4 Metabolic Interaction Between Folate, Vitamin B12, and Polyunsaturated Fatty Acids in Pregnancy

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Abstract

Fetal growth and development are influenced by maternal nutrition and gestational weight gain. Adequate intake of nutrients such as folate, vitamin B12, and docosahexaenoic acid (DHA) is essential for healthy fetal and placental development. Many countries have a national flour fortification program with folic acid (FA), together with pre-pregnancy supplementation of FA (400 μg/day) during the first trimester of pregnancy. The latter has been recommended by the WHO and adapted to local requirements by perinatal guidelines. On the other hand, in population studies, many women of childbearing age have vitamin B12 deficiency (<148 pmol/L), which can be additionally masked by high FA intake and maternal pregestational obesity. Under these conditions, these patients could be having pregnancies in a folate/vitamin B12 imbalance, which is associated with higher adiposity, insulin resistance, altered lipid metabolism, and low DHA levels in their offspring. However, if these neonatal consequences of maternal pregestational obesity and folate/vitamin B12 imbalance can be reverted by DHA supplementation during pregnancy has not been addressed. This chapter reviews the literature and exposes the current gaps in knowledge and challenges in maternal nutrition with a life-course perspective.

4.1 Introduction

Growth and fetal development are complex processes influenced by genetics and environmental factors, including maternal nutrition, weight gain during pregnancy, maternal and fetal hormones,

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placental function, and maternal stress, among others. Both fetal growth restriction and overgrowth are associated with an increased risk of developing phenotypes that compromise the offspring's health in the neonatal period and later in life (Sibley et al. 2010). The Developmental Origins of Health and Disease (DOHaD) concept, named Barker's hypothesis, first reported the association of low birth weight and the risk of Type 2 Diabetes Mellitus (T2DM) and cardiovascular disease. DOHaD proposes that environmental exposures of the mother prior to or during gestation induce changes in the metabolism, functionality, and growth of systems and organs of the developing fetus by regulating the expression patterns of specific genes whose effects will be revealed in the early stages of the extrauterine life (Gluckman et al. 2007; Godfrey et al. 2007). In adverse intrauterine conditions, the fetus and the placenta adapt to limit their growth and prioritize the development of essential tissues like the brain, heart, and pancreas (Sandovici et al. 2012; Zhang et al. 2015).

According to studies of survivors of the Dutch famine, where a population of the north Netherlands was exposed to profound food deprivation due to the Second World War, maternal pregestational nutritional status and weight gain during pregnancy are essential to ensure adequate fetal growth and development (Barker 2007; De Boo and Harding 2006). The food shortage lasted nearly 6 months, affected the whole population of that country, and was called "The Dutch Hunger Winter Study" (1944–1945) (De Boo and Harding 2006; Jiménez-Chillarón et al. 2012; Schulz 2010; Yajnik 2014). The children conceived and gestated during the hunger months have been followed up in several cohort studies. As a result, many chronic health problems were significantly increased in this population, including type 2 diabetes, obesity, lung disease, and altered coagulation. In addition, several epigenetic mechanisms (DNA methylation) changes were observed in those exposed in fetal life to these severe nutrient restriction conditions. These studies have concluded that there are critical windows of sensitivity during human development to establish metabolic syndrome and systems physiology later in life (Rinaudo and Wang 2012).

In an epidemiological study in rural Gambia, Waterland et al. evaluated that mothers' periconceptional nutritional status influences their offspring's epigenome (Waterland et al. 2010). Maternal food intake during the rainy season in rural Gambia is characterized by reduced nutrient availability, whereas during the dry season, it is characterized by high nutrient availability. For the first time in humans, this study evidenced that epigenetic regulation occurs at specific genomic loci, resulting in epigenetic modifications that affect gene expression in tissues and persist into adulthood (Waterland et al. 2010). The increased risk of disease later in time induced by adverse environments during periconceptional and intrauterine development may be explained by epigenetic mechanisms affecting the expression of specific genes through DNA methylation, histone modifications, and non-coding RNAs (Bianco-Miotto et al. 2017).

Several studies have evaluated the effects of maternal nutrition, including macronutrients and micronutrients, on the offspring, emphasizing their role in DOHaD (Jiménez-Chillarón et al. 2012). Adequate nutrient intake (in quantity and diversity) during pregnancy is essential; among the critical micronutrients, B complex vitamins are essential. The bioavailability of micronutrients like methionine, choline, betaine, and vitamins B (folate, B2, B6, and B12) may influence DNA methylation by modifying the activity of the one-carbon cycle and the production of S-adenosyl methionine (SAM) (Jiménez-Chillarón et al. 2012). The critical role of vitamin B has been extensively described in the prospective Pune Maternal Nutrition Study. The small and thin Indian babies presented a higher fat mass at birth than the larger English babies. The latter showed lower adiposity at birth (Yajnik 2014). Furthermore, Indian babies had an increased risk of developing diabetes later in life due to higher insulin and leptin and lower adiponectin levels at birth. These effects were associated with high maternal folate and low vitamin B12 (Yajnik 2014).

4.2 Maternal and Fetal Folates and Vitamin B12 Requirements During Gestation

Folate requirements during pregnancy are 600 μg DFE/day (Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline 1998). This vitamin has been strongly associated with preventing neural tube defects (NTD) (Czeizel and Dudás 1992). Therefore, the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) recommended food fortification with 1.4 mg of folic acid per kg of product (Bailey et al. 2015). Furthermore, women of childbearing age should consume 400 μg/day of folic acid (supplements or fortified foods) in addition to natural dietary folate to prevent NTD and 5 mg/day if they have a previous history of NTD (Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline 1998; Organización Mundial de la Salud 2014). Based on this, in 2000, Chile started the "Program of Flour Fortification" with 1.8 mg/kg of FA (accepted range: 1.0–2.6 mg/kg) according to the last report (Subsecretaría de Salud Pública. Ministerio de Salud. Instituto de Salud Pública de Chile 2011). Additionally to the food fortification, the perinatal guide of the Chilean Ministry of Health (Ministerio de Salud – Gobierno de Chile, Subsecretaría de Salud Pública, División Prevención y Control de Enfermedades, Departamento de Ciclo Vital, Programa Nacional Salud de la Mujer 2015) recommends prenatal supplementation with 1000 μg/day of folic acid, 3 months prior to pregnancy and until the first trimester of pregnancy, to decrease NTD rates (Ministerio de Salud – Gobierno de Chile, Subsecretaría de Salud Pública, División Prevención y Control de Enfermedades, Departamento de Ciclo Vital, Programa Nacional Salud de la Mujer 2015).

Despite the WHO recommendation and according to the last report about the food fortification program, Chile has the highest folic acid fortification in wheat flour (3.4 mg/kg) (Subsecretaría de Salud Pública. Ministerio de Salud. Instituto de Salud Pública de Chile 2011). In other Latin American countries, the folic acid levels in fortified food are below 1.8 mg/kg of product (David 2004). On the other hand, the Chilean National Survey of Food Consumption (Ministerio de Salud, Gobierno de Chile 2009) showed that women (14–64 years) consume 427 μg/day (CI 95% 416–438) of dietary folate equivalent (DFE). The DFE is the folate plus folic acid, reaching the intake recommendation for adult people of 400 μg DFE/day from all foods. In Chile, bread intake is between 73 and 184 g bread per day, contributing 128–323 μg of folic acid per day, without considering the intake of other foods fortified or supplementation with folic acid (1000 μg/day). This analysis is relevant since the Food and Agricultural Organization of the United States (FAO) and the Institute of Medicine (IOM) established a maximum tolerable level (upper level, UL) for folic acid of 1000 μg/day (considering only fortified foods and supplements). Intakes over this concentration (1000 μg/day) can mask the deficiency of vitamin B12, correcting the hematological symptoms without improving the neurological symptoms distinctive of vitamin B12 deficiency (Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline 1998). In 2003, 3 years post-fortification with FA in a sample of 751 women of childbearing age, Hertrampf et al. found a 300% increase in plasma folate concentrations (from 9.7 ± 4.3 to 37.2 ± 9.5) in red blood cells (from 290 ± 102 nmol/L to 707 ± 179 nmol/L) and the absence of folate deficiency (Hertrampf et al. 2003).

The requirements of vitamin B12 increase from 2.4 to 2.6 μg/day during pregnancy. According to the National Survey of Food Consumption (ENCA 2010), vitamin B12 intake by Chilean women (14–64 years) was 1.6 μg/day (CI 95% 0.7–3.0) (Ministerio de Salud, Gobierno de Chile 2009). The study previously described showed that after folic acid fortification, 10% of

these women presented a vitamin B12 deficiency (<148 pmol), and 13% had depleted (148–221 pmol) vitamin B12 (Hertrampf et al. 2003). Unfortunately, there are no data on folate or vitamin B12 intake or blood levels in Chilean pregnant women. Recently, the National Health Survey (ENS 2016–2017) reported the folate status in a small sample of women of childbearing age in the metropolitan area of Chile (n = 222), showing that only 0.9% of women presented folate deficiency and 7% of them had supra-physiological levels (>45 nmol/L) (Busso et al. 2021). Therefore, it is likely that pregnant women are consuming much higher folic acid quantities than the recommended (600 μg DFE/day) and even more than the tolerable UL (1000 μg FA/day), with unknown consequences for themselves and their children. This condition is paralleled with the prediction of low vitamin B12 plasma levels in women of reproductive age (Hertrampf et al. 2003).

4.3 Biological Functions of Folate and Vitamin B12

Folates are transported from the mother to the fetus through the placenta by three specific transporters (RFC, FOLR1, and PCFT/HCP1) (Solanky et al. 2010) and other non-specific transporters belonging to the ABC superfamily (Keating et al. 2011). In a previous study from our group, differential expression of the placental FOLR1 receptor was reported according to birth weight (Caviedes et al. 2016) and gestational age (Castaño et al. 2017). Vitamin B12 needs to be bound to proteins to be transported in the blood. Transcobalamin (TC) and haptocorrin are the primary transporters of vitamin B12 in plasma; TC binds over 70% of vitamin B12 transported across the placenta (Layden et al. 2016). Vitamin B12 bound to TC constitutes the HoloTC complex (Hughes et al. 2013). All maternal vitamin B12 is transported through the placenta by the specific transcobalamin receptor (TCblR/CD320) that recognizes HoloTC but not haptocorrin (Abuyaman et al. 2013; Quadros et al. 2009; Schneider and Miller 2010). Both folate and vitamin B12 are taken up by placental cells to participate in the synergic metabolic pathways and be transferred to the fetus (Fig. 4.1).

The metabolism of folate and vitamin B12 is present in most organs, including the placenta (Shin et al. 2014). Folates uptake occurs in the syncytiotrophoblast (the specialized cell of the placenta) in the form of 5-methyltetrahydrofolate (5-MTHF), the main circulating form of folate in the body (Scott 1999), or like folic acid. First, folic acid is converted into dihydrofolate (DHF) and 5-MTHF. Folate and vitamin B12 participate in the remethylation of homocysteine (Hcy) to methionine (transmethylation pathways) through methionine synthetase (MS), with vitamin B12 (in the form of methylcobalamin–MetCbl) as a co-factor and 5-MTHF as the methyl donor (Hoffbrand 2014; Scaglione and Panzavolta 2014). SAM is produced in the transmethylation pathways after the previous methionine synthesis and ATP (Hoffbrand 2014). SAM is the primary methyl group donor in the methylation reactions in the body and, consequently, participates in epigenetic mechanisms that include DNA and histone methylation (Anderson et al. 2012; Molloy 2012). Moreover, SAM is an allosteric activator of cystathionine β synthase (CβS) (Ereño-Orbea et al. 2014), which requires vitamin B6 as a co-factor in the transsulfuration pathway of Hcy, where cysteine, an important precursor of glutathione (γ glutamyl-cysteinyl-glycine, GSH), is produced (Hoffman 2011). The transsulfuration pathway constitutes an essential pathway for Hcy degradation (Scaglione and Panzavolta 2014) (Fig. 4.2).

On the other hand, vitamin B12, or cobalamin (Cbl), in the adenosylcobalamin form (AdoCbl or AdoB12), is a co-factor of the methyl malonyl-CoA mutase (MMCoAM) in the mitochondria (Obeid et al. 2015). The enzyme MMCoAM converts methyl malonyl CoA (MMCoA) to succinyl-CoA to enter the Krebs cycle (Adaikalakoteswari et al. 2016). The MMCoA and MMCoAM are regulators of the enzyme carnitine palmitoyl transferase-1 (CPT-1) (López-Viñas et al. 2007; Takahashi-Iñiguez et al. 2012), which is responsible for transporting fatty acids into the mitochondria to be β-oxidized

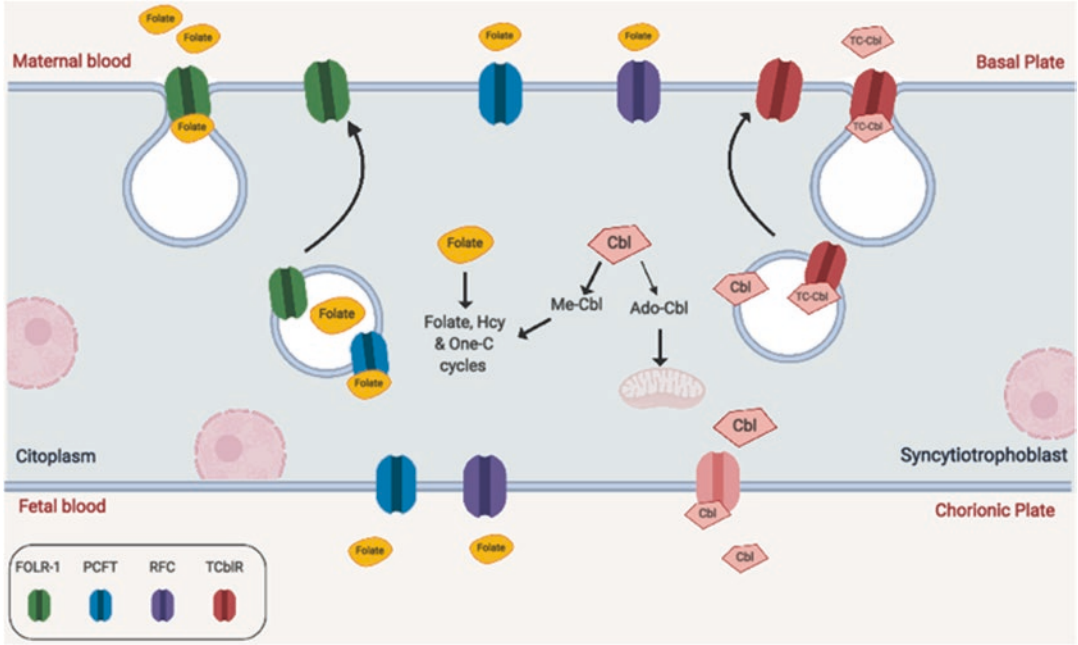

Fig. 4.1 Placental transport of folate and vitamin B12 from mother to fetus Folates are transported from the mother to the fetus through the placenta by three specific transporters (RFC, FOLR1, and PCFT/HCP1). Vitamin B12 bound to TC is called HoloTC. Maternal vitamin B12 is transported through the placenta by the specific transcobalamin receptor (TCblR/CD320)

(Nsiah-Sefaa and McKenzie 2016). In this way, an imbalance of folates (excess or deficit) and vitamin B12 (deficit) may conduce to Hcy levels above normal values, decreasing the synthesis of SAM and methionine and altering the energetic balance by inhibiting the CPT-1 and then affecting the β-oxidation. Consequently, these vitamins are related indirectly to lipid metabolism (Fig. 4.2).

4.4 Folate and Vitamin B12 Imbalance During Pregnancy

The imbalance of folate and vitamin B12 means that circulating folate concentrations (in plasma or erythrocytes) are higher and vitamin B12 levels (in plasma) are lower when analyzed as a ratio (folate/vitamin B12) regarding normal levels. The WHO established the cut-off points for the adult non-pregnant population: for serum folate as a hematological indicator, concentrations are classified as high >45.3 nmol/L, normal 13.5–45.3 nmol/L, and deficit <6.8 nmol/L; in erythrocytes: depletion <362 nmol/L and anemia 226 nmol/L (Organización Mundial de la Salud 2012); for plasma vitamin B12 levels as: normal >221 pmol/L, depletion 148–221 pmol/L, and deficit <148 pmol/L (Allen 2009). Some authors indicated that elevated circulating Hcy (>13 μmol/L) levels indicate a metabolic alteration 16 and that MMA above 0.37 μmol/L and Hcy above 21 μmol/L are indicators of vitamin B12 deficit (Green et al. 2017). There is no consensus about the cut-off points for these vitamins and their relationship with metabolic alterations during pregnancy. In the National Health and Nutrition Examination Survey (NHANES) of 1991–1994 ($n = 4940$) and 1999–2002 ($n = 5473$) with the general population in the United States, aged 20 years and older, it was shown that a high folate/vitamin B12 ratio, such as high plasma folate (>45.3 nmol/L) and low vitamin B12 (<148 pmol/L) levels, was associated with higher

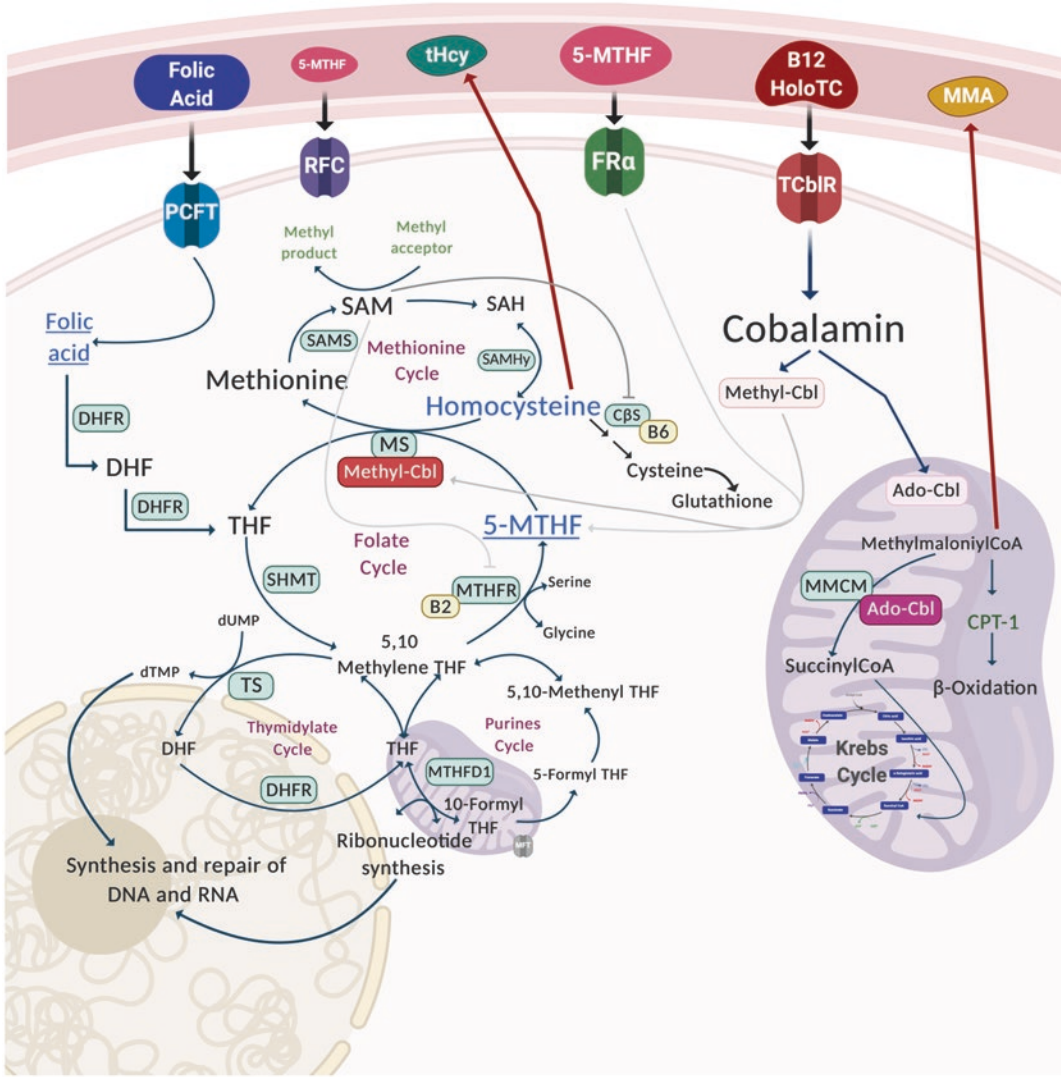

Fig. 4.2 Folate and vitamin B12 metabolism Folate uptake occurs in the syncytiotrophoblast in the form of 5-MTHF, or folic acid. Folate and vitamin B12 participate in the remethylation of homocysteine to methionine (transmethylation pathways) through methionine synthetase (MS), with vitamin B12 (in the form of MetCbl or MetB12) as a co-factor and 5-MTHF as a methyl group donor. SAM is produced in the transmethylation pathways after the previous methionine synthesis and with ATP. SAM is an allosteric activator of cystathionine β synthase (CβS). Vitamin B12, or cobalamin (Cbl), in the AdenosylCobalamin (AdoCbl) form is a co-factor of the methyl malonyl-CoA mutase (MMCoAM) in the mitochondria. The enzyme MMCoAM is responsible for the conversion of methylmalonil CoA (MMCoA) to succinyl-CoA to enter in the Krebs cycle. In turn of this, MMCoA and MMCoAM are regulators of the enzyme Carnitine Palmitoyl Transferase-1 (CPT-1)

concentrations of Hcy (>20 μmol/L) and MMA (>0.8 μmol/L) (Selhub and Rosenberg 2016).

In an observational study from India, a high maternal intake of folic acid and a low vitamin B12 level were related to small for gestational age (SGA) infants (Dwarkanath et al. 2013). A previous study found (median, 25th–75th centile) higher serum folate levels (66, 25–219 nmol/L) with low serum vitamin B12 (219, 97–868 pmol/L). Therefore, a more elevated serum folate/vitamin B12 ratio was found in the cord blood of preterm newborns (305, 181–428)

compared to term newborns (141, 86–216; $p = 0.002$) (Castaño et al. 2017). These results were unexpected because preterm births have been associated with low maternal folate levels in plasma or RBC folate (Tamura and Picciano 2006). Therefore, an explanation for a higher folate/vitamin B12 ratio in preterm newborns may be related to the high levels of folic acid intake in the Chilean population.

Similarly, in a cohort of pregnant women and their children in Pune, India, the authors found that children from mothers with high erythrocyte folate (>1144 nmol/L) along with low plasma vitamin B12 levels (<114 pmol/L) during pregnancy showed higher homeostasis model assessment for insulin resistance (HOMA-IR) at 6 years of age (Yajnik et al. 2008). Similar results were found in Indian children at 9.5 and 13.5 years (Krishnaveni et al. 2014). In summary, high folate and low vitamin B12 intake or blood levels have been related to adverse pregnancy outcomes and metabolic alterations.

On the other hand, increased cholesterol and Hcy levels were produced by the adipocyte cell line Chub-S7 and subcutaneous adipose tissue collected at the time of cesarean section, cultured under low or no B12 concentrations (Adaikalakoteswari et al. 2015). The induction of cholesterol biosynthesis was associated with a reduced SAM/SAH ratio, an indicator of the methylation potential. In the proximal promoter regions of SREBF1 and LDLR, the binding sites for PPARγ and C/EBPα were hypo-methylated, and their transcripts and cholesterol biosynthesis were significantly increased in vitamin B12-deficient conditions. Therefore, low B12 plasma levels (<148 pmol/L) increase cholesterol biosynthesis in human adipose tissue (Adaikalakoteswari et al. 2015).

Studies in non-pregnant (Arias et al. 2017) and pregnant women (McNulty et al. 2013) supplemented with folic acid have observed that vitamin B12 levels decreased after supplementation, suggesting that high blood levels of folic acid may affect the vitamin B12 metabolism. Selhub et al. indicated that a possible mechanism for this could be that folic acid can oxidize the cobalt of vitamin B12, which should be in a highly reduced state (Cob I) to accept the methyl group from 5-methyl tetrahydrofolate, and for this, the plasma vitamin B12 levels decrease (Selhub and Rosenberg 2016); however, this hypothesis has yet to be tested.

The effect of folate/vitamin B12 imbalance has also been studied in the human placenta. In BeWo and JEG-3 placental cell lines cultured with high folic acid (2000 ng/mL), the tumor necrosis factor-alpha (TNFα) gene transcript was overexpressed. Also, higher levels of Hcy and malondialdehyde (MDA-lipoperoxidation marker) were found. However, by treating these cells with the two active forms of vitamin B12 (adocobalamin and methylcobalamin), MDA, Hcy, and inflammation levels decreased significantly (Shah et al. 2016).

4.5 DHA in Pregnancy and Biological Functions

Unlike vitamins, the estimation of the requirements of polyunsaturated fatty acids (PUFAs) intake during pregnancy lacks sufficient studies to establish recommendations (Flock et al. 2013; Kris-Etherton et al. 2009). However, the WHO and the Food and Agriculture Organization of the United Nations (FAO) recommend 300 mg/day of EPA+DHA intake for pregnant and lactating women, of which 200 mg should be DHA (FAO 2010). The benefits of long-chain PUFAs in different metabolic diseases have been widely studied (Flock et al. 2013). Especially in pregnancy, LC-PUFAs positively affect women with gestational diabetes mellitus (GDM), preeclampsia, and intrauterine growth restriction (IUGR) (Wadhwani et al. 2018). From a fetal perspective, maternal DHA is the only supply source (Rogers et al. 2013).

PUFAs are classified into two main series (*n*-6 and *n*-3), considered essential fatty acids, since the body does not synthesize them. Linoleic acid (LA, 18: 2*n*-6) and alpha-linolenic acid (ALA, 18: 3*n*-3) are the main PUFAs in the diet. From these fatty acids, important LC-PUFAs such as arachidonic acid (AA 20: 4*n*-6), eicosapentaenoic acid (EPA, 20: 5*n*3), and docosahexaenoic acid (DHA, 22: 6*n*3) are derived (FAO 2010).

These LC-PUFAs have essential functions in developing the nervous system of the fetus and the child. These fatty acids are part of the structure of cell membranes, and their primary location is in the brain; therefore, they are related to cognitive development (Colombo et al. 2017; Jones et al. 2014; Scifres and Sadovsky 2011). In addition to their structural and energy source functions, these fatty acids are precursors of eicosanoids such as prostaglandins, prostacyclins, thromboxanes, and leukotrienes (FAO 2010). These molecules regulate physiological and pathological processes, gene expression, cell differentiation, immunity, and inflammation (FAO 2010; Scifres and Sadovsky 2011).

Circulating fatty acids are mainly bound to albumin, triglycerides, and phospholipids; therefore, different proteins participate in their transport and release. Lipoprotein lipases (LPL) or epithelial lipase (EL) participate in their release, as do other transport proteins in the uptake of fatty acids into the cytoplasm, like fatty acid translocases (FAT), fatty acid-binding proteins of the plasma membrane (FABPpm), and fatty acid transport proteins (FATP) (Jones et al. 2014; Wadhwani et al. 2018). The FABPs direct these fatty acids towards the nucleus, lipid droplets, or fetal circulation (Scifres and Sadovsky 2011). The placenta also expresses the same hepatic mechanisms of cholesterol transport and lipid metabolism genes (Scifres and Sadovsky 2011).

LC-PUFAs regulate the energy metabolism by acting as ligands of the nuclear receptor family of transcriptional regulators involved in lipid metabolism, such as peroxisome proliferator-activated receptors (PPARα, PPARγ, and PPARδ). PPARs form a heterodimer with retinol alpha receptor X (RXRα) and bind the peroxisome proliferator response element (PPRE) on target genes (Nakamura et al. 2014). PPARs also interact with hepatic alpha receptor X (LXRα) and protein binding of sterol regulatory elements (SREBP-1c) (Gil-Sánchez et al. 2011; Meher et al. 2014; Nakamura et al. 2014; Scifres and Sadovsky 2011); this interaction has also been described in the trophoblast (Scifres and Sadovsky 2011). PPARγ and RXRα participate in systemic and cellular metabolism, increasing lipid uptake and accumulation. LXRα is activated by oxysterols and derivatives of cholesterol and induces the transcription of genes required for reverse cholesterol transport and de novo lipogenesis in the liver, such as SREBP-1c, which responds to increased insulin levels. LC-PUFAs such as DHA inhibit the induction of SREBP-1c by the LXRα agonist and suppress de novo lipogenesis. PPARα is predominantly expressed in the liver and gastrointestinal tract and regulates the fatty acid β-oxidation by inducing genes like CPT-1, ETFDH, and HADHA that are involved in mitochondrial β-oxidation of PUFAs (Nakamura et al. 2014). However, little is known about lipid metabolism in the placenta of women with obesity or the effects of maternal DHA supplementation.

DHA has been mostly studied concerning child neurodevelopment since it is an essential nutrient for the central nervous system (Morse 2012). In a pregnant cohort from Canada, cord plasma fatty acid levels were lower in newborns of women with gestational diabetes compared to non-diabetic pregnancies, and a lower cord plasma DHA was associated with lower fetal insulin sensitivity, even after adjustment for maternal and newborn characteristics (Zhao et al. 2014). The Generation R Study showed a higher maternal *n*-6: *n*-3 PUFA ratio associated with higher total body and abdominal fat mass in childhood. Higher DHA was associated with a lower childhood total body fat percentage, without changes in BMI and abdominal fat mass (Vidakovic et al. 2016).

In the GUSTO cohort, maternal (26–28 w) plasma DHA levels were associated with a higher postnatal length/height ratio at 12 months and 5 years of age. Linoleic acid was positively associated with birth weight, body mass index, head circumference, and neonatal abdominal adipose tissue volume (Bernard et al. 2017). Additionally, in a randomized, triple-blind, placebo-controlled trial from Iran, participants received 1000 mg of fish oil (120 mg of DHA) from week 20 of gestation to birth. No significant differences in the maternal outcomes were found (Ostadrahimi et al. 2017). Maternal DHA is essential during

pregnancy, although the offspring's effects are inconclusive, suggesting that studies evaluating DHA interactions with other nutrients are required.

4.6 Interaction Between Folate, Vitamin B12, DHA, and Maternal Obesity

According to the WHO, the body mass index (BMI, kg/m^2) in adults is normal weight BMI: 18.5–24.9; overweight BMI: 25–29.9; and obesity BMI ≥ 30 (WHO 2000). The prevalence of obesity has increased worldwide and is currently considered a public health problem (Ng et al. 2014). In addition to this prevalence, insulin resistance (IR), type 2 diabetes, and cardiovascular diseases (CVD) are associated with obesity Chile is no stranger to this phenomenon, where adults presented an excess weight (BMI >25) prevalence of 74% in 2017, according to the National Health Survey (MINSAL 2017). This prevalence has been related to the nutritional transition, which began in the 1970s and increased the NCDs' prevalence (Atalah et al. 2014). In this scenario, women of childbearing age and pregnant women have a higher risk of developing this condition, affecting their offspring's health. In the country, more than 50% of women between 15 and 44 years of age are overweight (BMI > 25), and 23% of the pregnant population (classified by Atalah recommendations (Atalah et al. 1997)) are obese (BMI > 30) (Farías 2013). Pregnant women treated in the public health system have a prevalence of obesity of 32.4% (MINSAL 2016).

Maternal obesity represents an increased risk for the mother and her offspring in developing metabolic complications such as impaired lipid metabolism, inflammation, and oxidative stress, among others (Hrolfsdottir et al. 2016; Madan et al. 2009; Malti et al. 2014). Studies in pregnant women with obesity have found that more significant weight gain during pregnancy is related to an altered lipid profile in the mother. Nevertheless, obesity before pregnancy affects the mother, the offspring (Cinelli et al. 2016), the placental nutrient uptake, the metabolism, and the lipid profile (Segura et al. 2017). Furthermore, oxidative stress represents a risk factor in the decrease of maternal levels of DHA, and on the contrary, the supplementation of this fatty acid could improve the antioxidant response (Leghi and Muhlhausler 2016). According to this, if obesity before and during pregnancy alters micronutrients and lipids in the mother, it would be a risk factor in the fetus's adequate supply of these nutrients, affecting both placental and offspring metabolism.

Regarding the relationship between obesity and micronutrient levels, it has been found that folate and vitamin B12 levels in plasma are lower and red blood cell (RBC) folate levels are higher in women of reproductive age and pregnant women with obesity compared to normal-weight women (Berglund et al. 2016; Bird et al. 2015; Bjørke-Monsen et al. 2016; da Silva et al. 2013; Knight et al. 2015; Park et al. 2017; Shen et al. 2016; Sukumar et al. 2016; Tinker et al. 2012; Wang et al. 2016). However, it is unclear how obesity can affect folate and B12 levels. Some authors say that high or low folate levels in obesity may be due to a redistribution from plasma towards the other tissues, as evidenced by the higher folate levels in RBC (da Silva et al. 2013).

Few studies in humans have evaluated the effects of the interaction of these vitamins with DHA. In a prospective study, maternal folate, B12, and DHA levels were lower, and Hcy was higher in the last trimester than in the first trimester of pregnancy. A negative correlation between Hcy and DHA in the third trimester was found. A positive correlation between folate, DHA, and birth weight was observed in the third trimester of pregnancy (Wadhwani et al. 2015). This study provides evidence about the association of the levels of these nutrients with birth weight, suggesting that a balance in their intake could be beneficial for the mother and her offspring.

In the HELENA study with non-pregnant adolescents, a negative correlation between Hcy and DHA levels and a positive correlation between these vitamins' biomarkers, mainly folate and B12 with EPA and DHA, were described. An increase of 10 nmol/L of erythrocyte folate, or HTC, in adolescent women produced a rise of

15.85 μmol/L of EPA but not of DHA, while an increase of 10 nmol/L of Hcy in men had a decrease of 2.06 μmol/L of DHA (Iglesia et al. 2017). These results suggest that LC-PUFA levels can be affected by other nutrients, such as B-complex vitamins. Therefore, it is necessary to study this relationship between pregnant women and their offspring.

In addition, Wistar rats fed with diets containing different folate levels and low or deficient vitamin B12 showed decreased DHA levels in maternal plasma and placenta (Kulkarni et al. 2011; van Wijk et al. 2012). These tissues also had lower global methylation, and these values were restored by supplementing with *n*-3 (Kulkarni et al. 2011). Kumar et al. found that pregnant rats fed with a folate- and B12-deficient diet showed altered body weight and lipid profiles. Their offspring presented low birth weight, higher body fat at 3 months, altered lipid profile at 12 months, higher levels of TNFα, IL-6, leptin, and lower levels of adiponectin and IL-1β, higher activity of lipogenic enzymes like FAS and acetyl CoA-Carboxylase (ACC) (Kumar et al. 2013, 2014). Meher et al. found that pups from pregnant rats with a restrictive diet in folate and B12 showed lower DHA and ARA levels and lower expression of PPARα (β-oxidation marker) and PPARγ (lipogenesis marker) in the liver. When these mothers were supplemented with *n*-3, the expression of these transcription factors was normalized, and the expression of SREBP-1c, LXRα, and RXRα was reduced (Meher et al. 2014). In summary, low maternal B12 levels affect lipid metabolism by increasing cholesterol levels, lowering DHA levels, and a low mRNA expression for CPT-1 (β-oxidation marker) and ACC1 (lipogenesis marker in the liver of offspring). Notably, the supplementation with *n*-3 restored these effects (Khaire et al. 2015).

The mechanisms behind the imbalance of folate/vitamin B12 and DHA levels could be explained by two potential mechanisms: (1) folate and vitamin B12 are involved in synthesizing SAM, the substrate of methyl reactions. One of the main acceptors of methyl groups are phospholipids; phosphatidylethanolamine (PE) and PE methyltransferase (PEMT) catalyze the methylation of PE to phosphatidylcholine (PC). PE methylation to PC is essential for DHA mobilization from the liver to plasma and other tissues (Wadhwani et al. 2018); (2) the opposite effects of DHA supplementation over low B12 could be explained since DHA regulates lipid metabolism through PPARγ, SREBP-1c inhibition, and PPARα stimulation. In conclusion, imbalanced folate/vitamin B12 alters lipid metabolism due to greater lipogenesis and less β-oxidation. This interaction between folate/vitamin B12 and its effects on lipid metabolism remains unknown in pregnant women, their placentas, and their offspring.

Additionally, it has been shown that maternal obesity modulates the lipid metabolism in the placenta by modifying the expression of genes involved in the transport and storage of lipids (Hirschmugl et al. 2017). In a population-based prospective cohort study at 20 weeks of gestation, obese women had higher total saturated fatty acid concentrations and lower total *n*-3 PUFA concentrations than normal-weight women (Vidakovic et al. 2015). A similar study found that pre-pregnancy BMI was inversely associated with maternal MUFA, LA, and DHA and fetal *n*-6 and DHA after adjusting for maternal lipids (Cinelli et al. 2016).

In studies with maternal supplementation with DHA, total lipid content was significantly lower in the placentas of obese women supplemented with *n*-3 PUFAs (800 mg DHA). The mRNA expression of placental FAS and diacylglycerol O-acyltransferase 1 (DGAT1), two enzymes involved in the accumulation and esterification of fatty acids, was negatively correlated with maternal plasma enrichment in DHA and EPA (Calabuig-Navarro et al. 2016). Another study evaluating the effect of maternal DHA supplementation on child adiposity found a significant increase in erythrocyte DHA levels at 36 weeks of gestation but no significant differences in the neonate's adiposity at birth, 2, or 4 years (Foster et al. 2017). Maternal obesity influences lipid metabolism; however, the interaction of maternal obesity in the presence of folate/vitamin B12 imbalance and DHA supplementation is unknown (Fig. 4.3).

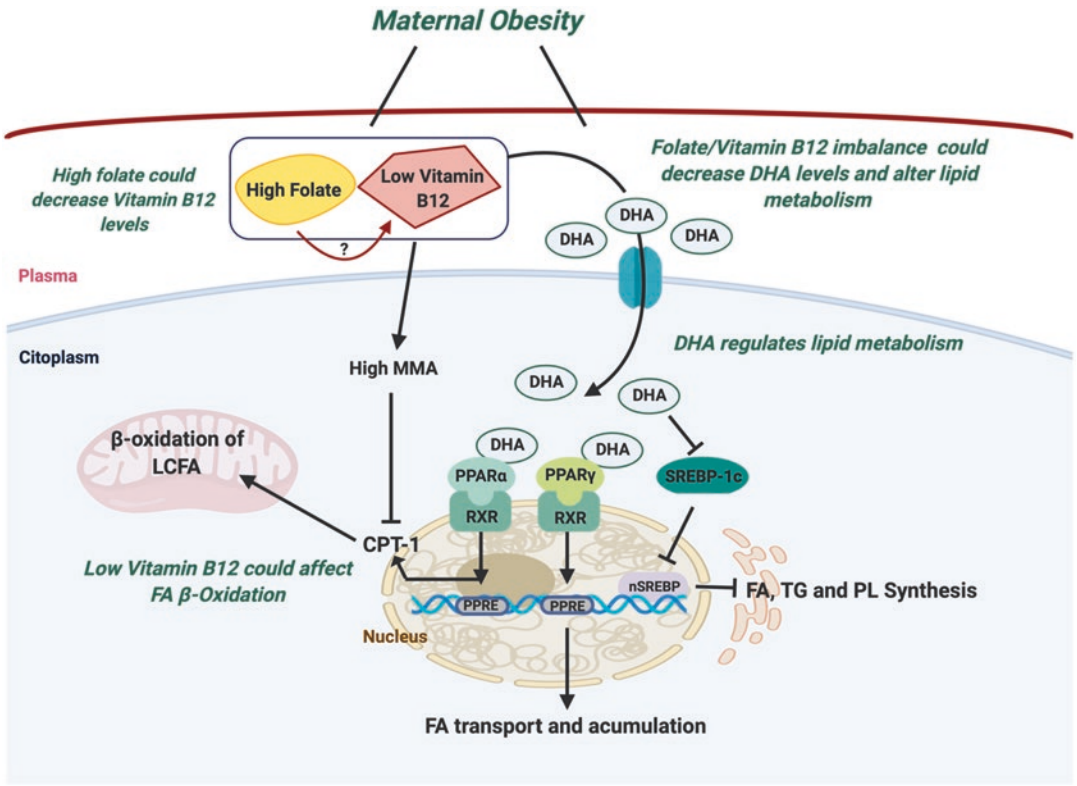

Fig. 4.3 Folate, vitamin B12, and DHA interactions in maternal obesity Maternal obesity increases folate/vitamin B12 imbalance, which increases methylmalonic acid (MMA), which downregulates the enzyme carnitine palmitoyltransferase 1 (CPT-1). Consequently, the β-oxidation is blocked. Maternal obesity decreases the transfer of DHA to the fetus. Lower DHA levels in the placenta may affect the fatty acid β-oxidation, transport, accumulation, and synthesis

In synthesis, it is clear that fetal programming related to altered maternal nutritional status is a risk factor for the early development of non-communicable chronic diseases, and some pregnant women, particularly in Chile, may be exposed to a high folic acid intake and, presumably, to low vitamin B12 levels. We have the following premises: (1) there is a relationship between maternal folate/vitamin B12 imbalance and low DHA levels over pregnancy outcomes and lipid metabolism in the offspring, both humans and animals; (2) maternal obesity influences folate and B12 levels; and (3) this interaction (folate, vitamin B12, and DHA) has not been thoroughly studied in the placentas of pregnant women with obesity.

The future is very challenging from the DOHaD point of view. As a broad mean, maternal and fetal exposome are the most critical factors that can be considered, modified, and optimized to improve the health of our next generation. Important moments of the life cycle need to be considered in the prevention strategies of the different countries, and the most relevant ones are those in the reproductive cycle. Starting with healthy adolescents with balanced nutrition and lifestyle practices, including physical activity, to optimize gamete biology and later pregnancy and the first 1000 days of life. Many knowledge gaps still need our best efforts to unravel the best choices and interventions to optimize development and health for future generations.

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