SREBPs regulate the transcription of ACC and FAS genes, which encode enzymes critical for fatty acid biosynthesis [130, 131]. The regulatory activity of the SREBP-1 in targeting FADS2 seems responsible for regulating the LCPUFA biosynthesis. Insulin is also known to act via SREBP1c by increased nuclear activities, while LCPUFAs inhibited the expression of this transcription factor [132]. SREBP and other transcription factors, such as PPARγ, regulate insulin resistance by controlling fatty acid metabolism.

The placental transport critically determines the net transfer of nutrients to the developing fetus. Altered placental fatty acid transport reflects increased cardiometabolic risks [133] and impaired neurodevelopment in infants [134]. For example, offspring shows altered rhythmic sleep maturation when born from GDM mothers with reduced DHA in their cord blood [135]. Due to limited endogenous conversion and synthesis of DHA, the human fetus mainly depends on the placental supply of DHA from the maternal side. Uptake of maternal free fatty acids by placental trophoblasts is mediated by transmembrane and intracellular proteins such as FAT/CD36, FATPs, FABPpm, and FABPs [136, 137]. The

recent reports suggest that a novel transporter can involve DHA uptake and transport in the fetus [25, 134, 138]. The MFSD2A, major facilitator superfamily domain-containing 2A, is localized at brain endothelial vasculature, which acts as a nutrient-transport facilitator across the blood–brain barrier. It has been marked as selective transporter of lysophosphatidylcholine fraction of DHA from the circulation into the brain [139–142]. The precise roles of MFSD2A in placental DHA transport are not established yet. However, the membrane transporter, MFSD2A, is expressed lower in the GDM placenta than a healthy one suspects an impairment of maternal–fetal DHA transport. MFSD2A was recognized as a syncytin-2 receptor, and the latter is involved in trophoblast fusion during gestation [143]. Syncytin-2 protein is involved in embryo implantation in the human womb, encoded by highly conserved endogenous retrovirus group FRD member 1, envelope (ERVFRD-1) gene. MFSD2A signal was localized in the cytoplasm and plasma membrane of the trophoblast cells. All evidence indicates multiple roles of MFSD2A that could be used as a biomarker of placental development and DHA transfer during pregnancy.

Available pre-clinical data suggest that PUFA deficiency affects the placental expression of fatty acid metabolic mediators [2, 26, 144]. The maternal PUFA deficiency can regulate the placental adaptive capacity of fatty acid transfer to the offspring. However, further studies involving clinical outcome are required for a definitive conclusion.

The Placenta Harbors the Epigenetic Fingerprint of a Pregnancy Outcome

Both mother and fetus share a placenta, but it is primarily regulated by the fetal (epi) genome. Thus, the placental epigenetic marks can affect fetal gene expression and act as an essential determinant for the fetus' growth and development. Genome imprint implies an epigenetic process that suppresses one parental allele results in monoallelic expression. Evidence support that paternally expressed imprinted genes are growth-promoting for the placenta and fetus while maternally expressed imprinted genes dampen the growth [145]. In humans, the autosomal chromosome contains two copies of alleles of a gene where one copy is maternally inherited, and the other copy is inherited paternally. Although the DNA sequence of imprinted gene alleles is identical, each allele carries epigenetic marks of their distinct inherited parent. Majorities of the imprinted genes are expressed in the placenta, and several of these function as control of fetal growth and development. The imprinted genes directly influence fetal growth and affect nutrient supply through optimal functional development of the placenta. For example, imprinted gene *Phlda2* showed a marked change in the placental development associated with IUGR [145, 146]. The mechanism that changes imprinted gene expression involves differential DNA methylation, histone modification, and non-coding RNAs at the transcriptional and post-transcriptional levels. The well-studied imprinted gene *IGF2* controls the growth of the placenta and fetus in mice depending on the parent-of-origin manner [147]. Genome imprinting plays a crucial role in fetal growth regulation as altered allelic expression of an imprinted gene correlated with birth weight [148]. The placenta harbors epigenetically regulated parent of origin-specific gene expression [149].

DNA methylation plays a significant role in regulating placenta-specific gene expression, including inactivated X-chromosome and monoallelic expression [150]. DNA methylation (DNAm) is the most predominant carrier of epigenetic regulation in response to diet and environmental cues since DNA is a stable molecule. DNA molecules are modified directly by the DNAm process. DNAm involves the covalent addition of a methyl group to the DNA nucleotide cytosine of a cytosine-guanine dinucleotide (CpG) 5-carbon of the cytosine ring by DNA methyltransferase, resulting in 5-methylcytosine (5mC). The biological importance of DNAm as a significant epigenetic carrier in modulating phenotype and gene expression is being studied extensively. The epigenetic alterations in the placenta are strong predictor of disease risks in adult life [151]. A methyl group to/from the cytosine base was added by DNA methyltransferase, DNMTs (DNMT1, DNMT3A, DNMT3B) or ten-eleven-translocases, TETs (TET1, 2, 3), without changing the sequence of DNA. DNMT3-dependent-de novo methylation occurs in both embryo and placenta, while DNMT1 controls DNAm during replication. DNAm plays a defined role in expressing several printed genes in response to maternal nutritional status. DNAm levels remained lower during placental development [152]. The dysregulated DNAm induces morphological and biochemical changes in the placental cells [153]. The expression and localization of DNMT3A are dysregulated in the preeclamptic placenta [154]. Alteration in DNMT3A expression is associated with placental telomere length [155]. Increased DNMT3B correlates with decreased expression of HLAG in the PE placenta [156].

Genome imprinting can involve DNA methylation during placental development. For example, monoallelic DNAm can lead to monoallelic repression/expression of one of the two copies of parent-of-origin imprinted genes randomly. These imprinted genes enriched with "imprint control region (ICR)" and methylation of ICR can lead to monoallelic gene suppression. For example, the DNMT1 gene exhibited placenta-specific imprinting of maternal allele DNAm (suppression) and paternal allele expression in the placenta [157]. Since the imprinted ICR/DMRs are tightly controlled in normal development thus, DNAm can be a potential tool in detecting chromosomal aberrations in the placenta [158]. Exploring DNAm profiles of

the placenta with an unbalanced share of parent-of-origin imprinted genes (in case of triploidy, hydatidiform mole) could identify the novel imprinted gene of parental original [159]. Although monoallelic DNAm can also happen in the absence of genome imprinting, modulating such methylation is helpful in fine-tuning gene expression.

The global DNAm of a healthy placenta shows a sequence resemblance with cancer invasion [160]. The chorionic villi within the placenta have a distinctive DNAm profile compared to embryonic tissue, decidua, amnion, and chorion. DNA hypomethylation is more prevalent during early mammalian embryo development than subsequent developmental stages [161]. Chorionic villi (derived from trophectoderm) showed ~ 10–25% less methylated than fetal tissues. Placental DNA hypomethylation seems to be supported by downregulated expression of DNMT1 via monoallelic promoter DNAm in trophoblast cells and placental tissues [162]. In contrast, stressors like n-3 PUFA deficiency showed increased expression of DNMT1 proteins and hypermethylated global DNAm in mice's placenta [26]. Placental DNAm loci are associated with birth weight. The CpG methylation of the placenta was correlated with the transcript levels of the genes (for example, lipid metabolism, inflammation, oxidative stress), which are dysregulated in impaired fetal growth [148]. DNA methylation (DNAm) is being linked with prenatal environmental expression of placental epigenome [163]; periconceptional exposure [164] indicates the roles of epigenetic programming in contributing to the association of "maternal nutritional & environmental exposure and placental adversity" in adult health and diseases [165]. Table 1 collected human studies that shows the effects of placental epigenetic changes in DNA methylation and its influence on fetal growth and development outcome.

Maternal n-3 PUFAs and Placental Epigenetic DNA Methylation

Data are emerging that DNAm changes in the placenta when exposed to in utero PUFA diets. The n-3 LCPUFA intake during gestation seems to influence the DNAm of FADS gene promoter in the offspring [166]. The difference in the DNAm is identified between individuals with low and high n-3 PUFA intakes [167]. In addition, n-3 PUFAs can directly induce changes in the methylation at specific CpG sites [168]. The LCPUFA levels profoundly change the methylation pattern on CpG loci of fatty acid desaturases (FADS), key enzymes responsible for the endogenous conversion of PUFA into LCPUFA [169]. The FADS methylation clusters correlate to the circulating and tissue LCPUFA content [170]. The FADS-mediated conversion of n-3 PUFAs to n-3 LCPUFAs targets epigenetic DNAm [168, 171].

The n-3 fatty acids can modify DNAm globally or through gene-specific promoter sequences. Changes in DNMT3A or 3B expression could be a potential mechanism underlying modulated DNAm during n-3 PUFA deficiency. The n-3 PUFAs modulate epigenetic DNAm by activating transcription factors [172]. In addition, it regulates metaflammation by changing the DNAm of PPARγ, PGC1α genes. Higher circulatory n-3 PUFAs negatively correlate with IL6 promoter methylation [173]. The changes in DNAm of the Elovl5 gene (encodes elongation of very long-chain fatty acids) are associated with n-3 PUFA deficiency [174]. In rats, dietary n-3 PUFAs modulate maternal dyslipidemia-induced DNAm and histone acetylation in the placenta and fetal liver [175]. Data showed that maternal n-3 PUFA deficiency increased the 5-methylcytosine level in the F0 placenta while such aberration was continued to the F1 placental epigenome [26]. The n-3 PUFA deficiency significantly increased global DNAm in F0 and F1 mice placenta compared to n-3 PUFA-sufficient mice. However, the changes in DNAm were unaffected in liver and brain tissue. Thus, the placenta is more susceptible to epigenetic modification than other organs, especially when n-3 PUFA levels are deficient. It was further noted that these epigenetic alterations were associated with increased DNMT3A and DNMT3B expression in F0 and F1 placentas. Thus, the expression of DNA methyltransferases was possibly involved with the changes in epigenetic DNAm since their expression was upregulated along with 5-mc hypermethylation.

Supplementation of n-3 PUFA shows a decreased LINE-1 DNAm, a surrogate of global DNAm [176]. Large for gestational age is correlated with increased global placental DNAm [177]. The upregulation of DNMT3A and DNMT3B in the ARA(-)/DHA(-) mice indicated that de novo DNAm was affected in the n-3 fatty acid-depleted placenta [178]. Moreover, ARA-derived prostaglandin E2 (PGE2) alters global DNAm by increased DNA methyltransferase activities [179]. Changes in DNMT3A or 3B expression could be a potential underlying mechanism modulated DNAm during the n-3 deficiency state. The mice DNA shares ~ 98% similarity with humans. Thus, the changes in DNAm due to n-3 PUFA deficiency can correlate with epigenetic instability and dysregulated gene expression in humans under such conditions. Thus, an adequate maternal n-3 PUFA diet can protect epigenetic stability by regulating placental DNA methylation.

A cross-sectional study examined the changes in DNAm of diabetes and CVD-related traits associated with n-3 PUFA intake of Yup'ik Alaska natives ($n = 185$). Among the differentially methylated cytosine-phosphate-guanine (CpG) sites, the genomic regions on chromosomes 3, 10, and 16 significantly associated with n-3 PUFA intake. The intake of EPA and DHA is generally

Table 1 Changes in placental DNA methylation and fetal outcome: clinical studies

Study objective and design	EpiAssay	Epigenetic changes and fetal outcome	Ref
A causal association between maternal hyperglycemia, placental *LEP* DNAm, & neonatal adiposity; Mother–child dyad ($n=259$); Gen3G birth cohort;	DNAm	Maternal hyperglycemia was associated with DNAm of 3 CpG sites of the placental leptin gene and neonatal leptinemia	[184]
Placental epigenome-wide association between birth weight and DNAm linked genetic loci of cardiometabolic risk factor; Pregnancy cohort ($n=301$)	DNAm	Placental CpG methylation was correlated with the lipid metabolic, inflammation and oxidative stress-related transcript levels, those are dysregulated in altered fetal growth	[148]
Placental epigenetic marker of PE and its sex specific infant association from Mother–child dyad ($n=194$); Harvard Epigenetic Birth Cohort;	DNAm & gene expression	Infant sex-specific PE-associated DMPs among singletons; Association of PE and its correlation with differential DNAm of NAPRT1 gene;	[185]
Global placental DNAm ($n=1023$) in relation with birth size for gestation age as SGA or LGA	DNAm	LGA offspring displayed significantly higher global placental DNA methylation compared to appropriate for gestational age	[177]
Association between mode of conception and epigenetic risks in assisted reproduction technology, ART ($n=51$), and spontaneous ($n=48$) pregnancy; Placenta and cord blood DNAm;	DNAm & gene expression	DNAm was lower in IVF-placenta compared to spontaneous; Potential dysregulation of syncytin encoded gene; Expression of ERVFRD-1 gene positively correlated with birth weight and placental weight in the control group;	[186]
DNAm pattern of fetal serotonin transporter gene (SLC6A4) in placenta of GDM ($n=18$) and control ($n=32$);	DNAm & gene expression	Placental SLC6A4 mRNA levels inversely correlated with average DNAm indicate an epigenetic role in placental regulation of gene expression	[187]
Genome-wide DNAm changes in PE ($n=27$) and GDM ($n=28$) or normal ($n=30$) placenta	DNAm	64% of genes with DMPs are altered both in PE and GDM belongs to cell adhesion and differentiation cluster functions	[188]
Genome wide DNAm of gene promoters in placenta from first ($n=18$), second ($n=11$), and third ($n=14$) trimesters with a progressive increase in average methylation;	DNAm	Most differentially regulated genes are immunomodulatory. Changes in DNAm was higher in the third as compared to the first trimester	[162, 189]
Placental DNAm and birth outcome in relation with maternal smoking during pregnancy (MSDP) of PACE consortium data ($n=344$)	DNAm	Placental MSDP linked CpGs are inflammatory growth factor signaling genes; Placenta alter epigenetic response due to adverse maternal environment during pregnancy	[163]
Placental DNAm of LINE1, HSD11β2, IGF2 genes, and its correlation with fetal growth and pollutant exposure; Nested case–control birth cohort of FGR and healthy ($n=130$) pairs;	DNAm	Placental DNAm changes of HSD11β2 and IGF2 were correlated to both lower pollutant exposure and fetal growth	[190]
Prenatal cigarette smoking, DNAm, and gene expression of xenobiotic CYP1A1 and AHRR in first trimester placenta ($n=39$) & liver ($n=43$);	DNAm & gene expression	First trimester smoking was linked with changes in DNAm and gene expression of CYP1A1 and AHRR genes in the placenta but not in the liver	[191]

DNAm, DNA methylation; *DMPs*, differentially methylated position; *SGA*, small for gestational age; *LGA*, large for gestational age

Table 2 PUFA modulates pregnancy and fetus outcome by epigenetic DNA methylation

Model	Study objective and design	EpiAssay	Epigenetic changes and consequences	Ref
Human	Preconception ($n=346$) and early trimester ($n=374$) maternal plasma fatty acids (FAs) and their relevance to newborn DNAm of CpG sites; EAGeR trial	DNAm	Preconception but not early trimester fatty acid levels are related to newborn DNAm	[192]
Human	Maternal intake of low, medium, and high n-3 PUFAs at birth as measured by plasma FAs and cord blood PBMC-DNAm of mother-newborn ($n=118$) pair	DNAm	Four differentially methylated genes correlate to the onset of insulin resistance, adiposity, immune response, craving, and sugar addiction	[144]
Human	Double-blind RCT of DHA supplemented (800 mg/d) in utero from week 20 until delivery and infant's ($n=369$) differentially methylated regions (DMRs) as epigenome units	DNAm	The numbers of DMRs are higher in males (127) than females (72); Maternal DHA intake causes small effects of DNAm difference (< 5%);	[193]
Human	Genotype matched FADS2 promoter methylation and its relation to memory performance of the 16mo toddler using cord blood ($n=71$)	DNAm & gene expression	Maternal methylation correlates delayed memory performance while toddler methylation relates immediate memory performance	[166]
Human	Prenatal supplementation of DHA (400 mg/d) from 18 to 22 wks. of gestation to parturition and DNAm of cord blood PBMC	DNAm	DNAm of IGF2 is higher in the DHA group of pre-term than control; Maternal DHA intake during gestation affects infant growth by modulating DNAm of IGF imprinted genes and inflammatory response of Th1/Th2 balance	[194, 195]
Mouse	Weanling female mice of n-3 PUFA deficient (0.13%enALA) or sufficient (2.26%en ALA) diet for two generations	DNAm	n-3 PUFA deficiency increased global DNAm, DNMT expression in placenta but not in the liver for successive generations as compared to n-3-sufficient group	[26]
Mouse	Intrauterine excess of n-6 PUFA enriched Western diet (n6/n3 = 15/1) and control diet and epigenetic programming of brain tissue	DNAm	Genome-wide DNAm of fetal male brain (E18.5) showed hypermethylated CpG sites in n-6 PUFA-enriched mice	[196]
Mouse	Mice fed n-3 PUFA deficient (0.1%en) or sufficient (1%en) during pregnancy and lactation	DNAm	Maternal n-3 PUFA deficiency resulted in a decrease in BDNF expression and increase in DNAm of associated CpG sites in 3mo offspring	[197]
Mouse	Maternal LA and ALA availability during gestation and lactation and alteration in FADS2 DNAm in mother and offspring liver	DNAm	Increasing maternal ALA intake induces epigenetic changes by increasing both FADS2 promoter and intron-1 DNAm in mothers and pups	[198]
Rat	Weanling Wister female rats fed HFD/control diet contain unsaturated fatty acids from fish oil and sunflower for two months; gD 18–20 placenta and liver DNAm analysis;	DNAm	Maternal dyslipidemia increased global DNAm, DNMT1 in liver and placenta of pregnant rats	[175]

20-folds higher in these people than the average US population [180]. Therefore, these data provide clinical evidence on the functional roles of nutrigenomics in protecting the risks of adult health and diseases. Recent data suggest that FADS1-FADS2 genetic polymorphism of fatty acid metabolism susceptibility is mediated by the changes in DNAm and gene expression [181]. The association between maternal erythrocyte fatty acid composition (as an indicator of *n*-3 PUFA intake) and cord blood DNAm of mother-newborn ($n = 118$) pair suggests that the maternal n-3 PUFAs influences the DNAm of multiple gene pathways, including immune response and craving in the offspring [144]. Furthermore, maternal circulatory LCPUFAs at different trimesters are associated with infant DNAm suggesting that maternal lipid can influence the epigenetic programming of fetal development [182]. Emerging studies that report how dietary PUFA modulates pregnancy and fetal development outcomes by epigenetic DNA methylation are summarized in Table 2.

Summary and Future Directions

The human and rodent placental development is different. Thus, animal data may not extrapolate linearly with humans. However, animal models are helpful to study as the impact of long-term nutrient deficiency can track the stage-specific developmental effects within a short window. The maternal nutrient restriction models helped identify that a reduction in placental vascular development causes fetal growth restriction [183]. Therefore, the necessities of the models in characterizing the disease pathology are required. The significant takeaway is the data derived from mice models dealing with genotype and epigenetics changes. Mice have closer genetics in humans. On the contrary, mice can convert DHA endogenously from its EFA precursor, unlike humans. Thus, animal studies may support the mechanistic explanation of human physiology to a limited extent.

Adequate vasculogenesis and angiogenesis are critical to establishing placental circulation and thus help growth and development. If reduced placental vasculature is secondary to placental insufficiency is not known yet. Again, whether inadequate placental vasculature due to n-3 PUFA deficiency

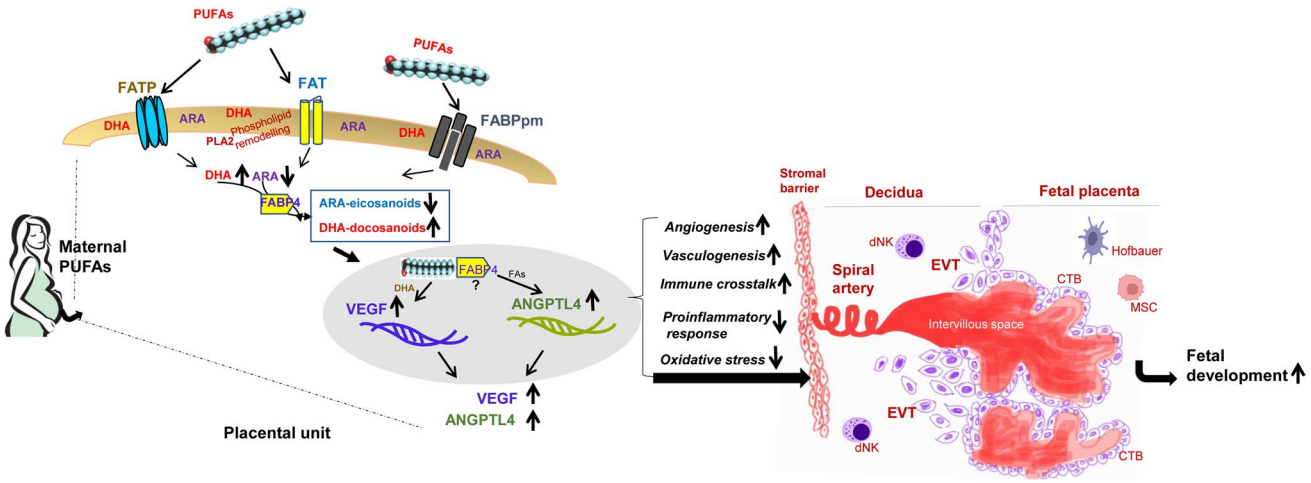

Fig. 2 Putative roles of maternal PUFAs on angiogenesis and vascular development, its implication to the decidual-placental interaction, and fetal development. Maternal PUFAs are actively transported across the plasma membrane via a set of transporters, including FATP, FAT, and FABPpm. Higher maternal plasma n-3/n-6 PUFAs increase the DHA-derived docosanoids while decreasing the ARA-derived eicosanoids in the circulation, which collectively leads to a less pro-inflammatory microenvironment. These albumin-bound fatty acids are traversed intracellularly by FABP4. Data showed DHA (n-3)-stimulated transcription and secretion of a pro-angiogenic growth factor VEGF in the trophoblast cells. At the same time, other n-6 fatty acids stimulated the expression and secretion of ANGPTL4 in these cells. Furthermore, in vivo data demonstrated that n-3 PUFAs promoted placental vasculogenesis, angiogenesis, favored maternal–fetal immune competence, reduced the activities of oxidative stress, and pro-inflammatory mediators. Collectively, these events facilitated adequate nutrients and blood flow across the decidua-placental interface by improving feto-placental health and establishing an optimal microenvironment for improved fetal development. FABP4, fatty acid-binding protein-4; VEGF, vascular endothelial growth factor; ANGPTL4, angiopoietin-4; dNK, decidual natural killer cells; EVT, extravillous trophoblast; CTB, cytotrophoblast; MSC, mesenchymal stem/stromal cells

is a cause for placental dysfunction is not established clinically. Incremental data suggest that insufficient placental vascular development due to reduced angiogenic, invasive, and adhesion factors may be appended due to n-3 PUFA deficiency. These could potentially influence uteroplacental remodeling, and blood flow, leading to altered fatty acid flow to the growing fetus (Fig. 2). Emerging data suggest that n-3 fatty deficiency inversely correlates placental angiogenesis, vasculogenesis, and epigenetics, which collectively can affect fetal growth and development. However, clinical data is required to establish the association between n-3 PUFA deficiency and placental functions.

Changes in global DNAm can reflect placental pathology and placental response to nutrient stimuli. Placental DNA is uniquely methylated than an embryo or extraembryonic tissue. The DNAm profile keeps changing throughout gestation due to dynamic cellular composition across the trimesters and differential gene expression due to different types of cells within the placenta. Again, significant DNAm changes were observed in the third trimester than in the first trimester due to an accumulative response. Placental adaptation to the nutritional cues also allows changing placental methylome due to compensatory response resulting in a cycle of programming-reprogramming in gene expression. The placenta becomes a hotspot for epigenetic regulation as it harbors genome imprints, allele-specific methylation, and gene expression. Thus, placental methylome could produce a unique signature bearing response due to nutrient deficiency, including n-3 fatty acid deficiency.

N-3 PUFA deficiency in the placenta stimulated altered DNA methylation profile, resulting in suppressed gene expression during feto-placental development. Maternal n-3 fatty acids during pregnancy are crucial for maintaining epigenetic stability in the fetus, starting from early development spanning through the whole life. Despite genetic/ethnic variation in PUFA metabolism globally, maternal intakes of n-3 fatty acids are required to sustain fetal brain development. Moreover, it can protect the fetus from altered epigenetic programs and minimize the disease risks in adulthood. Lower maternal n-3 fatty acid status may lead to the placenta's functional inadequacy, changes in fetoplacental growth, and fetal brain development. Therefore, pregnant women need to consider adequate essential n-3 fatty acids well before gestation to protect the structural and functional integrity of the feto-placental growth and optimal brain development.

Author Contribution SB: conceived, wrote, reviewed, edited, and finalized the content. AD: contributed to editing and revision of the work. Both have approved the final version for submission.

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Declarations

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