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FASD: Folic acid and Formic Acid - An unholy alliance in the alcohol abusing mother

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Abstract  Word limit - 200

Alcohol consumption during pregnancy remains a significant cause of preventable birth defects and developmental disabilities, however, the mechanism of toxicity remains unclear. Methanol is present as a congener in many alcoholic beverages and is formed endogenously. Since ethanol is preferentially metabolized over methanol, it has been found in the sera and CSF of alcoholics. Toxicity resulting from methanol has been attributed to formic acid. Formic acid is present in significantly higher quantities in biofluids of alcoholic patients. These higher levels can be cytotoxic and cause neuronal cell death. However, the adverse effects can be mitigated by adequate levels of hepatic folic acid, since formic acid elimination depends on folic acid.

During pregnancy, folate concentrations are at least two-fold higher in cord blood then in maternal blood due to increased folate requirements. The reverse has been demonstrated in alcohol abusing pregnancies, suggesting downregulation of folate transporters and low fetal folate levels. Moreover, formic acid can cross the placenta and its adverse effects can be mitigated by folic acid. Thus, the combination of low fetal folate levels and presence of formic acid form a potent cytotoxic combination that may play a significant role in the etiology of fetal alcohol spectrum disorder.

Key words: Formic acid, methanol, folic acid, FASD, neurotoxicity,
Fetal alcohol spectrum disorder (FASD) is an umbrella term used to describe a range of physical, behavioral, and cognitive effects resulting from in-utero exposure to alcohol. The most severe form of FASD is fetal alcohol syndrome (FAS), which is characterized by facial dysmorphology, growth restriction, and CNS neurodevelopmental abnormalities (Sokol et al. 2003). A wide spectrum of effects has been described, from the milder form known as alcohol-related neurodevelopmental disorder (ARND), to the extreme manifestation known as FAS. It has been demonstrated that both the pattern and timing of alcohol consumption play a significant role in the development of FASD. Alcohol is one of the most commonly consumed teratogens worldwide among women of reproductive age (Nevin et al. 2002), and since roughly half of pregnancies are unplanned (Edwards and Werler 2006; Finer and Zolna 2014), alcohol consumption in this cohort poses a significant public health concern. Light-to-moderate drinking in pregnancy is still controversial. Some studies have found that this level of alcohol consumption may lead to adverse neurodevelopmental effects (D’Onofrio et al. 2007; Flak et al. 2014b), while other studies have established no such effects (Falgreen Eriksen et al. 2012; Skogerbo et al. 2012; Underbjerg et al. 2012). Due to the demonstrated potential for harm to the fetus, most health organizations advocate abstinence from alcohol throughout pregnancy (Centers for Disease Control and Prevention 2005; Public Health Agency of Canada 2012). Although the total volume of alcohol intake is an important variable, the number of binge drinking episodes may have a greater effect on the risk for adverse effects (Maier and West 2001). Given that the etiology of FASD is not fully understood and that there is significant
heterogeneity in terms of fetal effects associated with any amount of alcohol consumption, no "safe" level of alcohol intake during gestation has been established (Centers for Disease Control and Prevention 2005; Charness et al. 2016; Flak et al. 2014a; Sowell et al. 2014). Axelrod and Daly demonstrated the endogenous formation of methanol (MeOH) from S-adenosylmethionine (SAM) in the pituitary glands of humans and various other mammals (Axelrod and Daly 1965b). In an elegant experiment, Majchrowicz and Mendelson (Majchrowicz and Mendelson 1971a) illustrated the rise in MeOH levels among subjects drinking MeOH-free alcohol, which corroborated the finding by Ericksen and Kulkarni that MeOH can be produced endogenously (Ericksen and Kulkarni 1963). Most alcoholic beverages also have a small amount of MeOH as a congener (Roine et al., 1989a; Sprung et al. 1988a). Both ethanol (EtOH) and MeOH are metabolized by the same enzymes, alcohol dehydrogenase (ADH), and aldehyde dehydrogenase (ALDH), with the final products being acetic acid and formic acid, respectively.

Since EtOH has a higher affinity for ADH than MeOH, it is preferentially metabolized (Mani et al. 1970). As a result, MeOH accumulation from endogenously produced MeOH and/or MeOH consumed as part of an alcoholic beverage has been reported in concentrations of up to 2 mmol/L (Majchrowicz and Mendelson 1971b) and confirmed by others (Iffland and Staak 1990; Jones and Lowinger 1988; Kapur et al. 2007; Roine et al. 1989; Sprung et al. 1988). Altered pharmacokinetic behavior of MeOH in the presence of EtOH has been demonstrated by various authors (Lesch et al. 1990; Martensson et al. 1988). The presence of MeOH has also been
suggested as a potential marker for chronic alcohol abuse (Iffland and Staak 1990; Roine et al. 1989)

Neurotoxicity resulting from MeOH consumption is extensively documented in both humans and animals, and has been attributed to the formation of formic acid from the metabolism of MeOH by ADH and ALDH (Jacobsen and McMartin 1986; Roe 1955; Wood and Buller 1904). Formic acid is eliminated through the one-carbon pathway with folic acid acting as a coenzyme. The rate of formic acid (formate) oxidation and elimination is dependent on adequate levels of hepatic folic acid, particularly hepatic tetrahydrofolate (THF) (Johlin et al. 1987; Tephly 1991). Indeed, to prevent metabolic acidosis in MeOH poisonings, folic acid is currently part of standard of care to drive formic acid oxidation into carbon dioxide and water. It has been demonstrated that significantly higher formate levels are produced when folate-deficient animals are exposed to MeOH, as compared to folate-sufficient animals (Lee et al. 1994; McMartin et al. 1975; Noker et al. 1980). Sokoro and colleagues determined that the half-life of formic acid was 40 minutes in folate-sufficient minipigs compared to 120 minutes in folate-deficient minipigs (Sokoro et al. 2008).

Our group demonstrated the presence of formic acid in the sera of alcoholic patients (Kapur et al. 2007), which was significantly higher (p <0.001) compared to non-alcohol controls. In an experiment using rat brain slice cultures, we further showed that formic acid concentrations achieved in alcoholic patients’ sera can be cytotoxic and can cause neuronal cell death. Formic acid cytotoxicity was shown to be both time- and dose-dependent and it was
found that neuronal cell death could be mitigated by folic acid.

There is an abundance of literature implicating acetaldehyde in the pathogenesis of alcohol-related disorders, but to our knowledge there is no literature that has questioned the role of formic acid in chronically alcohol abusing individuals. To better understand the relationship between EtOH toxicity and FASD, it is imperative to consider MeOH and its metabolite, formic acid, as potential contributors to the teratogenic effects associated with EtOH. Accumulation of MeOH suggests higher-than-normal levels of formic acid in alcohol-drinking populations. When folate levels are low, elimination of formic acid is slower and its levels are elevated (Sokoro et al. 2008; Noker et al. 1980). Women who are folate-deficient and consume alcohol may have higher levels of formic acid. Since animal studies suggest that formic acid may be toxic to the developing fetus (Brown-Woodman et al. 1995), should these women become pregnant, their fetuses are at greater risk. In this paper, we review the role of formic acid and folic acid in the pathogenesis of FASD.

**Formate Metabolism:**

Two non-free radical pathways have been proposed for the conversion of formate to carbon dioxide: oxidation through the catalase-peroxidative system (Chance 1950), and via the one-carbon pool. Formate enters the one-carbon pool by combining with THF to form 10-formyl-THF, a reaction catalyzed by formyl-THF synthetase (Johlin et al. 1987). This is followed by oxidation of 10-formyl-THF to carbon dioxide, mediated by formyl THF dehydrogenase (10-
Studies have shown that this is the major route of formate metabolism (Chiao and Stokstad 1977; Johlin et al. 1987; Makar and Tephly 1976; Palese and Tephly 1975) and the predominant one in primates (McMartin et al. 1977). Folic acid plays a major role as a coenzyme in one-carbon metabolism and is a key participant in the biosynthesis of DNA, RNA, and certain amino acids. Formate oxidation to carbon dioxide is dependent upon folic acid in rats, monkeys (McMartin et al. 1977; Noker et al. 1980), and humans (Johlin et al. 1989). Although the liver is the main source for folate, Neymeyer et al. showed the presence of folate and 10-FTHFDH in the retina, optic nerve, and various regions of the rat brain (Neymeyer et al. 1997; Neymeyer and Tephly 1994). In the brain, folate levels were found to be present at concentrations between 3% and 14% of those found in the liver. The presence of folate and 10-FTHFDH in the brain suggests that formic acid can be metabolized in this tissue.

Formate can cause oxidative stress by producing free radicals through the Fenton-like reaction (Dikalova et al. 2001; Walling 1975). In this reaction, a hydroxyl radical (·OH) is formed, which in turn oxidizes formate (HCO$_2^-$), forming the carbon dioxide anion radical (·CO$_2^-$). The ·CO$_2^-$ radical then reacts with molecular oxygen, forming carbon dioxide and the cytotoxic reactive oxygen species (ROS) ·O$_2^-$ superoxide radical.

\[
\begin{align*}
    \text{H}_2\text{O}_2 + \text{Fe}^{2+} & \rightarrow \cdot\text{OH} + \text{Fe}^{3+} + \text{OH}^- & \text{[1]} \\
    \text{HCO}_2^- + \cdot\text{OH} & \rightarrow \cdot\text{CO}_2^- + \text{H}_2\text{O} & \text{[2]} \\
    \cdot\text{CO}_2^- + \text{O}_2 & \rightarrow \text{CO}_2 + \cdot\text{O}_2^- & \text{[3]}
\end{align*}
\]
Experiments by Chance have shown that formate can be metabolized by the catalase-peroxidative system (Chance 1950). When anti-oxidants are depleted, increased ROS are formed (Treichel et al. 2004). Formic acid-induced cell damage has been attributed to the generation of the cytotoxic ROS species. Formic acid disrupts mitochondrial electron transport and energy production by inhibiting cytochrome oxidase activity (Nicholls 1975; Nicholls 1976; Sharpe et al. 1982), and causes cell death through the increased production of cytotoxic ROS, secondary to the blockade of the electron transport chain.

The rate of formate oxidation and elimination is dependent on adequate levels of hepatic folic acid, particularly hepatic THF (Johlin et al. 1987; Tephly and McMartin 1974; Tephly 1991). Thus, significantly higher formate levels are achieved when the folate-deficient animals are exposed to MeOH (Dorman et al. 1994; Lee et al. 1994; McMartin et al. 1975; Noker et al. 1980). Tephly has suggested that people with inadequate folate metabolism would be prone to the MeOH toxicity, since they would have difficulty metabolizing one-carbon units (Tephly 1991).

While there is a large body of literature relating maternal folic acid deficiency to fetal neural tube defects and mitochondrial dysfunctions, there are no references relating low dose formic acid to cytotoxicity, and only a few references relating formic acid to mitochondrial inhibition. The latter has been shown in the context of MeOH intoxication and retinal dysfunctions (Seme et al. 1999; Seme et al. 2001). The toxic effects of formate have also been demonstrated in dissociated primary mouse neural cell cultures (Dorman et al. 1993). The concentration of formate that resulted in 50% LDH leakage after 8-hour incubation was
estimated to be 45 mM. The total intracellular ATP concentration was significantly reduced
following exposure to either 20 mM or 40 mM of formate for 8 hours, and is consistent with the
hypothesis that formate may inhibit mitochondrial function, resulting in decreased intracellular
ATP and neurotoxicity.

**Folic Acid and Alcohol (EtOH):**

Human beings are fully dependent on dietary sources or supplements for their folate
supply. Folic acid is an essential coenzyme in normal purine, thymidylate, and methionine
synthesis, and in many other biochemical reactions involving single-carbon transfers. Chronic
alcohol ingestion has been shown to reduce the intestinal absorption of dietary folic acid leading
to a decrease in the folate metabolic pool (Halsted et al. 2002b). As a result, folate deficiency is
a common finding in chronic alcoholics (Axelrod and Daly 1965a; Eells et al. 2000a; Halsted et al.
2002b; Halsted et al. 2010). This malabsorption has been attributed to the downregulation of
folate transporters (Halsted 1980a; Halsted et al. 2002b; Hamid et al. 2007b; Hamid and Kaur
2007a; Thakur and Kaur 2015b). Folate deficiency can lead to a decrease in SAM levels (Miller et
al. 1994). The overall status of the one-carbon pathway is also dependent on the levels of
methionine and vitamin B6 and B12 (Bailey and Gregory 1999; Barak et al. 1991; Barber et al.
1999; Halsted et al. 2002a; Lucock 2000; McPartlin et al. 1993). A decrease in the folate pool leads
to prolonged formate blood levels, since the rate at which formate combines with THF is
reduced, which is the first step in its metabolism to carbon dioxide (McMartin et al. 1977). This
ultimately leads to formate-mediated cytotoxicity.
During pregnancy, folate requirements increase, as it is needed for DNA synthesis and cell division. Fetal folate levels (cord blood levels) are 2- to 4-fold higher relative to maternal blood levels (Economides et al. 1992; Guerra-Shinohara et al. 2002; Stark et al. 2007). Folate deficiency during pregnancy is associated with abnormalities in the fetus (Greenberg et al. 2011; Safi et al. 2012). The extent to which folic acid is transferred across the placenta and into the fetal circulation is mediated by placental folate transporters folate receptor-α at the microvillous membrane of the syncytiotrophoblast (Bisseling et al. 2004; Solanky et al. 2010), the reduced folate carrier, and the proton-coupled folate transporter (Prasad et al. 1995; Yasuda et al. 2008b; Yasuda et al. 2008a). Hutson and colleagues measured folate in both maternal and umbilical cord blood at the time of delivery in pregnancies with chronic and heavy alcohol exposure and in non-drinking controls. They found that among alcohol-exposed pairs, the fetal to maternal folate ratio was reversed with maternal levels being higher (Hutson et al. 2012). This suggests that chronic and heavy alcohol use in pregnancy impairs folate transport across the placental wall. Folate transporters in the alcohol using/abusing pregnant woman must be downregulated at the placental level. The mechanism is perhaps similar to the downregulation of folate transporters in the context of intestinal absorption of dietary folic acid (Halsted 1980b; Halsted et al. 2002b; Hamid et al. 2007a; Hamid and Kaur 2007b; Thakur and Kaur 2015a).

It is well established that ethanol can readily cross the placenta, and acetaldehyde can be been detected in fetal tissues (Karl et al. 1988). Interestingly, our group found formic acid in the CSF of three of four alcoholic patients, and in all four of the corresponding serum samples.
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(Kapur et al. 2007). The presence of formic acid in the CSF suggests that either it crossed the blood-brain barrier or is formed in-situ from the metabolism of water-soluble MeOH that crossed the blood-brain barrier. Thus, it is conceivable that formic acid has the physicochemical properties to cross the placental barrier. Using a human placental perfusion model, Hutson and colleagues showed that formic acid does indeed cross the placenta (Hutson et al. 2013) leading to decreased hCG secretion. A decrease in hCG suggests that formic acid may alter steroidogenesis and differentiation of cytotrophoblasts, and has the potential to be toxic to the developing fetus. The authors also showed that this adverse effect is mitigated by folic acid when it was added to their perfusion model (Hutson et al. 2013). Thus, altered folate concentrations within the placenta and the fetus, combined with increased formic acid, may in part contribute to the deficits observed in FASD. We hypothesize that low folate and high formate contributes to the development of alcohol related brain damage, and that the neuronal damage is caused by oxidative stress and mitochondrial dysfunction.

Ethanol and acetaldehyde can be metabolized in the brain, since the activities of ethanol-metabolizing enzymes – catalase, CYP2E1, ADH, and ALDH – are shown to be present in this tissue (Brzezinski et al. 1999; Kapoor et al. 2006; Roberto et al. 2006; Sun and Sun 2001; Upadhya et al. 2000; Vasiliou et al. 2006; Yadav et al. 2006; Zimatkin et al. 2006). However, placental CYP2E1 is inducible and its activity has been demonstrated to be variable among heavy drinking women (Rasheed et al. 1997). Using an animal model, Yadav and colleagues showed that CYP2E1 activity is present in different regions of the brain (Yadav et al. 2006). Vasiliou and colleagues suggest in the conclusion of their animal work that “although the
contribution and CYP2E1 and catalase in ethanol oxidation may be of little significance, these enzymes appear to play a significant role in ethanol metabolism in the brain” (Vasiliou et al. 2006). Using brain tissues from human abortuses, Brzezinski and co-workers found CYP2E1 activity in various regions of the developing fetal brain (Brzezinski et al. 1999). They stated that “relatively low levels of the P-450 isoform present in conceptal brain may be sufficient to generate reactive intermediates that elicit neuroembryotoxicity following maternal alcohol consumption”. Thus, in the context of alcohol consumption in pregnancy, both formic acid and CYP2E1 activity can mediate toxicity in the developing fetus.

In 1985, Stromland followed 30 children of nine mothers who had used alcohol or drugs during pregnancy and found defects of the outer eye region and/or intraocular abnormalities in 90% of these children (Stromland 1985; Stromland 1987). In a later publication Stromland and Sundelin, (Stromland and Sundelin 1996) described the abnormalities of these same children and found that the children born to mothers who abstained from alcohol during some of their pregnancies were born without birth defects. Anomalies of the outer eye region, like ptosis and strabismus, were frequently found in children with FAS, but were not common in controls. Malformations of the fundus were the most frequently occurring abnormalities in the children with FAS. Forty-eight percent of children showed hypoplasia of the optic nerve head and 49% had abnormal tortuosity of the retinal arteries.

Visual impairment is one of the most common ophthalmic abnormalities found in children who have been identified as having FAS (Hug et al. 2000; Pinazo-Duran et al.).
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The severity of toxic lesions varies among children and can be correlated to the degree of alcohol abuse during pregnancy (Eells et al. 2000b; Pinazo-Duran et al. 1997; Stromland and Pinazo-Duran 1994; Stromland and Sundelin 1996). Formic acid is known to be toxic to the optic nerve. It has been suggested that formate inhibits retinal mitochondrial function and increases oxidative stress (Seme et al. 2001).

Although formic acid and its association with visual impairment has been reported in both animal and human studies, to our knowledge, this association has not been made in children with visual impairment born to mothers who used alcohol during pregnancy.

Brown-Woodman et al. (Brown-Woodman et al. 1995) assessed the embryonic toxicity of formic acid in an in-vitro explanted rat embryo cultured in rat serum and showed that formic acid can be toxic to the embryo. Eells developed a non-primate (rat) model to study MeOH-induced visual toxicity (Eells 1991). The author stated, “relatively brief exposures to formate appear to produce reversible retinotoxic actions detectable by electroretinography (ERG). Furthermore these ERG alterations occur at formate levels lower than those required to affect flash-evoked cortical potential and lower than those associated with retinal and optic disc edema in humans and monkeys.” There are numerous animal studies that report on similar ophthalmologic abnormalities following MeOH administration (Ashwell and Zhang 1994; Eells et al. 1996; Murray et al. 1991; Pinazo-Duran et al. 1993).

Studies on non-rodent Xenopus (frogs) (Yelin et al. 2007) and zebrafish (Marrs et al. 2010) models show that ethanol exposure during early development causes a decrease in the
expression of eye marker morphogenetic genes \textit{Pax6} and \textit{Tbx3}, which are involved in the formation of the eye (Kozmik 2005; Li \textit{et al.} 1997). A reduction in the expression of these genes could be the result of overexpression of sonic hedgehog (\textit{shh}) signaling pathway (Ahlgren \textit{et al.} 2002; Yelin \textit{et al.} 2007). To elucidate the mechanism, Kashyap and colleagues (Kashyap \textit{et al.} 2011) designed a rescue experiment to target both sonic hedgehog (\textit{shh}) and retinoic acid (RA) signaling pathways that may be affected by ethanol. Ethanol is suggested to act as a competitive inhibitor of the enzyme retinol dehydrogenase, which converts retinol (Vitamin A) to RA, a morphogen for vertebrate limb and nervous system morphogenesis (Duester \textit{et al.} 1991). Their studies show that ethanol-mediated defects were independent of these pathways suggesting alternate pathways (Ahlgren \textit{et al.} 2002; Yelin \textit{et al.} 2007). Perhaps the effects of ethanol on these pathways are both tissue and stage specific (Kiecker 2016). In the zebrafish model, Muralidharan and colleagues (Muralidharan \textit{et al.} 2015), studied ethanol-mediated retinal tissue defects and folic acid’s potential for rescue. They showed that folic acid co-supplementation with ethanol significantly rescued optic nerve and photoreceptor defects.

Human visual functions are also impaired with chronic low-level exposure to MeOH (Frederick \textit{et al.} 1984). Toxicity to the visual system produced by either acute, subacute, or chronic MeOH exposure share a common mechanism, which is most likely mediated by formic acid (Eells \textit{et al.} 1996; Murray \textit{et al.} 1991). Formic acid toxicity is likely determined by folic acid and 10-formyltetrahydrofolate dehydrogenase concentrations, which have been shown to be present in both human and rat retinas (Martinasevic \textit{et al.} 1996).
The pattern and timing of maternal alcohol consumption play a significant role in the etiology of FASD. Although the total volume of alcohol intake is an important variable, the number of binge drinking episodes, four or more drinks in about two hours for women, may have a greater impact on the risk of adverse effects (Maier and West 2001). Binge drinking leads to high blood EtOH concentration and perhaps even higher levels of formic acid to which the fetus is exposed. Similarly, chronic drinking likely leads to sustained levels of formic acid in the fetus. Since brain development is continuous during the gestational period, chronic or episodic high-level exposure to formic acid may be a significant contributing factor to the development of FASD (Figure 3).

Several studies have shown that there is enzymatic activity in the brain, which can metabolize both EtOH and acetaldehyde (Brzezinski et al. 1999; Kapoor et al. 2006; Roberto et al. 2006; Sun and Sun 2001; Upadhya et al. 2000; Vasiliou et al. 2006; Yadav et al. 2006; Zimatkin et al. 2006). Yadav et al. measured NDMA-d activity in the presence and absence of alcohol (Yadav et al. 2006). They showed a regional difference in CYP2E1-dependent NDMA-d activity that was inducible when there was pre-treatment with EtOH. Significant increase in enzymatic activity was observed in the olfactory lobe, mid brain, hippocampus, hypothalamus, and cerebellum. Bell and co-workers reported a high prevalence of epilepsy and seizure history in children with FASD (Bell et al. 2010a). Impairments, such as cognitive dysfunctions and seizures are associated with the above mentioned regions of the brain (Matthews and Silvers 2004). Thus, it is conceivable that formic acid that crosses the placental barrier and/or is locally produced from MeOH can significantly impair brain function (Figure 4).
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**Other Mechanisms of Alcohol Teratogenesis**

A variety of other mechanisms have been proposed to explain the toxic effects of ethanol on the growing fetus. In addition to the link between folate deficiency, oxidative stress, and their respective associations with FASD discussed in this paper, ethanol consumption has also been associated with the augmented production of prostaglandins, which can adversely impact fetal development (Ylikorkala *et al.* 1988). Interestingly, it has been demonstrated that the administration of a prostaglandin synthesis inhibitor in mice prior to alcohol consumption is associated with a 50% reduction in malformations relative to mice that receive the inhibitor subsequent to alcohol intake (Randall and Anton 1984).

Oxidative metabolism – the primary mechanism by which ethanol is eliminated in adults – plays a minor role in the metabolism of ethanol in the fetus, especially earlier in gestation (McCarver and Hines 2002; Smith *et al.* 1971). Conversely, the enzymes involved in the non-oxidative metabolism of ethanol are present early in gestation (Bearer *et al.* 1992; Bearer *et al.* 1995; Krekels *et al.* 2012; Stanley *et al.* 2005). Consequently, the non-oxidative pathways of ethanol metabolism may play a compensatory role in early gestation, in the context of alcohol exposure (Zelner and Koren 2013). Fatty acid ethyl esters (FAEE) are non-oxidative metabolites formed via the breakdown of ethanol. These metabolites have been established as biomarkers of chronic excessive alcohol use in adults (Auwarter *et al.* 2001; Pragst *et al.* 2001) and in utero exposure to alcohol (Bearer *et al.* 1992; Bearer *et al.* 1999; Caprara *et al.* 2005; Hungund and
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Gokhale 1994; Klein et al. 1999). FAEE have also been established as mediators of toxicity, since they have been demonstrated to reduce cell proliferation, uncouple oxidative phosphorylation, increase the fragility of lysosomes, decrease protein synthesis, increase the formation of lipid droplets, and incorporate into organic bilayers leading to their disordering (Best and Laposata 2003; Pragst et al. 2001). FAEE have been shown to accumulate in tissues susceptible to the toxic effects of ethanol, such as the liver, heart, brain, and adipose tissue (Laposata and Lange 1986). The presence of FAEE is associated with liver, pancreatic, myocardial, mitochondrial, and CNS damage (Bora et al. 1996; Gubitsi-Klug and Gross 1996; Haber et al. 1993; Hungund et al. 1988; Lange and Sobel 1983; Ponnappa et al. 1994; Szczepiorkowski et al. 1995; Werner et al. 1997). Elevated levels of FAEE in the meconium of ethanol-exposed neonates have been linked to deficits in various CNS domains, such as executive functioning and cognition (Min et al. 2015; Noland et al. 2003; Peterson et al. 2008).

Ethanol has also been associated with the disruption of the endocrine system (Haley et al. 2006), impairment of neurogenesis (Cartwright and Smith 1995; Heaton et al. 2002), a rise in cellular apoptosis (Cartwright and Smith 1995), and alterations in gene expression (Vangipuram et al. 2008). There are a number of risks factors associated with development of FASD, including but not limited to improper nutrition, deficient prenatal care, higher maternal age, paternal drinking, history of substance abuse, having a previous child with FASD, and low education level and socioeconomic status (Esper and Furtado 2014; May and Gossage 2011). Genetic factors may also play a role in the development of FASD (Ramsay 2010). Genetic variants that lead to altered ethanol metabolism can function to modulate the risk of adverse effects (Chernoff 1980). For
instance, variants at the *ADH1B* (*ADH1B*2 and *ADH1B*3) in the mother or child are associated with more efficient ethanol metabolism, which in turn has been associated with a mild protective effect relative to those that do not possess these variants (Arfsten *et al.* 2004; Jacobson *et al.* 2006). Ultimately, it is likely that the interplay of various proposed mechanisms give rise to FASD (Cohen-Kerem and Koren 2003).

**Conclusions**

Studies show that methanol and formic acid levels are elevated in human subjects consuming alcoholic beverages. Alcoholics have low folate levels due to reduced absorption, and following alcohol consumption, formic acid levels rise and can be neurotoxic. FASD is associated with significant neurological problems such as behavioural, cognitive and motor impairment (Chokroborty-Hoque *et al.* 2014) depression (Pei *et al.* 2011), and epileptic seizures (Bell *et al.* 2010b; Nicita *et al.* 2014). Women who consume alcohol during pregnancy may also have low folic acid and higher formic acid levels that can be teratogenic to the fetus. (Iffland and Staak 1990; Jones and Lowinger 1988; Kapur *et al.* 2007; Roine *et al.* 1989). The role of folate in the pathogenesis of neural tube defects (Kim 1999) and cardiovascular disease (Stolzenberg-Solomon *et al.* 1999) is well established. Moreover, epidemiological studies have shown that folate supplements can significantly reduce the risk of pancreatic cancer (Fenech 2001) and risk for cardiovascular diseases (Jiang *et al.* 2003). Folate requirements increase during gestation and folate deficiency during pregnancy is associated with abnormalities in the fetus. Alcohol downregulates folate transport across the placenta resulting in low fetal folate levels. Sustained
levels of formic acid during chronic drinking, and perhaps even higher formate levels during
binge drinking, may explain why binge drinking is particularly harmful to the fetus (Figure 3). This
supports our hypothesis that low folic acid and high formic acid levels are a toxic combination in
pregnant women who drink.

Pregnant women who consume alcohol and are folate-deficient may accumulate formic
acid and produce a high-risk constellation for the fetus. We hypothesize that FASD and
associated neuronal damage occurs through formic acid-induced oxidative stress and
mitochondrial dysfunction. Folic acid and formic acid play a significant part in the etiology of the
very complex syndrome that is FASD.

To our knowledge there is no published literature linking folic acid deficiency and formic
acid to FASD, although numerous studies suggest alcohol-mediated oxidative stress as the
possible mechanism (Brocardo et al. 2011; Dreosti 1993; Gupta et al. 2016; Muralidharan et al.
2015; Sarmah et al. 2016; Sogut et al. 2017). Thus, treatment using nutrients that have anti-
oxidant properties such as folic acid appear promising.

Our hypothesis raises a number of questions that need to be addressed including: what
could be considered clinically relevant concentrations of folic acid and formic acid that cause
teratogenicity? What duration of formic acid exposure will result in fetal cytotoxicity? What role
might formaldehyde and ethanol-induced folic acid deficiency be on the neurodevelopmental
process? Studies in genomic, genetic, and epigenetic mechanism may help in elucidating the
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molecular mechanisms of this very complex disorder. It is clear that further research is required
to answer these and other questions.

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Reference List


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https://mc06.manuscriptcentral.com/bcb-pubs
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Ref Type: Abstract


Public Health Agency of Canada. A Sensible Guide to a Healthy Pregnancy. Public Health Agency of...
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Titles

Figure 1: Methanol metabolism

Figure 2: Metabolism of formate by folate-dependent pathway

Figure 3: FASD – Gestation continuum

Figure 4: Implications of different studi
Figure 1 Methanol metabolism

CH$_3$-OH $\xrightarrow{ADH}$ H$_2$C=O $\xrightarrow{ALDH}$ HO-HC=O $\xrightarrow{formyl THF synthetase}$ CO$_2$ + H$_2$O

methanol    formaldehyde    formic acid    CO$_2$ + H$_2$O
Figure 2: Metabolism of formate by folate-dependent pathway

139x139mm (300 x 300 DPI)
Figure 3: FASD – Gestation continuum

chronic ethanol $\rightarrow$ ↑ methanol, ↓ folic acid, ↑ formic acid
Formic acid is produced locally in the fetal brain, and/or it crosses the placenta from mother to fetus.

Increased formic acid concentration will lead to formation of lesions at the local site.

Lesions can have significant functional impact in these brain tissues/organs.

Figure 4: Implications of different studies

156x84mm (300 x 300 DPI)