Nutrition Implications for Fetal Alcohol Spectrum Disorder[1,2]

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ABSTRACT

Prenatal alcohol exposure produces a multitude of detrimental alcohol-induced defects in children collectively known as fetal alcohol spectrum disorder (FASD). Children with FASD often exhibit delayed or abnormal mental, neural, and physical growth. Socioeconomic status, race, genetics, parity, gravidity, age, smoking, and alcohol consumption patterns are all factors that may influence FASD. Optimal maternal nutritional status is of utmost importance for proper fetal development, yet is often altered with alcohol consumption. It is critical to determine a means to resolve and reduce the physical and neurological malformations that develop in the fetus as a result of prenatal alcohol exposure. Because there is a lack of information on the role of nutrients and prenatal nutrition interventions for FASD, the focus of this review is to provide an overview of nutrients (vitamin A, docosahexaenoic acid, folic acid, zinc, choline, vitamin E, and selenium) that may prevent or alleviate the development of FASD. Results from various nutrient supplementation studies in animal models and FASD-related research conducted in humans provide insight into the plausibility of prenatal nutrition interventions for FASD. Further research is necessary to confirm positive results, to determine optimal amounts of nutrients needed in supplementation, and to investigate the collective effects of multiple-nutrient supplementation. *Adv Nutr 2014;5:675–692.*

Introduction

Prenatal alcohol exposure results in a vast spectrum of teratogenic effects and life-long implications for a child. These detrimental effects are collectively grouped under the general term "fetal alcohol spectrum disorder" (FASD)[3], widely known as fetal alcohol syndrome (FAS), which classifies the overall alcohol-induced effects witnessed in a child whose mother consumed alcohol during pregnancy (1). Because FASD is not a genetic disorder and there is no treatment to reverse alcohol-induced damage to the central nervous system (CNS), earlier intervention in pregnant mothers may be a key to prevent or mitigate the severity of FASD.

Although negative consequences of alcohol consumption during pregnancy are well covered by the media, government promotion, and educational programs, 3.3% of pregnant women continue to consume alcohol frequently (defined as ≥7 drinks/wk) or binge drink (when ≥5 drinks are consumed per occasion) (2). The prevalence of FASD varies among populations studied, ranging from 2–7 per 1000 in the United States (3) to ~100 per 1000 children in South Africa (4). The estimated prevalence of FASD in populations of first-grade schoolchildren (~6.5–7.8 y old) is as high as 20–50 per 1000 in the United States and some Western European countries (3). In Canada, the estimated prevalence of FASD is 9 cases per 1000 live births (5) and in Canadian Aboriginal communities ranges from 7.2 to 190 cases per 1000 live births (1,6). However, the true prevalence of FASD is uncertain because it often goes underreported due to lack of awareness, lack of resources, and lack of trained and skilled professionals in diagnosis (4,5).

Optimal nutritional status is required in producing healthy offspring. When maternal nutritional status is compromised with alcohol, essential nutrients are displaced or not obtained, which results in suboptimal health outcomes in the developing fetus due to deprivation of essential nutrients required for growth. In general, women with poor nutritional status during pregnancy have children who are characterized with low birth weight, poor health, physical malformations and abnormalities, behavioral disorders, and delayed cognitive and physical development (7).

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[3] Abbreviations used: ADH, alcohol dehydrogenase; AI, Adequate Intake; ALDH, acetaldehyde dehydrogenase; ARBD, alcohol-related birth defect; CNS, central nervous system; CYP2E1, cytochrome P450 2E1; FAS, fetal alcohol syndrome; FASD, fetal alcohol spectrum disorder; GD, gestational day; GPx, glutathione peroxidase; MEOS, microsomal ethanol oxidizing system; PD, postnatal day; RAR, retinoic acid receptor; RXR, retinoid X receptor; SOD, superoxide dismutase.

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A population-based study in South Africa showed that mothers who had children with FAS were substantially smaller in size than mothers of healthy children with regard to height, weight, head circumference, and BMI (8). Although alcohol is the sole cause of FASD, poor nutrition can further exacerbate the development of FASD. Thus, maintaining optimal nutrition during pregnancy is critical, which raises questions regarding how much and what should be provided during pregnancy to alleviate the severity of the outcome of FASD.

Although maternal nutrition intervention appears to be a promising strategy, robust information on the role of nutrients and nutrition interventions for FASD is scarce. In this regard, this review sought to find evidence for potential target nutrients for prenatal nutritional support for FASD by focusing specifically on alcohol metabolism, alcohol effects on fetal development, and several nutrients and their interactions with alcohol consumption.

Current Status of Knowledge

FASD classification

FASD has numerous clinical presentations that vary according to the severity of the abnormalities; thus, it is difficult to obtain a global estimation rate of FASD. In 1996, the Institute of Medicine (9) published a set of specific recommendations for health professionals and physicians to use in the diagnosis of FASD by dividing FASD into 4 specific disorders: FAS, partial FAS, alcohol-related birth defects (ARBDs), or alcohol-related neurodevelopmental disorders. There might be differences in the prevalence of ARBDs depending on if FAS or FASD is reported. Among these, FAS is the most severe form of FASD and recognized as the leading cause of non–genetic-based mental retardation. It presents a characteristic pattern of facial anomalies, growth deficiency, and CNS dysfunction (10,11). Facial anomalies include short palpebral fissures, midface hypoplasia, an indistinct and broad philtrum, thin upper lip, cleft plate, and epicanthal folds (10–12). Growth deficiency, both prenatally, which may cause a low birth weight in relation to gestational age, and postnatally, is a key characteristic of FAS (10,11). Postnatal growth deficiency is characterized when the height-to-weight ratio of a child is at or below the 10th percentile or when there is a disproportionately low weight-to-height ratio (1). Alcohol produces a variety of devastating effects to the developing CNS, and these include microcephaly and other brain anomalies, decreased intellectual ability, behavioral abnormalities, and impaired development of social, mental, and motor skills (1,11,12). Partial FAS is diagnosed in infants with some facial components of FAS (short palpebral fissure length, smooth or flattened philtrum, thin upper lip) and evidence of at least 1 of the following abnormalities: growth restriction, CNS anomalies, or behavioral and cognitive abnormalities (1,10–12). Alcohol-related neurodevelopmental disorder is diagnosed on the presence of CNS abnormalities that are typically characterized in FAS, and ARBD refers to numerous congenital and physical malformations (1). Although not all children who are prenatally exposed to alcohol develop FAS, usually some form of ARBD exists.

Alcohol exposure and consumption pattern

It has been stated that the frequency of alcohol consumption, consumption pattern, and period of exposure during fetal development are critical factors linked with the severity and presence of the distinct FAS of FASD characteristics.

Frequency of alcohol consumption. Chronic, daily heavy alcohol use or frequent intermittent alcohol use is much more toxic to the fetus than acute, moderate alcohol consumption (7,13). It has been suggested that a major risk to the fetus is presented through the chronic consumption of ≥6 drinks/d. Consuming high, frequent amounts of alcohol during a single drinking episode, known as "binge drinking," has a large effect in contributing to elevated blood alcohol concentrations (7). Total brain weight and development have a strong positive correlation with peak blood alcohol concentration. As blood alcohol concentrations increase, total brain weight decreases (14). Because nutrients from the mother are delivered through the placenta, high blood alcohol concentrations often displace or reduce the transfer of essential nutrients required for fetal development (15).

Timing of alcohol consumption. Alcohol exposure during any stage of pregnancy produces some type of detrimental effect in the offspring. After fertilization, most of the organs are very rapidly formed during the embryonic period (3–8 wk of pregnancy) in the early stage of the first trimester, and the organs continuously develop during the fetal period until birth (**Fig. 1**). The teratogenic effects of alcohol are most significant during embryogenesis; however, development can be disrupted beyond the first trimester (16). In animal studies, exposure to alcohol in a stage equivalent to the first trimester in humans produced the facial dysmorphologies similar to humans with FAS (17). Alcohol exposure during the second and third trimester is associated with neuronal loss (16). Prenatal alcohol exposure to the fetus during the third trimester was also shown to produce serious effects, because this is the stage that correlates with a large increase in essential nutrients incorporated into the brain and retina (16,18). Considering the above factors, it is understandable why there are variations in the severity of FASD.

Alcohol metabolism and its effects on fetal health

Alcohol metabolism. Understanding alcohol metabolism is the first step to understanding the negative impacts of alcohol on human nutritional status. Alcohol contributes 7.1 kcal/g of energy; therefore, continued usage causes primary malnutrition as alcohol displaces the intake of essential nutrients. Secondary malnutrition also occurs as a result of maldigestion or malabsorption of nutrients due to gastrointestinal problems (19). Although most nutrients are affected by alcohol intake, specific nutrients noted from numerous studies are thiamin, riboflavin, vitamin B-12, vitamin E, selenium, vitamin A, vitamin C, folic acid, vitamin D, zinc,

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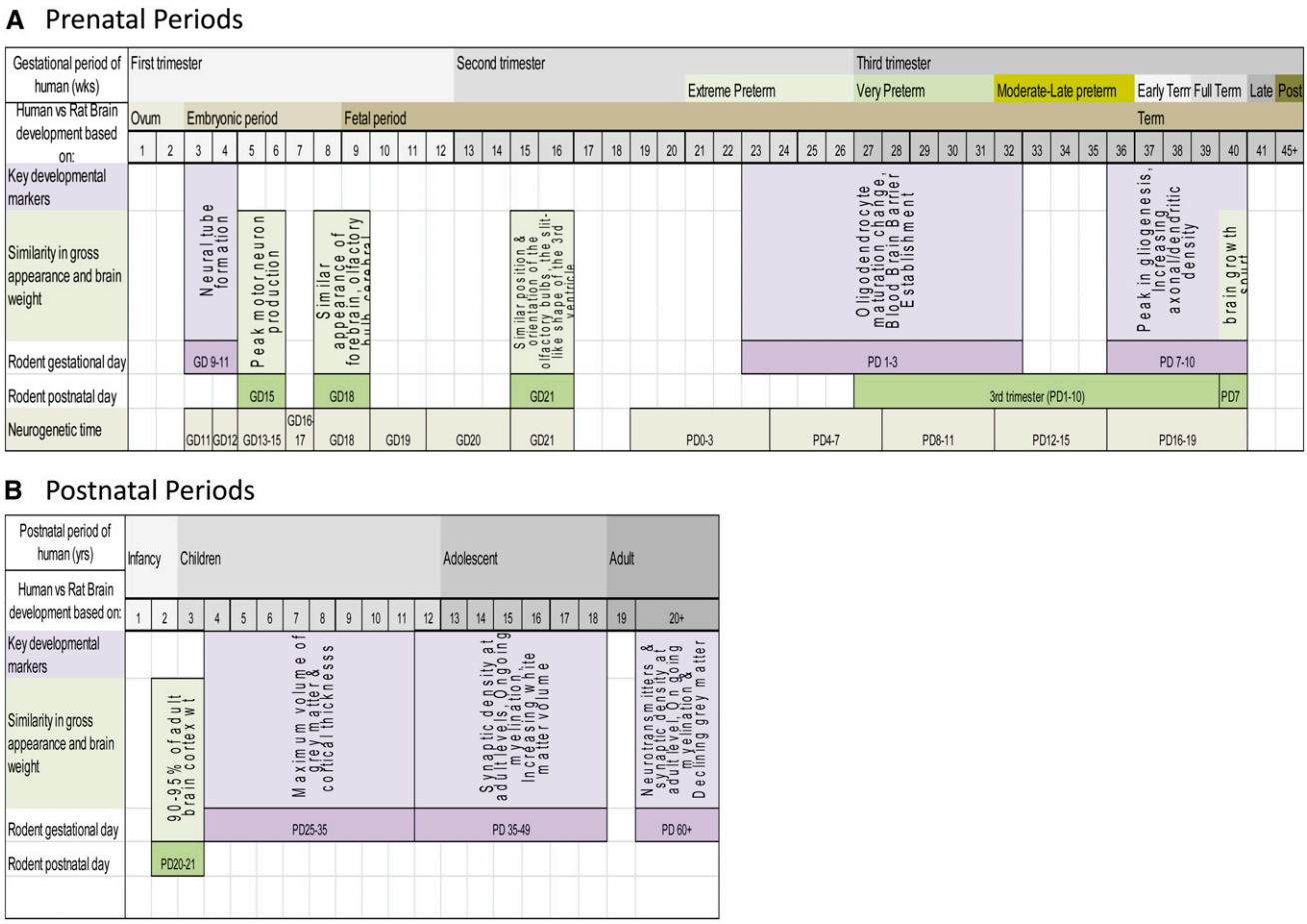

FIGURE 1 Comparison of brain development between humans and rodents during prenatal (*A*) and postnatal (*B*) periods. It is evident that the time scale differs considerably, whereas the sequence of key events in brain development was comparable between humans and rodents. Together with the early neural tube formation, studying a full gestation period in rodents provides some insights into earlier human brain development. GD, gestational day; PD, postnatal day.

and a few trace minerals. Alcohol is metabolized within hepatocytes by 1 of the 3 following pathways:

Alcohol dehydrogenase pathway (ADH): The first pathway, known as ADH, occurs in the cytosol of the hepatocyte (**Fig. 2**). ADH metabolizes ethanol to acetaldehyde, which is subsequently converted into acetic acid in mitochondria (20). In the ADH pathway, ethanol competes with vitamin A, or retinol, for metabolism because both substrates are metabolized by the same pathway (this is discussed later). Ultimately, ethanol is oxidized, which leads to the production of acetaldehyde and large amounts of NADH.

Microsomal ethanol oxidizing system (MEOS): The second pathway, MEOS, occurs in the endoplasmic reticulum (Fig. 2). The MEOS pathway activates cytochrome P450 activity, specifically cytochrome P450 2E1 (CYP2E1), which metabolizes and activates substrates to produce toxic byproducts (21). Chronic alcohol exposure causes increased cellular production of CYP2E1, therefore leading to an increased tolerance to ethanol (21). This pathway inhibits scavenger enzymes, such as glutathione, and causes the production of reactive oxygen species, such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), which results in lipid peroxidation. Therefore, the MEOS pathway has beneficial effects in individuals who consume alcohol acutely, yet also produces harmful effects because chronic alcohol exposure leads to increased enzyme activity, ethanol tolerance, and cellular oxidative stress.

Catalase: The third pathway is through the enzyme catalase (Fig. 2), which is located in the peroxisomes. In this mechanism, ethanol and H_2O_2 are converted to acetaldehyde and water. Oxidation of ethanol by catalase is more common in individuals who consume large amounts of alcohol. These individuals tend to accumulate higher amounts of FAs in the liver, causing hepatic steatosis, which may progress to hepatitis and cirrhosis. With the alcohol-induced fat accumulation, there is increased peroxisomal oxidation of FAs, and this may provide an explanation for the increased catalase activity (22).

Acetaldehyde, a highly toxic compound produced by all 3 pathways, is subsequently converted to acetate by acetaldehyde dehydrogenase (ALDH) in mitochondria and then eventually to carbon dioxide and water (Fig. 2). Evidence

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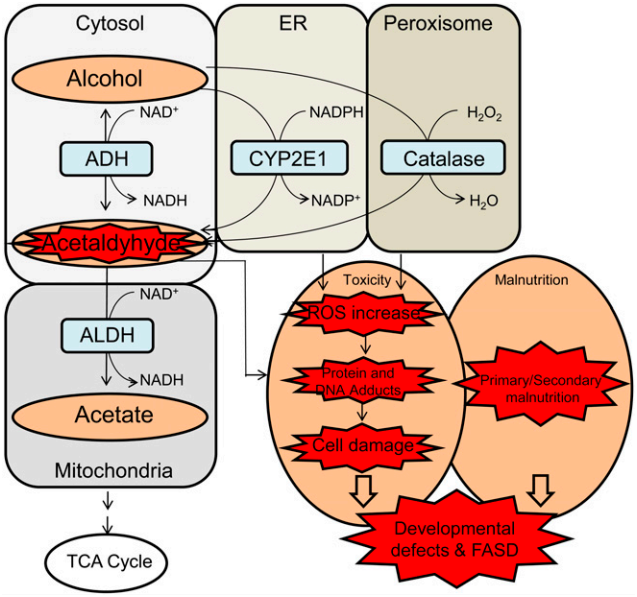

FIGURE 2 Metabolic pathway of alcohol and the toxic effects in vivo leading to developmental defects. Maternal alcohol intake produces direct and indirect consequences on fetal development. ADH, alcohol dehydrogenase; ALDH, acetaldehyde dehydrogenase; CYP2E1, cytochrome P450 2E1 (microsomal ethanol oxidizing system); ER, endoplasmic reticulum; FASD, fetal alcohol spectrum disorder; ROS, reactive oxygen species, TCA, tricarboxylic acid.

shows that when a genetic variation exists in *ALDH2* (e.g., common in Asians), acetaldehyde accumulates in the blood, exerting its harmful effects, which indicates the critical roles of this enzyme in alcohol metabolism. Understanding the polymorphism of this gene in different populations may further elucidate the mechanisms contributing to FASD development. The accumulation of acetaldehyde results in the increased production of free radicals, therefore causing oxidative stress, and contributes to metabolic disorders such as hypoglycemia, hypoproteinemia, and hyperlipidemia. In addition to poor dietary intake, the altered internal metabolic mechanisms work synergistically to further impair nutritional status and damage organs and tissues.

Alcohol effects on fetal development. The extent of alcohol-induced damage inflicted upon the fetus varies due to numerous factors, such as the amount of alcohol consumed, consumption pattern, length of fetal exposure, and specific stage of exposure during fetal development. Maternal alcohol intake produces direct and indirect consequences on fetal development (Fig. 1), as follows:

Direct effects on the fetus: Alcohol readily crosses the placenta and blood-brain barriers and rapidly diffuses into any aqueous compartment of the body, such as the neurons or lipid membranes (16). Exposure to alcohol during fetal development has been reported to reduce up to 12% of total brain weight, defined as microcephaly, due to decreased protein synthesis, which leads to decreased DNA translation (16,23). Both pre- and postnatal exposure of alcohol have shown impairment of the developing neurons in the hippocampus in rat, mouse, and human brains, leading to impaired learning and behavioral and memory function (24–26). Exposure for as little as 1 or 2 d during development causes irreversible brain damage and damage to the CNS due to neuronal death (16), indicating high susceptibility of the brain to alcohol. Impaired neuronal growth and widespread cell death provides an explanation for the sensory and motor deficits characteristic of children with FASD. Although ADH is present in the placenta and metabolizes alcohol when exposed to low amounts, activity in the placenta is much less in comparison to the activity present in the adult liver (~50,000 times less efficient) (27). Therefore, fetal blood alcohol concentrations obtained through the placenta are comparable to those in the mother.

Indirect effects on fetus: Alcohol induces maternal hypoxia, oxidative stress, and altered metabolism, affecting the growth and development of the fetus. It has been suggested that hypoxia is primarily responsible in altering cellular function, which causes the production of numerous morphologic abnormalities in the fetus, some of which are characteristics of FASD (28,29). The placenta is highly sensitive in detecting trace amounts of alcohol, and the umbilical vessels automatically vasoconstrict as a means to protect the developing fetus. In addition to lower oxygen in maternal serum after alcohol consumption, vasoconstriction further reduces oxygen transported across the placenta, which directly affects the hippocampus and cerebellum which are rich in neurons and neurotransmitters (16,27). When the neurotransmitters release glutamate and aspartate in high concentrations, it leads to neuronal damage of the CNS (7). At the cellular level, hypoxia leads to decreased ATP production, impaired sodium-potassium adenosine triphosphatase (Na^+/K^+ ATPase), increased lactate production, swelling of the mitochondria, and inhibition of protein synthesis (7). Specifically as a result of decreased ATP production and oxidative phosphorylation, growth retardation occurs and fetal acidosis may be induced, which has consequences involving fetal brain development and activity (16,30). In addition, decreased blood flow results in the reduced transportation of essential nutrients required for fetal growth.

Maintaining the balance between free radicals and antioxidants is essential in preventing cellular damage. As discussed previously, alcohol metabolism leads to the inevitable production of various free radicals, such as O_2^- and H_2O_2. During chronic alcohol consumption or binge drinking, the free radicals exceed the capacity of the internal antioxidants to neutralize them, resulting in oxidation of lipids. The consequences of lipid peroxidation include changes in membrane fluidity, membrane FAs, and glycolipid and phospholipid profile and decreased activity of certain enzymes, which all affect overall cell function and

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integrity (7). In vitro studies demonstrated that neural crest cells, which lack superoxide dismutase (SOD), are highly susceptible to oxidation when alcohol is consumed (31). Therefore, because neural crest cell death is attenuated with the addition of an antioxidant (vitamin E) or SOD (31), this key aspect may be the cause of the facial abnormalities that are characteristic of children with FAS. Similar to the placenta's ability to metabolize alcohol, fetal cells also contain specific enzymes and antioxidant systems required to neutralize free radicals. However, these mechanisms are less efficient because there are much lower amounts of antioxidants present and fetal cells are more sensitive to alcohol.

Nutrient and metabolic impairment in FASD

From the 1990s, studies have been conducted with aims to determine the importance of individual nutrients regarding their function, metabolism, and relevance to the development of FASD. Nutrients that have the most influence and relevance to neuronal development are vitamin A, choline, DHA (22:6n–3), folic acid, and zinc. Studies regarding vitamin E and selenium, which are important antioxidants, will also be reviewed. These nutrients will be discussed thoroughly in the following sections, and evidence from animal models (summarized in **Table 1**) and some human studies is provided.

It is important to note that the studies used various animal models, such as rodents, guinea pigs, and zebrafish, in this area of research. Because prenatal and postnatal brain maturity differs among species, including humans, direct translation of the study findings to humans is limited. Nevertheless, these models can help reveal the mechanisms of action of the nutrient in reducing alcohol's damaging effects, which may have some relevance to humans. For example, the zebrafish is a good model for studying embryonic development due its transparent embryos and external development, which makes it easy to follow the effects of ethanol or other nutrients. Guinea pigs have a longer gestation period (~65 d) than rodents and complete brain development at birth. Thus, it is easy to identify the prenatal insults, such as ethanol, for the birth defects. Because rodents are more commonly used, the differences in brain development between and human and rodents as a function of age are summarized below in more depth. Although there is no single best animal model mimicking human brain development, results from various species can provide some insights to FASD study. Considering the risk of nutrient toxicity in humans, nutrient supplementation for potential reduction of the negative effects of fetal alcohol exposure will require further, more in-depth study.

Comparisons of human and rodent brain development

Although rodents are the most common animal models to study cellular and molecular mechanisms of brain development, it is always challenging to relate these experimental findings to humans. Bayer et al. (32) compared developing rat brains with human brains on the basis of gross morphology by a detailed comparison of neuroanatomic data. The human embryonic period of 4–5 wk is similar to rat gestational day (GD) 11.5, 6 wk is similar to rat GD 15, the early fetal development period of 8–9 wk is similar to rat GD 18, and 15–16 wk is similar to rat GD 21 (Fig. 1). This study indicated that the morphologic development of the rat brain completed at birth matched the early second semester (~18 wk) of human brain. Neural tube formation occurs in midgestation (GD: 10.5–11) in rodents compared with between 3 and 4 wk during the embryonic period in humans (33,34). Dobbing (35) introduced "rules of thumb" for neural development with rat brain at postnatal day (PD) 1–10 being equivalent to the third trimester in humans, whereas the rat brain growth spurt at PD 7 equaled that of the human brain at birth. On the basis of the neurotransmitter γ-aminobutyric acid (GABA), a similar study by Romijn (36) suggested that PD 2–7 in rat corresponded to the human third trimester. Semple et al. (37) recently summarized the key postnatal developmental processes comparable between humans and rodents. PD 1–3 in rodents corresponded to 23–32 wk in humans. PD 7–10 in rodents corresponded to 36–40 wk in humans, and PD 20 in rodents corresponded to a 2- to 3-y-old human. All of these studies are summarized in Figure 1. It is evident that the time scale differs considerably between humans and rodents, although the sequence of key events in brain development is comparable. Studying the full gestation period of rodents provides insights about earlier human brain development before the second trimester.

Vitamin A

Vitamin A is essential for normal cell differentiation and normal growth and development in animals and humans. The RDA for vitamin A during pregnancy is 750–770 μg/d (38). Foods containing vitamin A precursors, such as β-carotene or retinyl esters, are hydrolyzed to form retinol. Retinol is absorbed and transported to the liver, where it is metabolized within the hepatocyte by retinol dehydrogenase to form retinaldehyde (retinal), which is subsequently converted to retinoic acid (16,20). If retinol is not required for metabolism, it is stored in the liver in the form of retinyl esters. When maternal intake of dietary vitamin A is sufficient, then the fetus receives an adequate supply of retinol required for growth and development.

Alcohol effects on vitamin A. Alcohol consumption during pregnancy depletes maternal vitamin A stores, which can interrupt normal cell growth of the fetus. The proposed mechanism for this is that when both retinol and alcohol are present, ADH involved in the rate-limiting step of retinol oxidation has a higher affinity to alcohol, therefore preferentially metabolizing alcohol instead of retinol. This results in a deficiency in retinoic acid synthesis (39,40), which is required to signal and control the cells involved in fetal development, organogenesis, organ homeostasis, cell and neuronal growth and differentiation, development of the CNS, and limb morphogenesis (16,40). Retinoic acid has 2

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TABLE 1 Studies of specific nutrient supplementation to alleviate prenatal or postnatal ethanol exposure effects[1]

Nutrient and animal model	Diet	Ethanol dose	Exposure period	Nutrient dose	Nutrient exposure period	Key results	Reference
Vitamin A							
Zebrafish embryos	N/A	100 mmol/L	3–24 hpf	1 nmol/L, retinoic acid	Concurrent to ethanol	− Rescued physical anomalies, but not to the control level	(40)
	N/A	100 mmol/L	2–48 hpf	1 nmol/L, retinoic acid	Concurrent to ethanol	+ Reversed small eye and body length defects − Did not reverse heart edema	(46)
Choline							
SD rats	Dams fed unspecified diet	Pups: 5.25 g · kg^{-1} · d^{-1} (bid, q2h) via intubation	PD 4–9	100 mg · kg^{-1} · d^{-1} choline chloride, subcutaneously	PD 11–20, 21–30, or 11–30; measured after PD 45	+ Mitigated deficits in spatial memory	(99)
SD rats	Dams fed feed pellets containing 2.25 g/kg choline chloride	Dams: 6.0 g · kg^{-1} · d^{-1} via intubation	Dam GD 5–20	250 mg · kg^{-1} · d^{-1} choline chloride via intubation	Concurrent to ethanol; measured after PD 45	+ Prevented alterations in behavior and memory − Did not prevent spatial learning task deficits	(98)
SD rats	Dams fed feed pellets containing 2.25 g/kg choline chloride	Pups: 5.25 g · kg^{-1} · d^{-1} (bid, q2h) mixed in the milk diet via intubation	PD 4–9	100 mg · kg^{-1} · d^{-1} choline chloride, subcutaneously	PD 4–30; measured at PD 30–33	+ Attenuated hyperactivity + Mitigated increase in M2/4 receptor density − Did not prevent decrease in muscarinic M1 receptor density in dorsal hippocampus	(101)
Long Evans rats	Dams fed unspecified diet	Pups: 3.0 g · kg^{-1} · d^{-1} via intubation	PD 2–10	100 mg · kg^{-1} · d^{-1} choline chloride subcutaneously	PD 2–20; killed at PD 21	+ Reduced hypermethylation in hippocampus and prefrontal cortex of the brain	(102)
SD rats	Dams fed feed pellets + liquid diet	1.7% to 5.0% v:v habituation period, 6.7% v:v (35% of the total dietary kcal)	GD 1–4 habituation period, higher dose GD 5–birth	642 mg/L choline chloride	GD 11–birth; pups: all group litters fostered from control lactating rats, PD 6—65, used	+ Prevented adverse effects on neurons + Normalized ethanol-altered hypothalamic proteins + Normalized ethanol-altered histone and DNA methylation in POMC neurons	(94)
DHA							
Guinea pigs	Adults fed feed pellets with/without tuna oil	6 g · kg^{-1} · d^{-1} in the diet	14 d before mating + entire pregnancy	0.5 g/d tuna (DHA, 130 mg/d)	14 d before mating + entire pregnancy	+ Recovered DHA concentrations in brain + Reduced high brain PC:PE ratio + Partially ameliorated motor function defects	(59)
SD rats	Dams fed liquid diet with/without DHA vs. feed pellet control	35.5% kcal from ethanol in liquid	GD 1–21	34.2% n–3 FAs (24.6% DHA) as food	Dams: GD 21–PD 22; pups: PD 22–60	+ Increased glutathione concentrations + Decreased lipid peroxidation + Reduced oxidative stress + Enhanced antioxidant capacity	(62)

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TABLE 1 (*Continued*)

Nutrient and animal model	Diet	Ethanol dose	Exposure period	Nutrient dose	Nutrient exposure period	Key results	Reference
Folic acid							
Guinea pigs	Sows fed Guinea pig feed pellets	4 g · kg^{-1} · d^{-1} via intubation	GD 2–65 (5 d treatment + 2 d no treatment per week)	2 mg/kg maternal wt via intubation	Dams: concurrent to ethanol, measured in fetus	− Did not prevent decrease in fetal folate concentrations + Prevented decrease in fetal hepatic folate levels	(71)
C57Bl/6 mice	Unspecified feed pellets with/without folate	2.9, 3.5, or 4.5 g · kg^{-1} · d^{-1}, bid, 1 d, i.p.	GD 6.75	10.5 mg/kg maternal wt	GD 0.5–15.5	+ Prevented myocardial wall changes and semilunar and atrioventricular valve defects	(73)
	Unspecified feed pellets with/without folate	2.9, 3.5, or 4.5 g · kg^{-1} · d^{-1}, bid, 1 d, i.p.	GD 6.75	10.5 mg/kg maternal wt	GD 0.5–15.5	+ Restored normal placentation/embryogenesis + Prevented alcohol-induced intrauterine growth restriction	(74)
Wistar rats	Dams fed feed pellets + water vs. ethanol	5.5, 7.8, and 8.9 g · kg^{-1} · d^{-1} for and 16.6 g · kg^{-1} · d^{-1} in tap water	Low doses, GD 1–21; high doses, GD 21–PD 21	152 µg/d	Entire pregnancy and lactation period	+ Reduced glutathione peroxidase activity and production of TBARS + Mitigated ethanol-mediated oxidative stress	(72)
Zebrafish embryos	N/A	100 mmol/L	2–48 hpf	75 µmol/L	Concurrent to ethanol	+ Rescued cardiac edema, small eye, and body defects	(46)
Zinc							
Mice	Dams fed feed pellets	2.9 g/kg (0.015 mL/g) bid, 1 d, i.p.	GD 8 at 0 and 4 h	2.5 µg Zn/g subcutaneously	GD8; killed at GD 18	+ Reduced physical abnormalities	(86)
C56Bl/6j mice	Dams fed AIN-93G dasein-based control diet	2.9 g/kg (0.015 mL/g) bid, 1 d, i.p.	GD 8 at 0 and 4 h	200 mg/kg in the diet	GD 0–18; measured at GD 18, PD 0, PD 60	− No significant differences in physical anomalies + Decreased cumulative mortality	(81)
SD rats	Liever-DeCarli liquid diet	5% ethanol, average 70 mL liquid diet/d	GD 2–20	5, 10 or 40 mg/L liquid diet	GD 2–20; GD 20 Zn transport measured	− No significant effect in zinc placental uptake	(85)
SD rats	Pups fed liquid formula (diet), artificial rearing system	4.5 g · kg^{-1} · d^{-1}	PD 4–9	0.54 g/L liquid diet	Concurrent to ethanol; PD 10 measured	− Did not attenuate ethanol induced Purkinje cell loss	(80)
Vitamin E							
Rats	Pups fed milk-based liquid diet	12% ethanol bid by intubation	PD 4 and PD 5	301 and 601 IU/100 mL liquid diet	Concurrent to ethanol; measured on PD 5	+ Reduced Purkinje cell loss + High dose completely prevented Purkinje cell damage and loss	(106)

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TABLE 1 (*Continued*)

Nutrient and animal model	Diet	Ethanol dose	Exposure period	Nutrient dose	Nutrient exposure period	Key results	Reference
Vitamin E and β-carotene							
Long Evans rat fetus hippocampal cultures	N/A	4, 8, 16, 18, 20, and 24 g/L in vitamin E experiments; 4, 16, and 20 g/L in β-carotene experiments	24 h	50 μmol/L	Concurrent to ethanol	+ Ameliorated hippocampal cell loss at low ethanol + Reduced cell loss at high ethanol	(47)
Selenium and folic acid							
Wistar rats	Dams fed basal diet (not specified) for 8 wk before pregnancy + food and water ad libitum	5% week 1, 10% week 2, 15% week 3, 20% from 8 wk before pregnancy until end of the lactation period	14 wk	8 μg/g of folic acid and 0.5 μg/g selenium	Concurrent to ethanol; measured pups at PD 21	+ Reduced selenium loss + Improved balance among oxidative enzymes	(109)
	Dams fed basal diet (not specified) for 8 wk before pregnancy + food and water ad libitum	5% week 1, 10% week 2, 15% week 3, 20% until end of the lactation period	14 wk	8 μg/g folic acid and/or 0.5 μg/g selenium	Concurrent to ethanol; measured pups at PD 21	+ Improved transport affinity for selenium-methionine absorption substrates + Minimized damage in the duodenal mucosa	(110)

[1] bid, twice a day; GD, gestational day; hpf, hours postfertilization; N/A, not applicable; PC, phosphatidylcholine; PD, postnatal day; PE, phosphatidylethanolamine; POMC, proopiomelanocortin; q2h, every 2 h; SD, Sprague-Dawley; +, results that reduced or alleviated ethanol exposure effects; −, results that did not reduce or alleviate ethanol exposure effects.

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classes of receptors: retinoic acid receptors (RARs) and retinoid X receptors (RXRs) (20,41). Binding of retinoic acid to the receptors is essential because it allows the transcription of genes that are required in regulating cell and neuronal differentiation for the limbs, brain, and nervous system to be transcribed (40). Kumar et al. (41) first demonstrated that in high concentrations of ethanol exposure in utero, RAR expression and its DNA-binding activity in the cerebellum of rat pups was decreased whereas RXR expression was increased, indicating that alcohol-induced disturbed cell differentiation exists in cerebella. The results indicate that it is essential to provide the fetus with an adequate supply of retinoic acid throughout pregnancy (41). A review by Ballard et al. (42) further supports that the retinoic acid–mediated pathway could be a major mechanism of the visible expression of FASD because of alcohol's role in inhibiting retinoic acid production in parts of the brain. When retinol is not oxidized, it will accumulate in nonhepatic tissues, such as in the kidney and the lungs (20). It has been suggested that the morphologic defects commonly seen in individuals with deficiency may also be due to an excess amount of retinol accumulating in nonhepatic tissues (16). Maintaining a balance of retinol and retinol metabolism is essential for proper growth and development, yet is disturbed due to maternal consumption of alcohol.

Vitamin A supplementation in animal and other models. With the use of *Xenopus laevis* frog embryos, Yelin et al. (43) found a high incidence of malformations such as microcephaly and microphthalia in embryos exposed to ethanol, which resembled the phenotypic anomalies induced in individuals affected by FASD. Yelin et al. (44) later provided further evidence for early molecular changes induced by exposure to ethanol, making this a useful model system for studying FASD. Kot-Leibovich and Fainsod (45) subsequently demonstrated biochemical evidence for the competition of alcohol for retinaldehyde dehydrogenase activity, which limited the formation of retinoic acid and the onset of retinoic acid signaling during gastrulation. Marrs et al. (40) conducted a unique study to examine in utero effects of retinoic acid supplementation (1 nmol/L); zebrafish exposed to 100 mmol/L ethanol, an amount correlated with the pathophysiologic concentration required to induce developmental changes in individuals with FASD, showed morphologic defects such as craniofacial cartilage formation, ear development, and early gastrulation cellular movements (anterior-posterior axis) (40) (Table 1). Retinoic acid supplementation corrected a few dysmorphologic signs compared with the ethanol-exposed group, but the rescued phenotype was not compatible to the control, which had not been exposed to either ethanol or retinoic acid (40). This indicates that retinoic acid can only partially rescue ethanol-induced physical defects. A more recent study tested specifically if retinoic acid could reverse ethanol-induced cardiac defects in zebrafish (46). Zebrafish embryos were exposed to 2 different concentrations of ethanol: 100 mmol/L (0.6% v:v) or 150 mmol/L (0.9% v:v) and cotreated with either folic acid or retinoic acid. During cardiogenesis, cosupplementation of 1 nmol/L retinoic acid with ethanol reversed small eye and body length defects but could not rescue heart edema (Table 1). The authors suggested that the findings of this study provide evidence that retinoic acid supplementation could restore ethanol-induced defects of gene expression during specification, heart cone formation, and cardiac looping but could not restore endocardial cushion formation defects. They had more promising results by using folic acid supplementation (75 μmol/L), which significantly prevented cardiac defects (Table 1) (46). Excess retinoic acid consumption during fetal development has been reported to produce numerous fetal anomalies that are similar to deficiency symptoms (20), indicating a narrow safe range of this nutrient. Although the data from fish and amphibians cannot be directly translated to humans, the findings suggest the importance of retinoic acid in FASD-normalizing cell formation. Further research is required to determine a defined amount of retinoic acid that can be consumed to alleviate alcohol-induced effects without producing other negative side effects. In other studies, β-carotene, the plant-based precursor of vitamin A, was studied to determine its effects as an antioxidant in alleviating free radical damage induced by ethanol in hippocampal cells of fetal rats (47). This study observed that β-carotene completely normalized free radical cellular damage in the hippocampus of fetal rats exposed to low amounts of ethanol 4g/L (Table 1).

Although both studies showed beneficial effects with vitamin A supplementation, they failed to consider the consequences of consuming excess vitamin A during pregnancy. Carefully designed dietary intervention studies are needed to investigate and determine the specific amount of vitamin A required in FASD because excess amounts of vitamin A produce morphologic defects.

Vitamin A supplementation in human subjects. Although there is limited research on vitamin A supplementation in pregnant human subjects who consume alcohol, 1 case study suggests that there could be a connection between maternal alcohol consumption, fetal vitamin A status after birth, and hydrocephalus (48). This is plausible because there is a well-documented connection between alcohol consumption and hydrocephalus, a buildup of the cerebrospinal fluid in the brain (49–52) and knowledge that vitamin A interacts with ethanol and affects brain development. The authors recommend further research and potential vitamin A supplementation for newborns whose mothers consumed alcohol during pregnancy (48).

DHA

DHA is highly important during fetal development because it plays an essential role in cognitive and visual development, as well as the development of the CNS (53,54). DHA is also a precursor of a potent neurotrophic factor (neuroprotectin D1), which protects the brain and retina against injury-induced oxidative stress and enhances cell survivals in these

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tissues. Thus, it is recognized as a conditionally essential nutrient for infants. There is no RDA for DHA, but the Adequate Intake (AI) for n–3 FAs for pregnancy is 1.4 g/d (55). DHA is esterified to membrane phospholipids to maintain optimal fluidity and cellular integrity. Among phospholipids, phosphatidylserine has been the most studied in association with CNS development (54,56,57). Optimal neuronal development of the fetus is dependent on maternal intake and dietary status of DHA. In humans, the accumulation and integration of DHA into phosphatidylserine and cell membranes occurs from 16 wk to term and continues into the early postnatal development period (53). It is specifically during the last trimester in which DHA is rapidly incorporated into phosphatidylserine synthesis and storage in the hippocampus, because it is during this period in which human brain growth rapidly occurs (57,58). Also, it is critical that maternal dietary intake and storage of DHA is sufficient, because this increase in DHA accumulation into the CNS occurs over this specific and limited period of development (59). Overall, deficiency impairs phosphatidylserine synthesis, leading to decreased neuronal development and survival, ultimately resulting in impaired cognitive and overall CNS development.

Alcohol effects on DHA. Maternal alcohol consumption during pregnancy has a significant effect on the DHA status of the developing fetus. Not only does alcohol decrease maternal intake of n–3 FA–rich foods, it also decreases maternal DHA status and reduces placental transfer of DHA to the fetus (58). Because of the decreased maternal stores and increased oxidation, brain DHA content, measured by amounts of total phosphatidylserine, is significantly reduced (15–20%) (40). In addition, alcohol promotes and influences apoptosis and neuronal cell death, which further impairs the growth, development, and survival of existing neuronal cells in the hippocampus (57,60). Fetal hippocampal cultures obtained from pregnant rats exposed to ethanol contained higher numbers of apoptotic cells when compared with the control group due to decreased DHA-dependent phosphatidylserine accumulation, because DHA provides a protective effect in preventing apoptosis (60). Therefore, it could be postulated that a fetus with insufficient DHA status in utero could be born with some form of behavioral, memory, attention, or learning impairment, as well as decreased visual acuity and fine motor function. Although there are some contrary results showing either no differences or rather increased DHA concentrations in umbilical cord serum exposed to alcohol (61), it is clear from the literature that chronic alcohol consumption reduces the availability and transportation of dietary DHA to the fetus. With this knowledge, intervention studies are needed to determine how these damaging effects may be alleviated or reduced with DHA supplementation.

DHA supplementation in animal models. Burdge (59) conducted a study on the effects of DHA-enriched tuna oil and phospholipid concentrations (phosphatidylcholine and phosphatidylethanolamine) in the brain of fetal guinea pigs exposed to ethanol during development. DHA concentrations were significantly reduced (57%) in the group exposed to alcohol (59). In the brain of fetuses exposed to both ethanol and tuna oil (130 mg DHA/d), DHA concentrations not only increased but were comparable to the control group (Table 1) (59). However, supplementation with the tuna oil alone had no effect in increasing brain DHA concentrations. This finding is important because providing increased concentrations of DHA may correct the damaging effects of alcohol. Determining an optimal concentration of DHA to consume during pregnancy may be difficult because the amount of DHA consumed would correlate to the amount of alcohol consumed. A more recent study was conducted to assess the effects of n–3 FA (including DHA) supplementation on glutathione concentrations and lipid peroxidation in rats (62). The study revealed that prenatal ethanol exposure caused a decrease in glutathione concentrations in the brain and an increase in lipid peroxidation, leading to oxidative stress. The n–3 FA–supplemented group (34.2% n–3 FAs including 24.6% DHA) showed an increase in glutathione concentrations and decrease in lipid peroxidation, thereby partially reversing the negative effects of prenatal ethanol exposure (Table 1) (62).This study suggests that n–3 FA supplementation could reduce oxidative stress and enhance antioxidant capacity in fetuses exposed to ethanol.

DHA supplementation in human subjects. There are no known studies on DHA supplementation in pregnant mothers who consume alcohol. Research regarding dietary DHA and its precursor, α-linolenic acid (18:3n–3) must be directed to determine the effects of these essential FAs in improving the mental, visual, and motor function of infants born with FASD. As presented in the literature, postnatal DHA supplementation has positive effects on mental and visual development; therefore, further research should be aimed at understanding the potential therapeutic effects of DHA on infants affected by alcohol. Currently, the RDA from all sources of n–3 FAs in pregnancy is 1.3 g/d (55). Perhaps providing increased amounts of essential n–3 FAs, such as DHA, for infants affected by alcohol would be beneficial in that it would help to replenish low DHA stores in the brain, because maximum brain development occurs from the last trimester until the first 3 mo of life (63). Similar to in utero DHA supplementation, research regarding a specified amount of DHA that would reduce the alcohol-related effects present in an infant is required. Such information is essential because potentially life-long consequences regarding infant growth and development could be corrected and decreased, therefore allowing the individual with FASD to grow and develop in more normalized conditions.

Folate (folic acid)

Folic acid, a water-soluble vitamin, has been identified as an essential nutrient that may provide a protective effect against

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gestational ethanol exposure. For folic acid to become metabolically active, it must be reduced to tetrahydrofolic acid (FH_4) as a carrier for single-carbon moieties. FH_4 is involved in the biosynthesis of the DNA and RNA precursors thymidylate and purine bases (64). Therefore, adequate maternal folic acid status is integral for optimal fetal growth and development. During pregnancy, the demand for folic acid is increased because it is not only required to support the mother for increased RBC formation but also to support the rapid growth of the fetus, including neural tube formation (65). The RDA for folic acid during pregnancy is 600 μg/d (66), and dietary sources are found in green leafy vegetables, beef, liver, pulses, and foods produced from whole wheat. Folic acid deficiency is a common occurrence in pregnant women who do not consume adequate dietary folic acid or supplements, which increases a higher chance of an infant born with neural tube defects, a set of congenital malformations in the brain and spine.

Alcohol effects on folate. Halsted et al. (67) reported that jejunal folate absorption is reduced to <20% in acute and chronic alcoholics, therefore resulting in deficiency. Under deficiency states, DNA and RNA synthesis is altered because of incorrect nucleotide incorporation, therefore causing instability and cellular apoptosis (65). Deficiency is also attributed to reduced hepatic folic acid storage and increased catabolism and urinary excretion. It is critical that blood folic acid concentrations be maintained at a constant level, because placental delivery and transfer to the fetus are dependent on plasma folic acid concentrations. When these concentrations are not maintained and intake is displaced by alcohol, deficiency-related birth defects, such as facial malformations, neural tube defects, cardiac dysfunction, and neurological symptoms such as microencephaly, are present in the child (65).

Folate supplementation in animal models. Intervention studies in mice have shown positive effects of folic acid supplementation in reducing cellular apoptosis and increasing cell proliferation and replication in fetal mice forebrain (68). Several other animal studies also produced similar results (69,70). However, very few studied the combined effects of folic acid supplementation and alcohol consumption on fetal development. Hewitt et al. (71) specifically studied the effects of chronic ethanol exposure and folic acid supplementation regarding folic acid status by using a maternal and fetal guinea pig model. The results showed that folic acid supplementation ($2\ mg \cdot kg^{-1} \cdot d^{-1}$) did not alleviate the typical effects seen with chronic ethanol exposure, such as a decrease in folic acid concentrations in the brain and hippocampus (71). However, maternal folate supplementation prevented a decrease in hepatic folic acid concentrations observed in both the mothers and fetuses (Table 1) (71). Oxidative stress can cause the development of neuronal defects; therefore, Cano et al. (72) conducted a study to investigate the protective effects of folic acid supplementation in the offspring of pregnant rats exposed to ethanol. Folic acid supplementation (152 μg/d) reduced oxidative stress and the formation of TBARS in the livers of the group consuming ethanol and folic acid (Table 1). Serrano et al. (73) also showed that folic acid (10.5 mg/kg maternal body weight) prevents the development of cardiac dysfunction in fetal mice exposed to ethanol in utero (Table 1). Another recent study found that folate supplementation (10.5 mg/kg maternal body weight) was able to restore normal placentation/embryogenesis and prevent alcohol-induced intrauterine growth restriction during pregnancy in mice (74). With regard to methylation, Downing et al. (75) found that placing dams on a methyl-supplemented diet (containing choline, betaine, folic acid, vitamin B-12, L-methionine, and zinc) before pregnancy and throughout gestation restored methylation concentrations after exposure to alcohol. In addition, methyl supplementation decreased prenatal mortality, increased prenatal growth, and decreased digit malformations. The authors summarized that the methyl-supplemented diet partially ameliorated ethanol teratogenesis in dams. Future studies should concentrate on determining an optimal supplementation value in animals that could help reduce or lessen the damaging effects of alcohol in the developing fetus or child. Once these preliminary studies are successful in animals, then the results could potentially be used to treat cases in which the presence of FASD is detected and in children who are born with FASD.

Folate supplementation in human subjects and observational studies. There are limited studies on folate supplementation related to prenatal alcohol consumption in human subjects. However, some studies provided valuable insight into folate supplementation in humans during pregnancy. A recent study looked at the effect of maternal diet on DNA methylation in maternal and cord blood (76). The authors looked specifically at methyl donor nutrients—vitamin B-12, betaine, choline, and folate—and found that intake of these nutrients did not increase DNA methylation in either the first or second trimester or in cord blood. The authors suggested various reasons for this including that most of the women studied had sufficient folate concentrations during pregnancy, especially by the second trimester, and that there is a possibility that there is a threshold of the effect of consuming methyl donor nutrients on methylation concentrations (76). Furthermore, Ballard et al. (42) recommended that folate be investigated further with regard to prevention of FASD in humans given its role in DNA methylation. They suggest that there could be a connection between folate deficiency and epigenetic changes that can occur with alcohol exposure, especially because poverty is a common risk factor for folate deficiency and FASD. In addition, recent studies have shown that folic acid consumption (from fruits and vegetables) and supplementation can reduce the risk of orofacial clefts (77) and gastroschisis (78), both of which are associated with prenatal alcohol consumption but not specifically FASD.

Zinc

Zinc plays an important role in the development of FASD because it is a mineral that is required for DNA and RNA

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stability and the activity of RNA polymerases, which are important for cell division (79). Zinc is an important cofactor for the synthesis of numerous enzymes, such as the antioxidative enzyme SOD, which prevents oxidation-induced cellular apoptosis in the brain (80). Because zinc is an important contributor to growth, the RDA for pregnant women is 11–12 mg/d (38). Red meats are an excellent source of dietary zinc, yet absorption is compromised when foods containing phytates are consumed. Zinc is absorbed in the lumen of the small intestine by both passive and active transport mechanisms, where it then enters portal circulation and is distributed to the liver and other body tissues. It is critical that maternal dietary intake of zinc is adequate during fetal development, because restricted overall body weight and brain growth have been reported to occur, resulting in poor cognitive function and delayed motor response (16).

Alcohol effects on zinc. Alcohol consumption on a chronic basis itself reduces the availability of zinc because there is decreased intake and absorption and increased urinary excretion. When acute zinc deficiency occurs as a result of ethanol exposure, metallothionein, a low-molecular-weight protein body, sequesters plasma zinc to the liver, resulting in a reduction in plasma zinc. This leads to decreased amounts available for placental transport, resulting in fetal zinc deficiency (81,82). For an in-depth review of metabolic alterations of zinc deficiency, see Keen et al. (79). Scholl et al. (83) reported that low dietary zinc intake during pregnancy increased the risk of delivering a low-birth-weight child and that low intake was also associated with a greater risk of preterm delivery. In pregnant women who consume alcohol, maternal plasma and fetal cord blood concentrations were significantly reduced in comparison to a non–alcohol-consuming group (84). Furthermore, low maternal and fetal plasma zinc concentrations were correlated with an increased risk of fetal dysmorphogenesis (84).

Zinc supplementation in animal models. Alcohol-induced deficiency and supplementation studies cannot be conducted in humans, and animal studies have yielded conflicting results. Pregnant rats fed an ethanol diet supplemented with 5, 10, or 40 mg zinc/L did not exhibit an increase in placental uptake and transport of zinc to the fetus (Table 1) (85). Ethanol-induced Purkinje cell loss in the fetal rat brain was not improved with maternal zinc supplementation (Table 1) (80). However, evidence does exist that that some zinc supplementation has a positive effect in the reduction in alcohol-related birth defects. When pregnant mice were injected with saline or 25% ethanol on the eighth day of gestation and were fed either a control zinc diet (35 mg/kg) or a zinc-supplemented diet (200 mg/kg), fetal mice from the ethanol treatment group had a significantly greater incidence of birth defects (26%) in comparison to the ethanol- and zinc-supplemented group (12%) (Table 1) (81). Abnormalities between the ethanol- and zinc-supplemented group in comparison to the saline groups did not significantly differ. Also, postnatal mortality was significantly higher in the ethanol treatment group in comparison to the saline and ethanol- and zinc-supplemented group, with a significant difference of 15%. The authors proposed that zinc supplementation altered maternal zinc homeostasis, because supplementation allowed greater amounts of zinc to remain in the plasma without being sequestered to metallothionein (81). Greater concentrations of plasma zinc contribute to higher concentrations of SOD in the plasma, which effectively scavenges oxidative products and free radicals produced from alcohol metabolism. In another study, the incidence of physical abnormalities in fetal mice from the zinc-supplemented ethanol group (250 μg zinc/mL of liquid diet) was significantly lower than in those exposed to ethanol alone in utero (Table 1) (86). From the evidence, it is difficult to conclude whether maternal zinc supplementation is protective against alcohol consumption during pregnancy. However, in all of the studies, even those that were inconclusive, there was no harm in zinc supplementation during pregnancy. Although it is not advised, pregnant women who do choose to continue and consume alcohol during pregnancy should maintain an adequate dietary intake of zinc to prevent malformation and development of the fetus.

Zinc supplementation in human subjects. Various human studies have been conducted to determine whether daily zinc supplementation (20–44 mg/d) from 20 wk of gestation until birth produces positive effects in children born to zinc-supplemented mothers (87,88). Plasma concentrations and anthropometric measures, such as birth weight and head circumference, were not significantly different in children born to mothers who consumed the supplements and those that consumed the placebo. However, in another study, zinc supplementation produced significant differences for these same outcomes when supplementation began at 19 wk of gestation (89), indicating earlier intervention may produce greater benefits. Given that zinc supplementation showed promising results in animal studies and that zinc deficiency in pregnancy is fairly common, zinc supplementation has been suggested as a possible protective factor for FASD in humans (79). However, no human studies have been conducted to prove the benefits of prenatal zinc supplementation on the severity of FASD.

Choline

Choline and its metabolites are invaluable in neurotransmission (acetylcholine), structural integrity of cell plasma membranes (phosphatidylcholine and sphingomyelin), and cell signaling and in folate-independent pathways as a methyl donor via its metabolite, betaine (42,90). This nutrient is the most-studied nutrient related to brain development and memory function and has been classified as an essential nutrient by the Institute of Medicine and National Academy of Sciences in the United States (66). The AI for choline during pregnancy is 450 mg/d (66). Although choline is found in many foods, the highest concentration of choline is found in eggs and organ meats such as liver. During pregnancy, women have an increased ability to form phosphatidylcholine, a source of choline, in the liver, because estrogen

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induces the process. Therefore, more choline can be delivered to the fetus via the placenta. Thus, it is clear that choline must play an important role in development (91). Animal and human studies have given further insight into the potential effects of choline, specifically with prenatal ethanol exposure.

Alcohol effects on choline. If alcohol is consumed, the ethanol competes with water for the phospholipase D–catalyzed reaction of phosphatidylcholine, and this affects protein expression and cell signaling (90,92,93). Choline-related mechanisms have been suggested to be a major part of the molecular etiology of FASD (91). Alcohol alters one-carbon metabolism, which causes less folate but more choline to be used. Furthermore, a low-choline diet or a genetic variation that increases the dietary demand for choline can decrease DNA methylation, increasing gene transcription and changing cell proliferation and differentiation, which can result in birth defects and abnormal brain development. Therefore, if alcohol creates an extra need for choline and choline deficiency causes abnormal development, it is possible that choline-related mechanisms could explain how prenatal ethanol exposure causes FASD (91). This promising area of research requires further study and careful consideration of the intricate balance of methylation mechanisms.

Choline supplementation in animal models. A recent study looked at the effect of choline supplementation on specific neurons that are altered in FASD (94). Pregnant rat dams were fed an alcohol-containing liquid diet or a control diet during GDs 7 and 21 with or without choline (642 mg/L choline chloride). The results showed that gestational choline supplementation prevented the adverse effects of alcohol on the neurons (Table 1) (94). Previous research from Thomas and colleagues (95–99) showed that perinatal choline supplementation can reduce the severity of FASD—specifically, hyperactivity and learning deficits in the rat model. The authors found that choline chloride supplementation (250 mg $\cdot$ kg^{-1} $\cdot$ d^{-1} choline chloride) prevented ethanol-induced alterations in tasks that require behavioral flexibility such as spontaneous alternation behavior and memory (Table 1) (98). The authors also showed that choline provision during the early postnatal period could reduce the behavioral effects of alcohol, specifically those that rely on the functional integrity of the hippocampus (95,99,100). Specifically, Ryan et al. (95) showed that 100 mg $\cdot$ kg^{-1} $\cdot$ d^{-1} choline chloride mitigated deficits in spatial memory (Table 1). A more recent study examined the effects of developmental alcohol exposure and perinatal choline supplementation on hippocampal M1 and M2/4 muscarinic receptors in rats, which would indicate alcohol-related behavioral affects (101). Choline supplementation (100 mg $\cdot$ kg^{-1} $\cdot$ d^{-1} choline chloride) reduced hyperactivity, did not have an effect on the muscarinic M1 receptors, but had an effect on M2/4 receptors (Table 1). The authors concluded that choline supplementation could reduce the alcohol-related behavioral changes due to developmental alcohol exposure that cause long-lasting changes in the hippocampal cholinergic system (101). Furthermore, another recent study found that choline supplementation (100 mg $\cdot$ kg^{-1} $\cdot$ d^{-1} choline chloride) reduced the hypermethylation caused by ethanol exposure in the hippocampus and prefrontal cortex of the brain (Table 1) (102).

These studies show promising results that prenatal choline supplementation could reduce some of the detrimental effects of alcohol consumption on the fetus. They also provide information on the specific mechanisms in which choline could be involved. However, further research should be undertaken on any adverse effects of choline supplementation, before it is used as a treatment for FASD in humans.

Choline supplementation in human subjects and observational studies. There is limited research on choline supplementation in human subjects who consume alcohol prenatally. However, a very recent study looked at whether choline intake for 12 wk, 480 or 930 mg/d with a daily DHA supplementation, affects indicators of choline-related lipid metabolism and phosphatidylcholine-DHA and phosphatidylcholine:phosphatidylethanolamine ratios in erythrocyte and plasma of pregnant and nonpregnant women. Choline and DHA intake increased the proportion of phosphatidylcholine-DHA in pregnant and nonpregnant women but did not affect the ratio of phosphatidylcholine:phosphatidylethanolamine (103). The authors concluded that increased phosphatidylcholine-DHA during pregnancy indicates an elevated phosphatidylethanolamine N-methyltransferase (PEMT) pathway using choline as a methyl donor. This study provides insight into the mechanisms of choline supplementation with ethanol exposure. An observational study examined the influence of choline during pregnancy and cognitive development of children at age 7 y (104). Choline intake among pregnant mothers was mildly associated with better child memory and nonverbal score at age 7 (104). It is not known whether similar effects can be found in children from mothers who consume alcohol during pregnancy.

Antioxidants

Antioxidants are compounds that are produced to scavenge free radicals and other compounds that threaten cellular oxidation. Cells can neutralize and scavenge reactive oxygen species through the enzymatic activity of SOD, glutathione peroxidase (GPx), and catalase. Nutrients such as folate, vitamin C (ascorbic acid), vitamin E (α-tocopherol), selenium, and zinc are important contributors to antioxidant activity. A detailed explanation regarding alcohol-induced oxidative stress was discussed previously. This section will focus on the nutrients mentioned above and their role in reducing oxidative damage caused by alcohol.

Vitamin E (α-tocopherol). Vitamin E, a fat-soluble vitamin known for its ability to prevent lipid peroxidation in the cell membrane, donates a single electron that converts the free radical into a stable form. Nuts and oils serve as rich sources of vitamin E. During pregnancy, an optimal amount of

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15 mg/d should be consumed to ensure adequate amounts for both the mother and the developing child (105).

Vitamin E supplementation in animal models. In vitro studies have shown that vitamin E supplementation is capable of ameliorating alcohol-induced oxidative effects at low levels of alcohol exposure in fetal rat hippocampal cultures (47). At higher concentrations (50 μmol/L), vitamin E increased the viability and survival when supplemented at various ethanol exposure concentrations, which ranged from 16 to 24 g/L (Table 1) (47). This finding is highly relevant to saving brain neural cells in FASD; however, it would be interesting to see if similar findings occurred in vivo.

Heaton et al. (106) investigated whether vitamin E supplementation would decrease Purkinje cell loss in neonatal rat pups fed a liquid diet containing vitamin E and ethanol. It is during the first postnatal week that the cerebellum is greatly affected by the presence of ethanol. Rat pups were fed a specified diet, composed of milk, milk and vitamin E, maltose dextrin, 12% ethanol, or 12% ethanol with vitamin E supplementation (301 and 601 IU) (106). It was observed that ethanol significantly reduced the number of Purkinje cells in the neonatal rat pups exposed to ethanol, whereas rat pups fed the diet containing ethanol plus 601 IU of vitamin E did not have this dramatic loss (Table 1) (106). The results of this study suggest that supplementation with high amounts of vitamin E are effective at preventing neuronal loss in the brain. Because the rat pups were killed on PD 5, the long-term effects of vitamin E supplementation and the effects on the CNS are unknown. The study may be more translatable to humans if pups were exposed to ethanol through maternal diet. Although these preliminary findings suggest the benefits of vitamin E supplementation, further research must be conducted to determine the long-term effects of supplementation, how other regions of the CNS benefit from supplementation, and how these findings can be used to prevent CNS alterations in utero and to correct or decrease the FAS effects typically seen in individuals exposed to alcohol.

Vitamin E supplementation in human subjects. There have been no studies on vitamin E supplementation in humans specifically relating to prenatal alcohol exposure and the potential mitigation of FASD to date.

Selenium. Selenium is a micronutrient that serves as an important component for the generation of the enzyme GPx. GPx inhibits oxidation because it is involved in scavenging free radicals, specifically hydrogen peroxide, and converting them to harmless products such as water. Selenium-based GPx primarily is active within the cytosol or the mitochondria. The amount of selenium obtained from the diet is based on the amount in the soil or water where the food source was grown. Once consumed, it is predominantly stored in the liver, because alcohol metabolism in the liver produces various reactive oxygen species and free radicals. The RDA for selenium during pregnancy is 60 μg/d (105).

Alcohol effects on selenium. Typically, selenium deposits and plasma concentratons are low in chronic alcoholics because of decreased dietary intake and increased production of free radicals resulting from alcohol metabolism (107). However, selenium concentrations in the plasma were reported to be increased and were significantly greater in women who drank heavily, defined as >140 g/wk, during their pregnancy in comparison to abstinent women and those who consumed alcohol moderately (108). In animal studies, it was shown that chronic ethanol consumption alters the overall selenium balance, yet does not produce significant differences in serum selenium concentrations (106). However, in the offspring of alcohol-consuming rat dams, serum selenium is significantly increased in comparison to control offspring. In rat dams with adequate selenium intake, GPx concentrations are maintained because there is sufficient selenium to maintain enzyme activity. In circumstances in which selenium intake is inadequate, the presence of alcohol promotes the depletion of hepatic selenium and sequesters it into the plasma as a means of maintaining high GPx activity to prevent oxidation (107,109). As a result, there is decreased maternal hepatic storage of selenium; therefore, less is available to be transported to the developing rat pup. In alcohol-exposed conditions, selenium transported to the developing pup is preferentially used for serum GPx activity and not stored in the liver, which explains why alcohol-exposed offspring have elevated GPx in the serum.

Selenium supplementation in animal models. In another study performed by Ojeda et al. (109), the effects of selenium supplementation (0.5 μg/g) on antioxidant activity with alcohol were investigated by using rat pups born to dams consuming alcohol during gestation and lactation (Table 1). The results of this animal study showed similar results, because plasma GPx activity increased with a subsequent reduction and storage of hepatic selenium, yet hepatic SOD, glutathione reductase, and catalase concentrations were increased. However, supplementation with selenium in the presence of alcohol alleviated these oxidative imbalances, because plasma concentrations of GPx were much lower in comparison to those in alcohol-exposed offspring (109).This research group continued their investigation by looking at selenium uptake in ethanol-exposed pups (110). Pregnant rats were treated with alcohol during induction (8 wk), gestation (3 wk), and the lactation period (3 wk) and either selenium (0.5 μg/g) or selenium plus folic acid was supplemented. Selenium concentrations in dams were restored to control levels with selenium supplementation. Pups born to dams fed either of the antioxidant diets had higher birth weights than those without supplementation, with or without alcohol exposure. Overall, the supplementations improved transported affinity for substrates needed for selenium-methionine absorption and minimized the ethanol-induced damage in the duodenal mucosa (Table 1). This is possibly because of an increase in GPx2 activity, which would prevent membrane lipid peroxidation (110). Duodenal selenium-methionine absorption and selenium

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bioavailability were higher after selenium-only supplementation than after selenium plus folic acid supplementation, possibly because of interactions between the absorption of these 2 nutrients (110). This study provided a more detailed explanation for how antioxidants such as selenium can prevent ethanol-induced damage.

Selenium supplementation in humans. There are currently no studies on selenium supplementation in humans specifically relating to prenatal alcohol exposure and potential mitigation of FASD. Supplementation with selenium during gestation and lactation may be beneficial only with the increased consumption of other antioxidants such as vitamin E, folate, and ascorbic acid, because all nutrients combined may have a greater impact than a single nutrient alone. Information on the long-term effects of supplementation is required with the use of animal studies before conducting such studies in human subjects.

Conclusions

FASD is a complex, multifactorial, and intriguing disorder. The consequences that can ensue from alcohol consumption vary on the spectrum from producing little to no effect to fetal mortality. Because numerous metabolic derangements can develop as a result of alcohol consumption during pregnancy, it is critical to find ways to minimize and reduce the physical and neurological malformations that develop in the fetus. Although robust information on the role of nutrients and intervention are scarce in FASD, potential nutrients have been reviewed, such as vitamin A, DHA, folic acid, zinc, choline, vitamin E, and selenium. The information garnered yielded mixed results regarding the impact of supplementation. Consequently, nutrient supplementation is only a part of a total strategy to ameliorate the impact of FASD.

Regardless of alcohol consumption, prenatal supplementation must be carefully planned to avoid the risk of human pregnancy complications. High concentrations of plasma vitamin A can cause teratogenic effects, and there is evidence of increased cancer risks with folic acid supplementation (111,112). Single nutrients should be more carefully monitored. Because FASD is the result of multiple metabolic impairments, supplementation with 1 nutrient may not be effective to fully reverse the damage induced by alcohol consumption. As stated in the Introduction, there are questions regarding how much and which nutrients should be provided during pregnancy to alleviate the severity of the outcome of FASD. It would be ideal if we could follow the nutritional status of alcohol-consuming pregnant mothers throughout pregnancy. However, this is difficult to accomplish because of significant underreporting by affected individuals.

The primary focus of future studies should be to determine the optimal amount of a nutrient needed to reduce a specific detrimental outcome of FASD and on the long-term effects of such supplementation. If results from these studies produce beneficial effects, then it would be prudent to examine the combined effects of multiple nutrients, which may have beneficial and synergistic effects. Also, the optimal timing for the intervention, whether during the first, second, or third trimester, needs to be established. Moreover, it is crucial to determine the nutritional status of the pregnant women at risk of alcohol consumption to establish the need for nutritional intervention. Although our review focuses on prenatal nutrition for the prevention of FASD, it is equally important to identify the nutritional status of children with FASD, which can provide the basis for nutrition intervention to ameliorate its signs and symptoms. Although the solution to the prevention of FASD is to abstain from alcohol, women consume alcohol during pregnancy for multiple reasons and causes. Thus, health professionals, educators, family members, the surrounding community, and government must offer support and education to vulnerable populations in order to reduce the prevalence rates and incidences of children born with FASD.

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