Early-life exposure to alcohol and the risk of alcohol-induced liver disease in adulthood

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Abstract

Alcohol consumption remains prevalent among pregnant and nursing mothers despite the well-documented adverse effects this may have on the offspring. Moderate-to-high levels of alcohol consumption in pregnancy result in fetal alcohol syndrome (FAS) disorders, with brain defects being chief among the abnormalities. Recent findings indicate that while light-to-moderate levels may not cause FAS, it may contribute to epigenetic changes that make the offspring prone to adverse health outcomes including metabolic disorders and an increased propensity in the adolescent-onset of drinking alcohol. On the one hand, prenatal alcohol exposure (PAE) causes epigenetic changes that affect lipid and glucose transcript regulating genes resulting in metabolic abnormalities. On the other hand, it can program offspring for increased alcohol intake, enhance its palatability, and increase acceptance of alcohol's flavor through associative learning, making alcohol a plausible second hit for the development of alcohol-induced liver disease. Adolescent drinking results in alcohol dependence and abuse in adulthood. Adolescent drinking results in alcohol dependence and abuse in adulthood. Alterations on the opioid system, particularly, the mu-opioid system, has been implicated in the mechanism that induces increased alcohol consumption and acceptance. This review proposes a mechanism that links PAE to the development of alcoholism and eventually to alcoholic liver disease (ALD), which results from prolonged alcohol consumption. While PAE may not lead to ALD development in childhood, there are chances that it may lead to ALD in adulthood.

Keywords: alcohol, drinking, exposure, liver, prenatal, programming effects

1 INTRODUCTION

Ethanol is a well-known teratogen. Its effect on the offspring is well documented (Caputo, Wood, & Jabbour, 2016; Hoyme et al., 2016), yet alcohol consumption during gestation and lactation remains prevalent. About 11.3% of pregnant women continue to indulge in alcohol

consumption despite recommendations to abstain (Denny, Acero, Terplan, & Kim, 2020) while 80% of those who abstain return to their usual drinking level after birth (Tran, Najman, & Hayatbakhsh, 2015). Alcohol-induced dysfunctions and abnormalities such as brain abnormalities, central nervous dysfunction, organ and body systems growth deficiencies observed in fetuses, and early childhood are broadly classified as fetal alcohol syndrome disorders (FASD) (Hoyme et al., 2016). FASD occur in offspring born to women who consumed alcohol during pregnancy (Conner, Bottom, & Huffman, 2020). Patients with brain abnormalities associated with FASD have been the primary focus of research; meanwhile, other body organs and systems may be affected (Caputo et al., 2016). Parental alcohol exposure (PAE) can program adverse metabolic (Amos-Kroohs et al., 2016; Castells, Mark, Abaci, & Schwartz, 1981) and neurological (Foltran, Gregori, Franchin, Verduci, & Giovannini, 2011; Gaztañaga, Angulo-Alcalde, & Chotro, 2020; Gibson & Porter, 2018) health outcomes in offspring.

Children with FASD have lower body weight, glucose intolerance, and insulin resistance in childhood (Amos-Kroohs et al., 2016; Castells et al., 1981). Furthermore, animal studies show that PAE can result in the development of obesity, Type 2 diabetes mellitus (T2DM), insulin resistance, and susceptibility to a high-fat diet in later life (Vaiserman, 2015). PAE may stimulate adolescent behaviors like cognitive and anxiety disorders, hyperactivity and alcohol use disorders, which together can lead to problematic drinking sequelae to alcoholic liver disease (ALD) development (Alati et al., 2006, 2008; Foltran et al., 2011).

According to the available literature, ALD may develop due to increased alcohol consumption that individuals with PAE may be more predisposed to. These findings are consistent with the theory of the developmental origins of health and disease (DOHaD) (Gluckman, Hanson, & Mitchell, 2010). The DOHaD hypothesizes that the fetus's physiology and anatomy adapt in favor of vital organs, particularly the brain, for short-term fetal survival, in response to adverse environmental or developmental factors (Gluckman et al., 2010). Such conditions, including poor nutrition, predispose the offspring to adverse health outcomes later in life. Human and animal studies indirectly support a DOHaD hypothesis for ALD. For example, the offspring of alcoholic mothers tend to crave and drink alcohol in later life. PAE causes a desire to drink alcohol in adolescence due to appetitive learning induced by the sensory and reinforcing pharmacological properties of alcohol (Gaztañaga et al., 2020). Notably, research reveals that, for every gram of alcohol consumed during adolescence, there is a 2% risk of developing severe liver disease (Hagström, Hemmingsson, Discacciati, & Andreasson, 2018). The mechanisms underpinning the developmental programming of ALD are not entirely understood. However, epigenetic changes offer a plausible mechanism for the relationship (Comasco, Rangmar, Eriksson, & Oreland, 2018).

This narrative review covers PAE metabolic programming in offspring and the mechanisms involved, summarizing preclinical and human studies' findings to highlight the possible pathways that link PAE to ALD development. Electronic databases and search engines including Pubmed, SCOPUS, and Web of Science were used to identify articles emphasizing the impact of PAE on adolescent alcohol use and disorder and the development of ALD due to prolonged alcohol use (starting in adolescence). Keywords searched within these databases, including but not limited to “prenatal alcohol use” AND “adolescent alcohol use” OR “adolescent alcohol abuse” were used. Human studies (birth cohort studies) that had examined the long-term effect of PAE on adolescent drinking and animal (rodents) studies that examined the underlying mechanisms were included. Relevant publications were gathered for the presented information. The information gathered was categorized into six

sections: First, we describe how PAE can lead to metabolic-related conditions in offspring at different developmental stages, the impact of alcohol consumption in adulthood on the metabolic system and provide the mechanism thereof. Next, we summarize the neurological effects induced by PAE, which can lead to prolonged alcohol use in adolescence and eventually to the development of ALD and other metabolic-related conditions. This review projects the concept that PAE programs metabolic diseases, including ALD, due to increased alcohol consumption in later life.

2 PAE AND METABOLIC PROGRAMMING

Metabolic programming is the interaction between inherited and environmental factors that results in epigenetic changes that can cause temporary or permanent alterations in physiological development and function (Agosti, Tandoi, Morlacchi, & Bossi, 2017). Subsequently, these alterations can lead to either improved or deranged metabolic outcome during the immediate neonatal developmental phase or adult life (Agosti et al., 2017). Metabolic programming outcomes may result from single-hit or multiple-hit exposure to an insult. In the single-hit theory of metabolic programming, the epigenetic changes cause the expression of phenotypic characteristics immediately in early life or later in adulthood (following a latency period) after a single exposure to the insult (Tamashiro & Moran, 2010). In the multiple-hit theory, the phenotypic changes are not expressed following a single-hit exposure to the insult. In early life, the first hit merely programs epigenetic alterations that increase the susceptibility to express the phenotypic changes if the organism gets exposed to suboptimal insults in subsequent developmental stages. Several mechanisms underpin the concept of DOHaD for metabolic programming; popular among them are the catch-up growth hypothesis, fetal insulin hypothesis, and hypothalamic–pituitary–adrenal (HPA) axis hypothesis (Okada et al., 2015).

Intrauterine growth restriction (IUGR) positively correlates with metabolic and cardiovascular disease risk in subsequent life stages (Itoh & Kanayama, 2018). Food and nutrient restriction leading to IUGR upregulates sterol regulating element-binding protein (SREBP) and fatty acid synthase (FASN) in rats (Yamada et al., 2011). Similarly, studies show that PAE causes IUGR with concomitant upregulation in SREBP1-c and FASN, among others (Shen et al., 2014). Changes in SREBP1-c persist into adulthood in rats, which may lead to dyslipidemia and obesity, as the activation of SREBP1c results in the induction of genes and enzymes involved in lipogenesis (Magee et al., 2008). PAE causes IUGR (Vaiserman, 2015). In excess maternal alcohol consumption, alcohol crosses the placenta and gets to the developing fetus. Unfortunately, the fetus cannot metabolize and eliminate alcohol at the same rate as adults. Consequently, a reduced elimination rate and reduced fetal metabolic enzyme (CYP2E1) provokes an increase in oxidative stress, leading to nutrient deprivation (Joya, Garcia-Algar, Salat-Batlle, Pujades, & Vall, 2015). It is noteworthy that alcohol becomes the liver's preferred fuel when present in the body, resulting in malnutrition (Volkow et al., 2015). Consistently, PAE induces IUGR in humans (Amos-Kroohs et al., 2016; Castells et al., 1981) and rodents (Akison, Reid, Wyllie, & Moritz, 2019). Furthermore, growth catch-up typically follows IUGR, resulting in increased body mass gain and increased risk for obesity and metabolic disease (Nam & Lee, 2018).

The fetal insulin hypothesis suggests that for the fetus to maintain normal metabolic rates in the face of hypoxia due to placental insufficiency, there are developmental adaptations of insulin sensitivity and β-cell function, resulting in glucose intolerance and diabetes later in life (Limesand & Rozance, 2017). In line with this hypothesis, adult guinea pigs (postnatal

day [PND] 150–200) prenatally exposed to ethanol had altered central and peripheral expression of insulin and insulin growth factor signaling molecules at the messenger ribonucleic acid (mRNA) level (Dobson et al., 2014). Furthermore, elevated fasted plasma glucose and elevated insulin (Akison et al., 2019; Harper, Tunc-Ozcan, Graf, & Redei, 2014) occur in PAE rat offspring in adulthood. However, other preclinical studies did not observe that PAE affects glucose and insulin concentration (Amos-Kroohs, Nelson, Hacker, Yen, & Smith, 2018; Elton, Pennington, Lynch, Carver, & Pennington, 2002; Probyn et al., 2013). The fetal insulin hypothesis postulates that both birth weight and T2DM are two phenotypes of the same genotype (Shields, Freathy, & Hattersley, 2010). Interestingly, single nucleotide polymorphism resulting in *alcohol dehydrogenase 2*3 (ADH2*3)* protects the fetus against IUGR, suggesting that the *ADH2* genotype somewhat impacted a higher risk for alcohol-related IUGR (Arfsten, Silbergeld, & Loffredo, 2004). This finding implies that genetic factors may predispose the association between birth weight and insulin resistance.

The HPA axis hypothesis postulates that overexpression of maternal glucocorticoids exposes the fetus to high cortisol levels, leading to IUGR and cause metabolic diseases in adulthood due to disturbance in the fetal HPA axis (Busada & Cidlowski, 2017). After birth, IUGR offspring shows a low basal activity and hyperactivity of the HPA axis along with glucocorticoid-associated disturbances in glucose and lipid metabolism (G. Liang, Chen, Pan, Zheng, & Wang, 2011). However, under chronic stress conditions, there is a gain rate in corticosterone and hepatic insulin resistance, indicating that PAE results in permanent damage to the hippocampus (G. Liang et al., 2011; Xia et al., 2020). There exist controversies regarding the HPA hypothesis because the postnatal environment can reset the HPA axis (Okada et al., 2015). Furthermore, a meta-analysis revealed a somewhat inverse association between IUGR and circulating cortisol level (van Montfoort et al., 2005).

Studies have often used a high-fat diet as the second hit in modeling PAE metabolic programming effect (Akison et al., 2019); however, alcohol has similar hedonic and metabolic effects as a high-fat diet. Food intake is essential for maintaining energy balance and homeostasis (Nakamura & Nakamura, 2018). Interaction between the key feed players (ghrelin, leptin, insulin) and the central appetite control center (hypothalamus) regulate motivated food intake (Brutman, Davis, & Sirohi, 2020). For instance, leptin serves as a “lipostat” sending signals to the brain about periphery energy storage to regulate energy expenditure (Y. Zhang & Chua, 2017). However, the homeostatic system may be dysregulated, leading to excessive caloric intake due to external factors such as palatability, availability, sensory cues, social, and environmental triggers (Brutman et al., 2020). A high-fat diet is highly palatable and often over-consumed both in humans and animals (Brutman et al., 2020). Excessive consumption of a high-fat diet is a form of nonhomeostatic eating referred to as hedonic eating (Hernández et al., 2018). Hedonic feeding activates the reward circuitry and the dopamine system (Coccurello & Maccarrone, 2018). The activation of dopamine signaling in the mesocorticolimbic reward circuitry is a common neural code that acts on the reinforcing component of the high-fat diet (polyunsaturated fatty acid) and alcohol (acetaldehyde [AA]) consumption to induce addiction (Coccurello & Maccarrone, 2018). In addition, all brain regions including the ventral tegmental area (VTA), DA-ergic signals to the ventral striatum (NAc), amygdala, prefrontal cortex (PFC), and the lateral hypothalamus are activated by palatable diets, alter the synaptic strength and increase dopamine strength similar to alcohol (Coccurello & Maccarrone, 2018). PAE programming for neurological effects is well researched; however, its role in metabolic programming has not received much attention, especially with alcohol as a second hit. Furthermore, heavy/chronic alcohol consumption in adolescence and young adulthood could result in cardio-metabolic effects due

to the toxic effects of alcohol and its intermediate metabolite, AA (Sandoval, Vásquez, Mandarim-de-Lacerda, & Sol, 2017). The harmful impact of alcohol on the cardiometabolic system and its mechanism is described in subsequent sections.

3 ALCOHOL CONSUMPTION, ALD, AND THE RISK OF METABOLIC SYNDROME

Metabolic syndrome (MetS) is a combination of metabolic abnormalities that increases cardiovascular disease risk and mortality (Sun et al., 2014). These metabolic abnormalities include abdominal obesity, dyslipidemia, hypertension, insulin resistance, and compensatory hyperinsulinemia (Gluvic et al., 2017). Globally, MetS is a public health concern as it has become prevalent, affecting 20–25% of the adult population (Vollenweider, von Eckardstein, & Widmann, 2015). The etiology of MetS remains elusive despite extensive research. However, both genetic and nongenetic factors have been implicated. External factors such as alcohol consumption, smoking, diet, and physical activity are modifiable factors that can potentially prevent the condition (Kaur, 2014). Studies show an association between alcohol consumption and MetS, albeit inconsistent: some report an inverse association (Kim, Hong, Chung, & Cho, 2017; Vidot et al., 2016); others report a positive linear association (Hirakawa et al., 2015; Oh, Kim, Han, Park, & Jang, 2018) while yet others report no association (W.-Y. Lee, Jung, Park, Rhee, & Kim, 2005; Santos, Ebrahim, & Barros, 2007). Interestingly, though, is the multifaceted relationship between alcohol consumption with the various components of MetS. Light-to-moderate alcohol consumption appears to reduce the risk and severity of T2DM and improve insulin sensitivity (Joosten et al., 2011; Metcalf, Scragg, & Jackson, 2014; Shimomura & Wakabayashi, 2013). Evidence provides that low-to-moderate alcohol consumption reduces the risk of developing hypertension (Briasoulis, Agarwal, & Messerli, 2012; Taylor et al., 2009). A J-shaped relationship exists between alcohol consumption and insulin resistance; light-to-moderate alcohol consumption decreases insulin production, and heavy drinking increases insulin resistance (Suarez, Beckham, & Green, 2017; Tatsumi et al., 2018). Furthermore, alcohol consumption has a positive association with high-density lipoprotein (HDL) levels (Vidot et al., 2016); Du, Bruno, Dwyer, Venn, & Gall, 2017), and triglycerides (Foerster et al., 2009; Klop, Rego, & Cabezas, 2013). With regard to obesity, the relationship is inconsistent (Traversy & Chaput, 2015). Overall, a meta-analysis from six prospective longitudinal studies shows that heavy drinking (>35 g/day) is associated with an increased risk of MetS. In contrast, light-to-moderate (0.5–5 g/day) alcohol consumption confers a reduced risk (Sun et al., 2014). This finding is consistent with a meta-analysis of cross-sectional studies (Alkerwi et al., 2009). Perhaps the intricate relationship between alcohol consumption and MetS is masked by elevated HDL (Sun et al., 2014). However, a recent longitudinal study found that HDL levels decrease with prolonged alcohol use (S. Huang et al., 2017). Furthermore, human studies may have confounding factors which might not have been accounted for in the analysis. For instance, in all the six studies used in the meta-analysis of Sun et al. (2014), some variables were consistently adjusted for (age, sex, body mass index) or baseline weight) while others were not, hence there was no uniformity.

There is a dearth of data on the presence of MetS in ALD patients. In a retrospective data of 81 patients with alcoholic cirrhosis due to heavy consumption of alcohol ≥80 g/day for more than 10 years, 53.4% of the patients had three or four components of MetS, 48.1% were obese, 25% had T2DM, 34.5% had hypertension, 27.2% had hypertriglyceridemia, and 64.2% had low HDL (Mehta et al., 2017). As this was a retrospective study, the authors assumed that NAFLD might have coexisted with ALD. Therefore, there is a need for

prospective studies that can provide clear answers. Vaiserman (2015) provides a mechanism that links PAE to MetS. Briefly, PAE causes IUGR and appetite dysregulation, resulting in adipogenesis, epigenetic changes, and beta-cell apoptosis that cause impaired glucose homeostasis ultimately leading to MetS.

4 THE PATHOPHYSIOLOGY OF ALCOHOL AND MetS

ALD is a term that describes disease manifestations of the liver that results from alcohol overconsumption. According to a WHO report, 3.3 million deaths (6% of all global deaths) are attributable to alcohol use, and alcohol abuse is a significant risk factor in about 50% of all cases of liver cirrhosis (Yoon & Chen, 2016). Alcoholic liver injury is divided into three stages. The first stage is hepatic steatosis (>5–10% fat accumulation in liver cells), usually accompanied by dyslipidemia, principally triglyceride (Cohen, Horton, & Hobbs, 2011). This stage is triggered by an imbalance in reduced nicotinamide adenine dinucleotide to oxidized nicotinamide adenine dinucleotide (NAD^+/NADH) ratio (Madrigal-Santillán et al., 2014). Phase 1 is usually benign and reversible. Steatohepatitis constitutes the second stage, and its development occurs in the setting of hepatic steatosis and is associated with inflammation, oxidative stress and organelle dysfunction (Seitz et al., 2018). A complex hepatotoxic effect of lipotoxicity activates steatohepatitis, reactive oxygen species, NADP, AA, endoplasmic reticulum stress, gut-endotoxin-mediated injury, and pro-inflammatory cytokine activation. The third stage is steatohepatitis's progression to fibrogenesis (fibroblast invasion and accumulation of fibrotic tissues); wherein hepatic regeneration and repair are compromised, leading to limited metabolic and homeostatic function of the liver (de la Monte & Kril, 2014).

Alcohol is a polar substance; at equilibrium, its movement is primarily directed by water content. It passes into small intestines by passive diffusion and into the liver where it is metabolized. The liver metabolizes about 95% of the alcohol consumed. Under physiological conditions, alcohol is metabolized by alcohol dehydrogenase into AA using nicotinamide adenine dinucleotide (NAD^+) as a cofactor (Kong et al., 2019). AA is further oxidized into acetate by AA dehydrogenase. Cytochrome P450 2E1 (CYP2E1) metabolizes about 10% of ethanol into AA (Kong et al., 2019). Catalase, located in the peroxisomes, also plays an accessory role in metabolizing ethanol to AA (Bradford et al., 1999). The CYP2EI and catalase systems are not usually activated until alcohol consumption is more than 10 mol/L (Ceni, Mello, & Galli, 2014). Light-to-moderate alcohol consumption may be beneficial since at that level ethanol induces hypoglycemia by inhibiting gluconeogenesis and suppressing the secretion of insulin from the pancreatic β-cells by downregulating glucokinase (Siler, Neese, Christiansen, & Hellerstein, 1998). Furthermore, increased NAD^+/NADH causes a decreased efflux of glucose from the hepatic cells leading to reduced insulin resistance (Siler et al., 1998). It is noteworthy that obesity and insulin resistance rules out this seemingly protective effect of light-to-moderate alcohol consumption (Traversy & Chaput, 2015; Yokoyama, 2011).

Insulin resistance results from a decrease in insulin capacity to suppress hepatic glucose production yet continue to stimulate lipogenesis, causing hyperglycemia, hyperlipidemia, and hepatic steatosis (Samuel & Shulman, 2016). Insulin resistance and the excessive efflux of fatty acids are largely to blame for MetS. The pathogenesis of insulin resistance involves oxidative stress and inflammation (Yaribeygi, Farrokhi, Butler, & Sahebkar, 2019). Efflux of glucose from the liver is controlled by various pathways, including the phosphatidylinositol-3-kinase (PI3K)/protein kinase B(AKT) signaling pathway. The β-cells of the islet of Langerhans of the pancreas secrete insulin in response to elevated glucose. Normally, insulin

binds to insulin receptors (INR) on the hepatocyte membrane surface. This results in automatic phosphorylation of the protein tyrosine kinase on the β-subunit. Activation of these INR phosphorylates the insulin receptor substrates, which leads to the activation of PI3K (F. Zhang et al., 2014). Phosphorylation of the third hydroxyl group on the inositol ring of phosphatidylinositol activates PI3K, catalyzing the conversion of phosphatidylinositol 4,5-diphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate (PIP3) (Y. H. Huang & Sauer, 2010). PIP3 is the primary substrate of phosphatase and tensin homolog deleted on Chromosome 10 (PTEN). PTEN dephosphorylates PIP3, converting it back to PIP2; reducing PIP3 production and signals dependent on it. Thus PTEN negatively regulates insulin (Chen, Dehart, & Sulik, 2004). The PIP3/AKT pathway plays four primary roles; stimulating glycogen production by inhibiting glycogen synthase, suppressing gluconeogenesis by inactivating Forkhead box O1 (FOX1), regulating fatty acid synthesis by regulating SREBP1-c and promoting glucose transport from the peripheral tissue into the cell for metabolism (Carr & Correnti, 2015). Ethanol induces hepatic insulin resistance by inhibiting the PI3K/AKT pathway, decreases INR density, inhibits the binding activity between insulin and its receptor and decreases receptor phosphorylation (Gunji et al., 2011; Pang et al., 2009).

In addition, in excess alcohol consumption, the induction of the alcohol dehydrogenase (ADH) system is accompanied by an increased NAD^+ to NADH ratio that results in a suppressed β-oxidation of fatty acids (Madrigal-Santillán et al., 2014). This results in an increased de novo lipogenesis leading to the production of diacylglycerol (DAG). The presence of DAG activates the c-Jun N-terminal kinase 1 (JNK-1) pathway (Y. J. Lee, Aroor, & Shukla, 2002). Once JNK-1 is induced, the inflammatory cascade is set into motion, resulting in the serine phosphorylation of hepatic insulin receptor Substrate 1. This renders the IRS inactive, resulting in insulin resistance (Aguirre, Uchida, Yenush, Davis, & White, 2000). The inactivation of IRS promotes hyperinsulinemia and deposition of fats (Lustig, 2013).

Furthermore, chronic alcohol consumption induces CYP2E1 which can break down gem-diol into AA leading to NADPH oxidation, and form hydrogen peroxide (H_2O_2) (Zakhari, 2006). Reactive oxygen species release is associated with the pro-inflammatory profile of liver damage by recruiting immune cells and inducing circulatory pro-inflammatory cytokines (Seitz et al., 2018). These pro-inflammatory cytokines reduce insulin sensitivity and compromise glycaemic control (Carr & Correnti, 2015). In addition, alcohol overconsumption enhances the excessive proliferation of gram-negative organisms leading to excess endotoxin production and AA (Purohit et al., 2008). This causes phosphorylation of the tight junctions of the intestinal wall, allowing endotoxins to cross the small intestines into the liver to induce inflammatory changes (Kong et al., 2019). Therefore, excess ethanol consumption leads to dyslipidemia, glucose dysmetabolism, and insulin resistance, all of which are components of MetS.

5 DEVELOPMENTAL ORIGINS OF ADOLESCENT DRINKING

The effect of PAE on cognitive and behavioral outcomes in domains of executive functioning, general intelligence, learning and memory, language development, academic performance, adaptive functioning, and concurrent psychopathology have received much attention. However, PAE effect on alcohol use in later life has received little attention, although evidence shows that PAE can affect alcohol use/abuse in later life.

In utero alcohol exposure mediates neurological effects in infants (Faas, March, Moya, & Molina, 2015). For example, infants whose mothers drank moderately during gestation show more appetitive facial expressions (suckling, smiling, and tongue protrusions) when stimulated with the odor of alcohol (Faas et al., 2015). These infants' reactions positively correlated with the mother's alcohol consumption level (Faas et al., 2015). The chemosensory effect of alcohol lasts beyond infancy in humans. Hanning and colleagues demonstrated that young adults prenatally exposed to alcohol found the odor of alcohol more pleasant than their control counterparts (Hanning et al., 2015). In addition, FASD children are more predisposed to alcohol use disorders in adulthood than healthy controls (9 and 2%, respectively) (Rangmar, Sandberg, Aronson, & Fahlke, 2015). Preclinical studies indicate that even light-to-moderate doses of alcohol during pregnancy may enhance ethanol intake in offspring later in life (Bordner & Deak, 2015; Nizhnikov et al., 2014). This suggests a two-hit or multiple-hit DOHaD hypothesis for ALD, prenatally and then at adolescence.

The few clinical prospective birth cohort studies on the developmental origin of adolescent alcohol use have been extensively reviewed by Foltran et al. (2011) and Gaztañaga et al. (2020). Both reviews explicitly indicate that PAE creates an affinity for alcohol in adolescence. According to the Mater-University study of pregnancy and its outcomes, findings revealed that the odds of drinking three or more alcoholic beverages at age 14 was 2.74 (1.70, 4.22) (Alati et al., 2008). In an earlier analysis of the same cohort examined at age 21, children of alcoholic mothers are four times more likely to drink alcohol than their counterparts not exposed to alcohol in utero (Alati et al., 2006). In the Seattle longitudinal study of alcohol and pregnancy, offspring of alcoholic mothers at ages 14, 21, and 25 showed a positive association between the early onset of alcohol use and prenatal exposure which remained after adjusting for other factors thought to influence alcohol use in adolescence (Baer, Barr, Bookstein, Sampson, & Streissguth, 1998; Baer, Sampson, Barr, Connor, & Streissguth, 2003). The positive association between prenatal exposure to alcohol and early onset of alcohol use was further substantiated in studies that evaluated whether a family history of alcoholism played a role. Observations proved that prenatal alcohol use is a significantly stronger factor in predicting alcohol use in offspring (O'Brien & Hill, 2014). However, it is noteworthy that an environment of alcoholics may be an equally significant predictor in offspring alcohol use (Rossow et al., 2016). Other studies that support the developmental origin of adolescent alcohol use stems from studies that showed high concordance for alcoholism among monozygotic twins (100%) compared to dizygotic twins (64%) (Streissguth, Barr, Sampson, & Bookstein, 1994). A meta-analysis of studies on twins showed alcohol use disorder (AUD) was 50% heritable (Verhulst, Neale, & Kendler, 2015). In addition, studies of adopted-out children of alcoholic mothers confirmed this assertion (Yates, Cadoret, Troughton, Stewart, & Giunta, 1998). Several animal studies support and explain the developmental origins of adolescent alcohol use in humans (Gaztañaga et al., 2020). Animal studies have shown that the effect of PAE transcends beyond the first generation. Nizhnilov and colleagues found that the sensitive sedation-hypnosis test decreased in the third-generation offspring of dams that received 1 g/kg bwt/day of alcohol at gestational days 17–20; an indication of higher risk of alcohol abuse/dependence in later life as a result of less sensitivity to the hypnotic effect of alcohol (Nizhnikov, Popoola, & Cameron, 2016). The sensitivity sedation-hypnosis test measures sensitivity to hypnosis induced by a drug (Wasilczuk, Maier, & Kelz, 2018). This may be performed by conducting a loss of righting reflex test. The animal is infused with a drug and observed until its losses capacity to stand on its four paws when placed on their back-loss of righting reflex (LORR) (Wasilczuk et al., 2018). The animals are maintained in this position until they regain LORR. The time interval between drug administration and LORR is known as latency, while the

period between loss and regain of LORR is known as the duration. Decreased LORR duration indicates decreased sensitivity to the hypnotic effect of the drug, which allows for an increased consumption (Popoola, Nizhnikov, & Cameron, 2017).

6 PROGRESSION OF ADOLESCENT ALCOHOL USE TO ADULT ALCOHOL-INDUCED LIVER DISEASE

During adolescence, it is usually at about 15 years of age that alcohol consumption begins (Spear, 2015). While at this young age, the youth drink less frequently than adults; however, binge drink when they do (Hingson & White, 2014). Early initiation of alcohol use and high binge drinking rates in late adolescence are significant factors that increase vulnerability for alcohol abuse and dependence in adulthood (Windle, 2010). According to the "sensitive-period hypothesis," individuals who start drinking alcohol at an early age (<15 years) are more likely to abuse alcohol in later life (Windle, 2010). This theory postulates a sensitive period within adolescence, particularly "early adolescence" that increases the chronicity of alcohol use due to brain growth and neural remodeling (Nixon, Morris, Liput, & Kelso, 2010). Grant and colleagues found that the likelihood of adult alcohol abuse and dependence increases significantly and linearly with each earlier year of onset of regular drinking (2–3 drinks per week) in a 12-year prospective longitudinal study (Grant, Stinson, & Harford, 2001). Pitkänen, Lyyra, and Pulkkinen (2005) also found that drinking before the age of 16 was a significant risk factor for problematic and excessive alcohol consumption in adulthood. More recent studies have shown that initiation of alcohol consumption during mid-adolescence (15–17 years) have an equal impact on later alcohol dependence (Enstad, Evans-Whipp, Kjeldsen, Toumbourou, & von Soest, 2019; Silins et al., 2018). On the other hand, other studies suggest no vulnerable period within adolescence during which susceptibility to later alcohol dependence is highest. Guttamannova and colleagues showed that rather than age bracket, regular alcohol use before the age of 21 was a better predictor for AUD in later life (Guttmannova et al., 2011). Furthermore, Rossow and Kuntsche (2013) indicated that early onset per se was not responsible for adulthood alcohol dependence unless part of a broader array of conduct problems. Finally, while there is no doubt, some divergent findings in the literature indicate that alcohol consumption in adolescence impacts alcohol abuse in adulthood.

Although controversy exists between the amount of alcohol consumed and ALD development, studies indicate that the quantity of alcohol consumed and its duration are closely associated with liver cirrhosis (Singal & Anand, 2013). Most patients are likely to develop alcoholic hepatitis (AH) when consuming about 100 g/day of alcohol equivalent to 6–7 drinks/day. However, patients who consume 30–50 g/day of alcohol for 5–10 years are at increased risk for AH (Singal & Anand, 2013). Studies that focused on the risk of liver disease resulting from overconsumption of alcohol have been cross-sectional and had short follow-up periods (Bellentani et al., 1997; Kamper-Jørgensen, Grønbaek, Tolstrup, & Becker, 2004; Liu et al., 2010). However, a recent prospective study (follow-up, 37.8 years) found that alcohol use in late adolescence is associated with an increased risk of severe liver disease development in later life; occurring in a dose-dependent manner with no sign of threshold effect (Hagström et al., 2018). After 37.8 years of following 43,296 men, 243 (9.1%) of the 2,661 who received an alcohol abuse diagnosis, developed a severe liver disease (Hagström et al., 2018). It is noteworthy that alcohol abuse/dependence is not synonymous with ALD; the percentage of heavy drinkers who develop steatosis (90%) do not all progress to advanced stages of the condition (only 20–40% develop alcoholic steatosis, 8–20% may progress to

cirrhosis, and 3–10% eventually develop hepatocellular carcinoma (Ohashi, Pimienta, & Seki, 2018).

7 PRENATAL ALCOHOL CONSUMPTION: MECHANISMS TO ALD

The endogenous opioid system plays a significant role in reward and reinforcement (Méndez, Hernández-Fonseca, & Abate, 2019). There are three distinct families of endogenous opioid system defined by their precursor molecules: pro-opiomelanocortin is the precursor for β-endorphins, pro-enkephalins for met-enkephalins, and prodynorphins gives rise to dynorphins. Three major classes of opioid receptors have been identified to be associated with these opioid peptides. β-Endorphins bind with equal affinity to mu (μ) and delta (δ) receptors, Met- and Leu-enkephalins bind with greater affinity to δ receptors than μ receptors. In contrast, the dynorphins bind selectively to kappa receptors (κ) (Toubia & Khalife, 2019). The endogenous opioid system's central role includes pain perception, reward and reinforcement, and homeostatic adaptations to food, water, and temperature (Shenoy & Lui, 2020). Research demonstrates that interactions between the endorphins and enkephalins with μ and δ receptors activate dopamine release and initiate reward and reinforcement processes. In contrast, the interaction between dynorphin and κ produce an aversive state and decrease dopamine release in adults (Shenoy & Lui, 2020).

The μ and δ-opioid systems are involved in ethanol acceptance in adulthood (Méndez et al., 2019); the κ-opioid system causes the aversive effects of alcohol in older infants and adults (Pautassi, Nizhnikov, Spear, & Molina, 2012). However, in early postnatal life, the κ-opioid system confers an appetitive impact (Barr, Wang, & Carden, 1994). The κ-opioid system is detectable around gestation day (GD) 13, while the delta-opioid system receptors becomes detected around PND 21 (Bordner & Deak, 2015). The majority of the elements in the opioid system are upregulated in the early postnatal life, an indication that there is continuity of development of these systems (Bordner & Deak, 2015), allowing neuroplasticity both in the fetus and the neonate. Alteration in the neurochemical system may be responsible for the effect of PAE. It is suggested that the voluntary consumption of alcohol is related to the monoaminergic system (Belmer, Patkar, Pitman, & Bartlett, 2016), while alcohol habits and sensitivity are mediated by the dopaminergic system (O'Tousa & Grahame, 2015).

PAE reduces opioid system peptide proteins but increases opioid system receptors in infancy and adolescence (Bordner & Deak, 2015; Fabio, Macchione, Nizhnikov, & Pautassi, 2015). Furthermore, Abate, Reyes-Guzmán, Hernández-Fonseca, and Méndez (2017) demonstrated increased met-enkephalin concentrations in different brain regions of adolescent rats that were prenatally exposed to alcohol which resulted in alterations in the stability of pro-enk mRNA, posttranslational processing of the precursor affecting the maturity of the peptide and its release in response to alcohol administration. These findings suggest a reduced sensitivity to alcohol's sedative effect, resulting in high acceptance of the drug, which subsequently leads to increased intake and palatability. Diaz and colleagues found that blocking the μ-opioid system receptors reduced palatability drastically compared to blocking the kappa-opioid system receptors (KOR) (Díaz-Cenzano, Gaztañaga, & Gabriela Chotro, 2013). Notably, Hausknecht et al. (2015) reported an enhanced excitatory synaptic strength in the ventral tegmental area dopamine neurons associated with drug addiction due to PAE that persists from infancy through to adulthood in rats (2–12 weeks). This implies that PAE modifies the reward system such that its plastic responses are persistently altered (Granato, 2020). Hence, the mu-opioid receptor (MOR) may be involved in the programming effect of

PAE as alcohol-induced β-endorphins stimulate the MOR believed to cause the release of dopamine in the brain reward system (Herz, 1997).

Alcohol can pass directly into the placenta and breast milk (D'Apolito, 2013). Around GD 17–20 rat fetuses can detect, modify intake and response to alcohol (Abate, Pepino, Spear, & Molina, 2004; Chotro & Arias, 2003) just as the newborn can detect differences in flavor in maternal breast milk (Koffman, Petrov, Varlinskaya, & Smotherman, 1998); indicating that amniotic fluid and breast milk can induce positive appetitive response. Habituation to neophobia facilitates the initial acceptance of the drug (alcohol) in early postnatal life following prenatal exposure (Díaz-Cenzano & Chotro, 2010; Spear & Molina, 2005). Other research focused on the chemosensory effect of alcohol odor indicated that PAE alters neurophysiological olfactory responses to ethanol which can last beyond early postnatal life (PND 42/43), but this effect disappeared in adulthood (Eade, Sheehe, & Youngentob, 2010; Middleton, Carrierfenster, Mooney, & Youngentob, 2009; Youngentob et al., 2007; Youngentob, Kent, & Youngentob, 2012). However, other studies have shown that this effect does not last beyond early postnatal life (Fabio et al., 2015). The fetus develops an appetitive conditional response to alcohol such that it forms an association between the flavor and its pharmacological effects (Bordner & Deak, 2015; Gaztañaga et al., 2020). In the brain, AA, a metabolite produced from the detoxification of alcohol mediates the production of appetitive properties of alcohol as well as serves as its reinforcing agent by acting on the opioid system (Gaztañaga, Angulo-Alcalde, Spear, & Chotro, 2017). The presence of AA, as well as that of opioid system receptors, is critical in increased alcohol intake. Studies show that the absence of AA and the blockade of the opioid system receptors resulted in lower consumption of alcohol (Gaztañaga et al., 2017; Youngentob et al., 2012).

Alcohol deprivation after birth may account for the increased alcohol consumption in rat offspring. The reinforcing capability of AA seems operational when rats are forcefully deprived of alcohol for some time (Israel, Quintanilla, Karahanian, Rivera-Meza, & Herrera-Marschitz, 2015). Animal studies indicate that short (4 hr) and prolonged (28 days) deprivation periods induced increased voluntary consumption of alcohol (Wegner et al., 2017); (Díaz-Cenzano, Gaztañaga, & Gabriela Chotro, 2014). Khisti, Wolstenholme, Shelton, and Miles (2006) observed 50–100% ethanol consumption in C57BL/6NCrl and C57BL/6J mice exposed to ethanol for periods of 4 and 14 days, respectively. Similar observations had previously been reported in other studies with different deprivation periods (Oster et al., 2006; Rodd et al., 2003). However, in all these studies, it was apparent that although repeated deprivation period induced increased consumption of alcohol, the magnitude of the first bout was much higher than subsequent periods of deprivation. Of interest was that the rats would go to greater lengths to achieve this feat, implying that the reward value increases postdeprivation and reaccess to ethanol. In most of the preclinical studies that observed increased alcohol consumption resulting from PAE, rats were exposed before birth, deprived for a period and access reinstated (Fabio et al., 2015; Oster et al., 2006; Rodd et al., 2003). This pattern is evident in human adults recovering from alcoholism who find themselves relapsing now and then (Hirth et al., 2016).

Besides PAE inducing increased alcohol consumption at adolescence due to chemosensory stimulation, dopamine activation and alcohol deprivation, studies provide that PAE also causes behavioral problems that contribute to increased alcohol consumption. Emerging evidence points to a relationship between PAE and social, cognitive and affective behavior in adolescents' rat offspring (Marquardt & Brigman, 2016). PAE induces an increased anxiety-like behavior in adolescent rats (G. Liang et al., 2014; Rouzer, Cole, Johnson, Varlinskaya, &

Diaz, 2017) and this phenotypic expression appears to affect males more both at adolescence and in adulthood (Diaz, Mooney, & Varlinskaya, 2016; Rouzer et al., 2017) Furthermore, PAE affects social behavior (Holman, Ellis, Morgan, & Weinberg, 2018; Mooney & Varlinskaya, 2019). Social function deficits and anxiety are associated with adolescent alcohol use (Diaz, Johnson, & Varlinskaya, 2020), which may be an attempt to alleviate stress under social circumstances and enhance interactions with peers. However, other studies found a somewhat attenuated alcohol reward effect even though PAE induced anxiety-like behavior (Cantacorps, Alfonso-Loeches, Guerri, & Valverde, 2019). The neural network that drives social behavior is vast, but some areas have been identified to associate with social processing the amygdala and prefrontal cortex and the hippocampus (Felix-Ortiz, Burgos-Robles, Bhagat, Leppla, & Tye, 2016; Gradin et al., 2012; Tang et al., 2013). Importantly these areas are significantly affected following PAE (Baculis, Diaz, & Valenzuela, 2015; Díaz-Cenzano et al., 2014; Fabio et al., 2015; Louth, Bignell, Taylor, & Bailey, 2016; Skorput & Yeh, 2016). Few preclinical studies have attempted to unravel the molecular mechanisms underpinning how PAE affects offspring behavior, leading to increased alcohol intake in adolescence. Other studies have demonstrated that PAE increases expression of inflammatory chemokine C□C motif ligand 2 (CCL2) or its receptor CCR2 with the orexigenic (appetite-stimulating) neuropeptide, melanin-concentrating hormone (MCH), to promote ethanol drinking behavior–anxiety, in adolescent rat offspring (Chang et al., 2018; Chang, Karatayev, & Leibowitz, 2015) (Orexigenic is a drug or naturally occurring neuropeptide that increases hunger and enhances food consumption). Additionally, chemokine C□C motif receptor (CCR2) enhances dopamine neurotransmission by modulating the potassium channels (Apartis, Mélik-Parsadaniantz, Guyon, Kitabgi, & Rostène, 2010).

Figure 1 provides a schematic diagram of the mechanism linking PAE to ALD.

Table 1 presents studies using rodent models that explored the molecular mechanisms between PAE and increased alcohol intake or preference in adolescence.

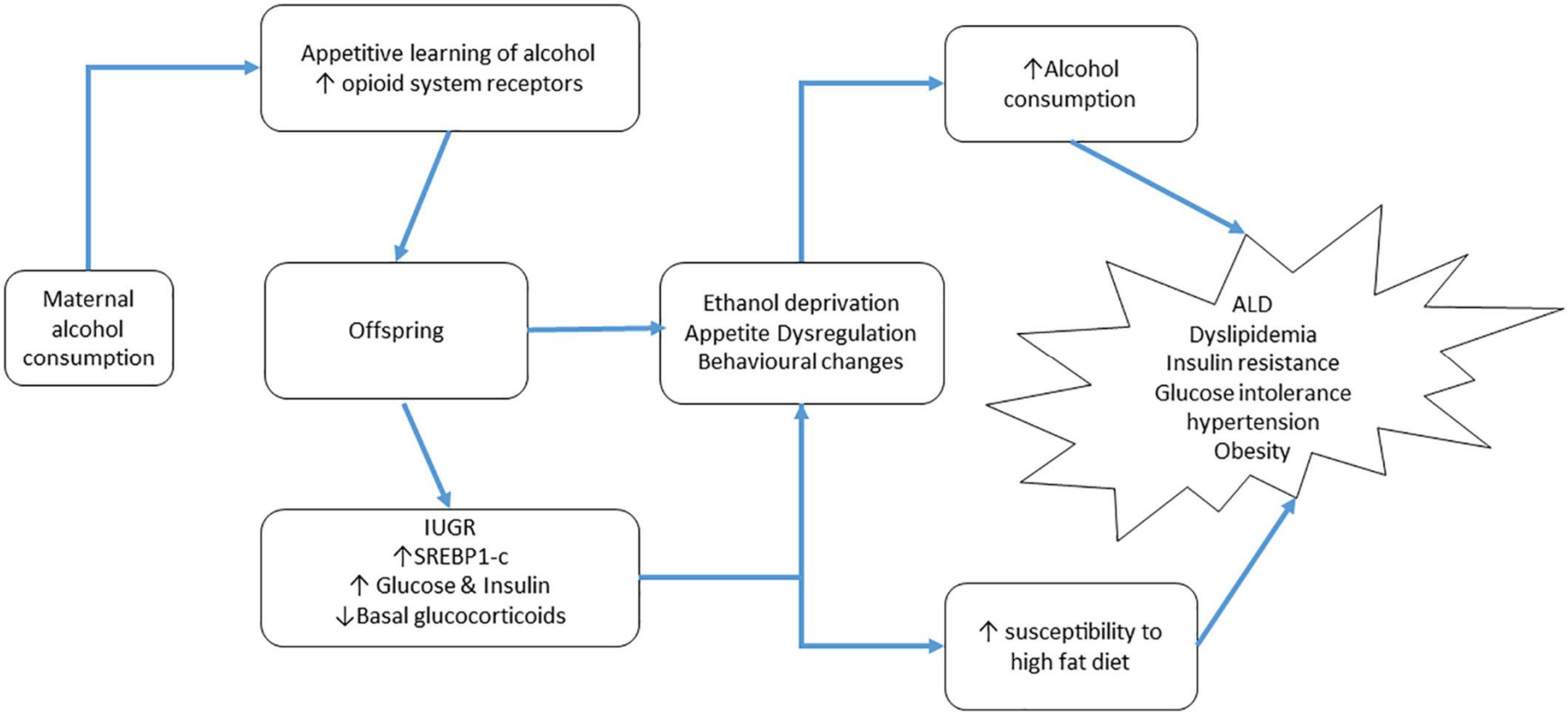

FIGURE 1 Schematic diagram

TABLE 1. Rodent models: unraveling the molecular mechanism for increased alcohol consumption in adolescence following prenatal alcohol consumption

Study	Prenatal study	Adolescent test day/treatment	Measurement/results	Proposed mechanism
Abate, Hernández-Fonseca, Reyes-Guzmán, Barbosa-Luna, and Méndez (2014), Abate et al. (2017)	Wistar rat 2 g/kg GD 17–20	PND 14 10% Intraoral infusion PND 30 Intraoral infusion	Increased intake Increased Met-enk concentration in the PFC, CP, hypothalamus and the hippocampus Decreased Met-enk in VTA	Induced anxiety-like behavior
Chang et al. (2015)	Sprague Dawley 1 g/kg GD 10–15	PND 15, 40 (M only) 20% Intermittent access (14 hr, 6 mins and 30 min)	Increased intake Increased MCH/MCH+, CCR/CCR2+/BrdU+	Behavioral disorders that induces increases alcohol consumption
Chang et al. (2018)	Sprague Dawley 2 g/kg GD 10–15	PND 35 (F), PND 40 (M) 20% Intermittent access (14 hr, 6 min and 30 min)	Increased alcohol intake Increased MCH/MCH+, CCR/CCR2+	Induced anxiety-like behavior
Díaz-Cenzano and Chotro (2010)	Wistar rat 2 g/kg G17-20	PND 14, 26–27 Intraoral infusion 6% (2 BC)	Increased intake Increased preference	Appetitive learning induced by the opioid system

Study	Prenatal study	Adolescent test day/treatment	Measurement/results	Proposed mechanism
Fabio et al. (2015a, 2015b)	Wistar rat 2 g/kg G17-20	PND 35–37 1.25, 2.5, and 3.25 g/kg PND 37–62 4 weeks (3 session/week, 18 hr/session) 5% (2 BC)	Increased ethanol intake Decreased positive c-fos cells in the infralimbic prefrontal cortex Increased intake Increased preference increase dopamine activation in the VTA induced c-FOS activation in AcbSh and AcbC Increase MOR mRNA expression in the VTA	PAE reverses the valence of KOR activation from aversive to appetitive EE was associated with insensitivity to the aversive effects of ethanol, and alterations in MOR mRNA expression in the VTA.
Nizhnikov et al. (2014, 2016)	Sprague Dawley 1 g/kg G17-20	PND 14 PND 42 Intraoral infusion (5, 10, and 20%)	Increased intake Decreased kappa opioid expression in the nucleus accumbens, amygdala and hippocampus	Attenuation of sensitivity of ethanol-induced hyponosis
Eade et al. (2010), Middleton et al. (2009), Youngentob et al. (2007)	Long Evans rats 35% of daily calorie intake G11-20	P30 P42–48 0.313, 0.625, 1.25, 2.5, 5% Vapor saturation	Increased unconditioned response to ethanol odor Decreased GABA receptor, mGluR2 receptor, Caskkin and SOX11 genes	Attenuated aversion by making it taste and smell better

Note: Preclinical studies are ordered alphabetically by the authors.

Abbreviations: AcbC, nucleus accumbens core; AcbSh, nucleus accumbens shell; BC, bottle choice; BrdU, 5-bromo-2-deoxyuridine; CCR/CCL2+, chemokine-C□C motif ligand/receptor; CP, caudate putamen; GABA, gamma-aminobutyric acid; GD, gestational day; KOR, κ-opioid receptor; MCH/MCH+, melanin-concentrating hormone/receptor; Met-enk, met-enkephalin; mGluR2, Group II metabotropic glutamate receptors; MOR, μ-opioid receptor; PFC, prefrontal cortex; PND, postnatal day; SOX11, SRY-related HMG-box gene 11; VTA, ventral tegmental area.

8 CONCLUDING COMMENTS AND FUTURE DIRECTIONS

The endocrine system and neurons in the brain are plastic and can be programmed adversely following prenatal insults during early development. The effect of PAE on the endocrine system results in metabolic dysregulation, while its impact on the brain results in alcohol use disorders. PAE alters the brain's reward system such that a withdrawal from the drug will stimulate the offspring to crave it, which could result in ALD. AA has been found to reinforce these behaviors and attitudes toward ethanol. Given that amniotic fluid and breast milk can program positive appetitive responses in offspring due to the presence of AA, it will be essential to determine if breast milk alcohol is sufficient to program offspring to increased alcohol intake. In this regard, a first step has been taken to ascertain whether breast milk alcohol can program increased alcohol consumption and ALD in later life. It will also be satisfying to understand the immediate effect (before birth) of PAE on opioid system receptors. Adolescence is a critical stage of development when brain growth continues yet it is also a period of vulnerability and stress. Given that an individual's genetic make-up, stress, and anxiety have been found to increase alcohol intake, a predisposition to alcohol in early life will likely exacerbate the propensity to developing ALD. In addition, the current obesity pandemic creates a robust environment which favors the development of the liver disease. Further, alcohol can create a susceptibility to a high-fat diet. Although PAE has often been used as the first hit in ALD, alcohol as a second hit has yet to be explored. Available literature shows that PAE can likely lead to the involuntary onset of alcohol consumption, progress to alcohol dependence and ultimately to ALD.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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