

Nonoxidative Ethanol Metabolism in Humans—From Biomarkers to Bioactive Lipids

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Abstract

Ethanol is a widely used psychoactive drug whose chronic abuse is associated with organ dysfunction and disease. Although the prevalent metabolic fate of ethanol in the human body is oxidation a smaller fraction undergoes nonoxidative metabolism yielding ethyl glucuronide, ethyl sulfate, phosphatidylethanol and fatty acid ethyl esters. Nonoxidative ethanol metabolites persist in tissues and body fluids for much longer than ethanol itself and represent biomarkers for the assessment of ethanol intake in clinical and forensic settings. Of note, the nonoxidative reaction of ethanol with phospholipids and fatty acids yields bioactive compounds that affect cellular signaling

pathways and organelle function and may contribute to ethanol toxicity. Thus, despite low quantitative contributions of nonoxidative pathways to overall ethanol metabolism the resultant ethanol metabolites have important biological implications. In this review we summarize the current knowledge about the enzymatic formation of nonoxidative ethanol metabolites in humans and discuss the implications of nonoxidative ethanol metabolites as biomarkers of ethanol intake and mediators of ethanol toxicity. © 2016 The Authors IUBMB Life published by Wiley Periodicals, Inc. on behalf of International Union of Biochemistry and Molecular Biology, 68(12):916–923, 2016

Keywords: nonoxidative ethanol metabolism; ethyl glucuronide; ethyl sulfate; phosphatidylethanol; fatty acid ethyl ester

Introduction

The consumption of ethanol has a widespread social tradition among many populations worldwide. Whereas moderate ethanol intake has been regarded beneficial to cardiovascular health, chronic alcohol abuse is associated with an increased risk of pancreatitis, cardiomyopathy, liver disease and cancer

(1). Although the cellular and molecular etiology underlying ethanol-associated diseases is incompletely understood a causative role has been attributed to the metabolic conversion of ethanol resulting in the generation of toxic intermediates and metabolic stress. After absorption by the oral, gastric and intestinal mucosae and distribution via the circulation the majority of ingested ethanol (95–98%) is metabolized and only a small fraction is excreted unchanged via breath, urine and sweat. The prevalent route of human ethanol metabolism is hepatic ethanol oxidation, which eliminates more than 90% of the ingested ethanol. The first step in the oxidative pathway is the conversion of ethanol to acetaldehyde catalyzed by cytosolic alcohol dehydrogenase and to a lesser extent by microsomal cytochrome P450 isoforms (especially CYP2E1) and catalase. In a second step, acetaldehyde dehydrogenase oxidizes acetaldehyde to acetate, which is mainly secreted into the circulation and converted to acetyl-coenzyme A (CoA) by extrahepatic tissues such as muscle, heart and brain (2). In addition to oxidation, several nonoxidative routes of ethanol metabolism have been described that result in the enzymatic conjugation of ethanol to endogenous metabolites such as glucuronic acid, sulfate, phospholipids and fatty acids (FAs). The resultant metabolites are termed ethyl glucuronide (EtG), ethyl sulfate (EtS), phosphatidylethanol (PEth) and fatty acid ethyl ester (FAEE).

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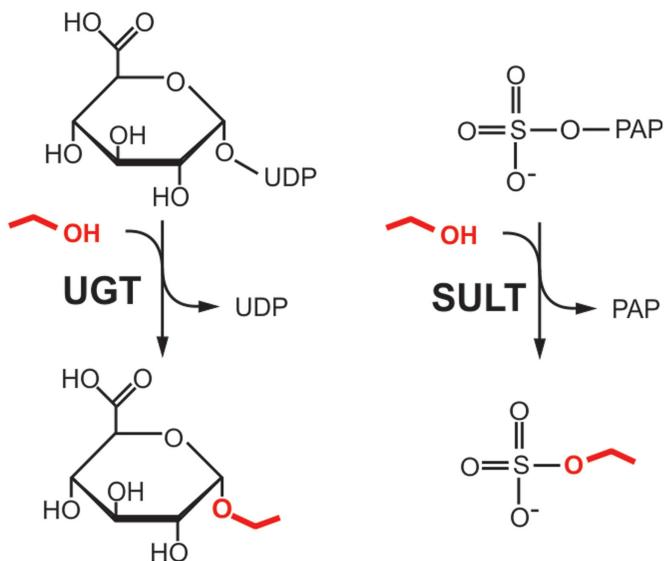


FIG 1

Enzymatic formation of EtG and EtS. EtG is formed by transfer of a glucuronyl moiety from UDP-glucuronic acid to ethanol (marked red) catalyzed by UGT. EtS is formed by transfer of a sulfonate group from 3-phosphoadenosine-5-phospho (PAP) sulfate to ethanol catalyzed by SULT.

In quantitative terms nonoxidative pathways constitute a minor fraction of total ethanol metabolism (2). However, due to slower elimination rates nonoxidative ethanol metabolites persist in body fluids and tissues for much longer than ethanol itself. This characteristic makes nonoxidative ethanol metabolites biomarkers that enable the retrospective assessment of ethanol intake even when ethanol itself is no longer present in the body. Moreover, accumulating evidence suggests that formation of specific nonoxidative ethanol metabolites interferes with cellular signaling pathways, disrupts organelle function and contributes to ethanol toxicity in organs with limited oxidative capacity. In the following sections we will review the biochemistry and biological significance of nonoxidative ethanol metabolism and discuss the implications of nonoxidative ethanol metabolites as biomarkers for ethanol intake and mediators of ethanol toxicity.

Ethyl Glucuronide and Ethyl Sulfate

Formation and Tissue Distribution

EtG is formed by transfer of a glucuronyl moiety from uridine 5'-diphospho (UDP)-glucuronic acid to ethanol (Fig. 1). This reaction is catalyzed by UDP-glucuronosyltransferases (UGTs), an enzyme family involved in phase II metabolism of xenobiotics as well as glucuronidation of endogenous metabolites (3). EtG was first identified in urine of ethanol-intoxicated rats and afterwards in blood and urine of ethanol-consuming humans (4,5). Blood EtG can be detected 1 h after beginning of ethanol intake, and peak concentrations are typically reached between 3.5 and 5.5 h (6,7). In addition to blood, detectable EtG levels have been reported in several other tissues including adipose

tissue, liver, brain, bone marrow, muscle and hair (8,9). Excretion of EtG occurs mainly via the urine and was shown to account for a minor part (<0.1%) of total ethanol elimination (10). EtS is formed by sulfonation of ethanol catalyzed by another class of phase II enzymes termed sulfotransferases (SULTs, Fig. 1). Like EtG, EtS was first observed in rats after ethanol administration and later detected in urine of ethanol-consuming human subjects (11,12). The formation, pharmacokinetics and elimination of EtS are similar to EtG and <0.1% of the ingested ethanol is typically excreted as urinary EtS (10). In addition to blood and urine, EtS has also been detected in liver, kidney, placenta, fetal tissues and hair (13–15).

Enzymes Catalyzing EtG and EtS Formation

The human genome encodes for 22 UGTs, which are divided into three subfamilies, termed UGT1A, UGT2A and UGT2B. UGTs are localized at the endoplasmic reticulum with active sites facing the lumen. Each UGT isoform shows a tissue-specific expression pattern. Liver, gastrointestinal tract and kidney express the highest levels of UGT isoforms (3). Consistent with the tissue expression and subcellular localization of UGTs, high ethanol glucuronidation activities have been measured in microsomal preparations of human liver and kidney (16). In addition, several UGT1A and UGT2B family members have been shown to catalyze EtG formation *in vitro*. Among them, UGT2B7 and UGT1A9 exhibited highest ethanol glucuronidation activities (16,17). Based on inhibitor experiments, these isoforms were estimated to account for ~50% of ethanol glucuronidation activity in human liver microsomes (16,18). Notably, the reported K_m values of ethanol glucuronidation exceed physiologically attainable ethanol concentrations (16,19). These observations likely reflect low affinity of UGTs for ethanol and may explain the limited contribution of glucuronidation to overall ethanol metabolism. The enzymatic sulfonation of ethanol was first observed in rat liver samples and was later also measured in tissue extracts of human liver, intestine and lung (11,20). To date 14 human SULT isoforms with different substrate specificities and tissue expression patterns have been described. SULTs catalyze the sulfonation of xenobiotics and endogenous metabolites using 3'-phosphoadenosine-5'-phosphosulfate as donor (21). According to *in vitro* studies using recombinant SULTs, members of the 1A, 1B, 1C, 1E and 2A subfamilies are able to catalyze sulfonation of ethanol. Based on its high expression in liver SULT1A1 has been suggested to be a major contributor to hepatic EtS formation (22). However, the relative contribution of SULT isoforms to EtS formation *in vivo* is currently unknown.

EtG and EtS as Biomarkers of Ethanol Intake

EtG and EtS exhibit extended half-lives in body fluids as compared to nonmetabolized ethanol and have been used as biomarkers for recent ethanol intake and abstinence monitoring. After a single event of ethanol intake the time frame of detectable serum EtG and EtS exceeds that of blood ethanol by 4–8 h (6,7). Moreover, urinary EtG and EtS remain detectable for 22–48 h after a single ethanol intake and for 40–130 h in heavy drinkers after withdrawal. As a consequence,

determination of EtG and EtS in serum or urine permits verification of ethanol consumption even after ethanol is no longer detectable (6,7,23,24). Further matrices for the detection of EtG (and EtS) are sweat, oral fluid, hair, meconium and placenta (14,25–28). The deposition of EtG in hair occurs through blood or sweat and is stable for several months thus permitting retrospective assessment of ethanol intake even after long periods of abstinence (29). Correlations between the amount of ingested ethanol and hair EtG concentrations have been observed in rodent models and human subjects, and threshold levels have been proposed to discriminate chronic excessive ethanol consumption from moderate drinking and abstinence (29–31).

Biological Significance of EtG and EtS

Phase II modifications increase water solubility and facilitate excretion of metabolites (3). Hence, EtG and EtS are not considered as bioactive metabolites. EtG as well as glucuronic acid have recently been demonstrated to activate toll like receptor (TLR) 4 signaling *in vitro* and to cause allodynia in rats after intrathecal administration (32). However, whether EtG concentrations obtained after ethanol consumption are sufficient to activate TLR4 signaling *in vivo* remains to be established.

Phosphatidylethanol

Formation and Tissue Distribution

PEth is formed by transphosphatidylation of phospholipids with ethanol, which was first observed by Alling et al. (33) in ethanol-intoxicated rats. PEth is detectable 2 h after acute ethanol intoxication in a multitude of rat tissues with highest levels in gastrointestinal tract, liver, lung and kidney (34). A similar tissue distribution of PEth deposition was also demonstrated in post-mortem tissue samples of human subjects intoxicated with ethanol (34,35). Furthermore, PEth can be found in blood of social drinkers and alcoholics and can thus serve as biomarker of ethanol intake (see below). After a single ethanol dose, blood PEth levels increase immediately reaching peak concentrations approximately 90–120 min after termination of drinking. With decreasing blood ethanol concentrations PEth levels decline with an estimated half-life of 3–5 days but remain detectable for up to 28 days after sobriety (36,37). The half-life of PEth is tissue-specific ranging from 1–2 h in pancreatic islets and perfused hearts to 10–17 h in brain (38–40).

Enzymatic Formation and Degradation of PEth

The transphosphatidylation of phospholipids and ethanol is catalyzed by phospholipase D (PLD) (41,42). Under physiological conditions, PLD catalyzes hydrolysis of membrane phospholipids (predominantly phosphatidylcholine, PC) to phosphatidic acid (PA). PLD-generated PA serves as lipid messenger and has been implicated in several important cellular processes such as membrane trafficking, cytoskeletal reorganization, endocytosis, proliferation and migration (43). In the presence of ethanol and other primary alcohols PLD performs a transphosphatidylation reaction resulting in the formation of PEth or other phosphatidylalcohols (Fig. 2). Interestingly,

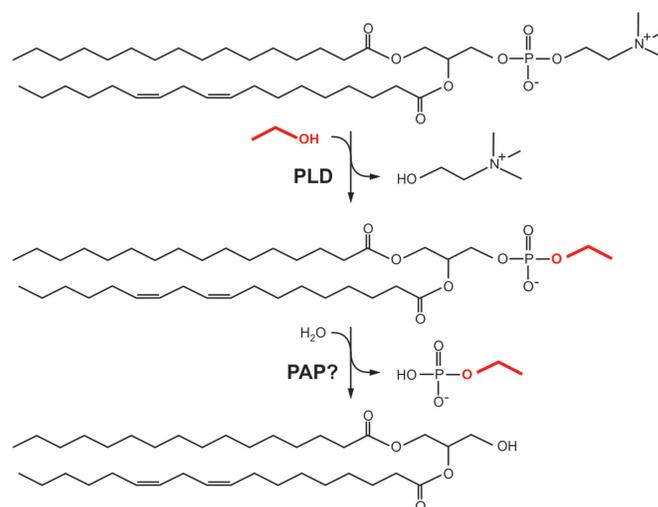


FIG 2

Enzymatic formation and degradation of PEth. PLD catalyzes transphosphatidylation of phosphatidylcholine and ethanol (marked red) resulting in the formation of PEth and the release of choline. PEth may be further metabolized by phosphatidate phosphohydrolase (PAP) to diacylglycerol and ethyl phosphate.

transphosphatidylation is more efficient than hydrolysis and has therefore been routinely used to measure PLD activity *in vitro* (43). In cultured cells, the rate of PEth formation correlates with ethanol concentrations in the medium and is promoted by pharmacological activation of PLD (44–46). Several studies indicate that PEth is mainly formed from PC (47,48). Both, PLD1 and PLD2, two major mammalian PLD isoforms, have been shown to catalyze PEth formation *in vitro* (49). However, the contribution of each isoform to PEth synthesis *in vivo* has not been addressed. Cell culture studies indicate that PEth turnover occurs at slower rates compared to PA indicating that PEth is more resistant to further metabolic conversions (38,50). Several phospholipases have been implicated in PEth breakdown including phospholipase A₂, PC phospholipase C and PA phosphohydrolase (38,51,52). Propranolol, an inhibitor of PA phosphohydrolase, has been shown to block PEth turnover in rat pancreatic islets and human hepatoma cells indicating a major role of this enzyme in PEth degradation (Fig. 2) (38,52). This notion is further supported by the presence of ethyl phosphate, a possible product of PA phosphohydrolase-mediated PEth hydrolysis, in ethanol-intoxicated rats (53).

PEth as Biomarker of Ethanol Intake

Due to slow PEth elimination rates, detection of blood PEth permits verification of ethanol intake even after several days of abstinence. The majority of blood PEth is associated with erythrocytes whereas only a minor fraction can be found in leukocytes and plasma (54). As an alternative to whole blood samples PEth was also successfully detected in dried blood spots (55). Blood PEth levels were found to correlate with reported ethanol intake of alcoholic subjects and threshold levels considered typical of chronic ethanol abuse have been

proposed to discriminate between moderate drinking and chronic ethanol abuse (10). Other matrices suitable for the detection of PEth are exhaled breath and umbilical cord (55,56).

Biological Significance of PEth

Because PEth formation occurs at the expense of PA upon activation of PLD it has been suggested that this reaction interferes with PLD-mediated cellular processes (44,45). In line with this notion, PEth formation was shown to inhibit PLD-dependent mitogenic effects of serum and carbachol in astrocytes (46,57). Conversely, PEth accumulation was shown to promote intestinal hyperplasia suggesting that cellular PEth formation can have promotive and antimutagenic effects (58). Moreover, PEth formation has been associated with increased basal levels of inositol 1,4,5-trisphosphate, which is derived from the action of PLC indicating that PEth may influence other phospholipid signaling pathways apart from the PLD/PA axis (59). In line with this notion, several studies indicate that PEth directly affects structural properties of biomembranes and alters the function of membrane-associated proteins. For example, inclusion of PEth in artificial or natural phospholipid membranes increases membrane fluidity and alters vesicle fusion in response to Ca^{2+} (60,61). Furthermore, PEth was shown to affect the activity of several transporters and enzymes *in vitro* including Na^+/K^+ -ATPase, protein kinase C, phospholipase C and cytosolic phospholipase A_2 (60, 62–65). Taken together, these findings suggest that PEth formation affects cellular signaling pathways by multiple mechanisms including competition with PA synthesis and functional disturbance of biomembranes and membrane-associated proteins.

Fatty Acid Ethyl Ester

Formation and Tissue Distribution

FAEEs are formed through the enzymatic esterification of ethanol with FAs. These ethanol metabolites have been described first by Goodman and Deykin in total body lipid extracts of rats acutely intoxicated with ethanol and were later found in multiple tissues of rodents subjected to acute or chronic ethanol exposure (66–68). In the 1980s, the presence of FAEEs was first detected in post-mortem tissue samples of human subjects acutely intoxicated with ethanol (69) and up to now, a range of different tissues has been shown to possess FAEE synthetic activity *in vitro* and to accumulate FAEE *in vivo* after ethanol intake. Among them, highest FAEE levels have been consistently reported in liver and pancreas. Detectable amounts of FAEE are formed within minutes in cultured cells and perfused organs and dose–response relationships have been observed for FAEE synthesis rates and extracellular ethanol concentrations (70–72). Consistent with this, oral intake of ethanol rapidly increases blood FAEE levels in humans within several minutes, and peak concentrations are usually reached within a few hours closely paralleling blood ethanol levels (73). Rapid onset of FAEE formation and deposition was demonstrated also in other tissues such as placenta, heart, liver and

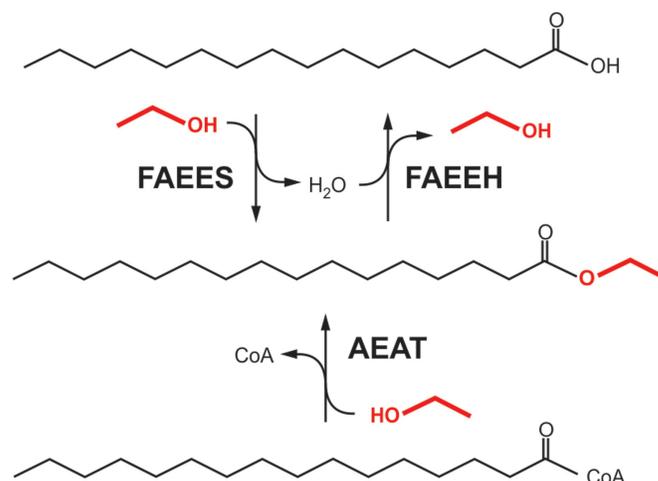


FIG 3

Enzymatic formation and degradation of FAEE. FAEE is formed by esterification of FA with ethanol (marked red) catalyzed by FAEES or by transfer of an acyl moiety from acyl-CoA to ethanol catalyzed by AEAT. FAEH catalyzes hydrolysis of FAEE to FA and ethanol.

brain (74–76). With the elimination of ethanol from the body, FAEE levels in blood and other tissues decline (73,76). Yet, detectable amounts of FAEE persist in human blood for 24 h (social drinkers) to 99 h (heavy drinkers) and in rodent tissues for several days after termination of ethanol intake (73–75,77).

Enzymatic Formation and Degradation of FAEE

Two enzyme classes catalyzing FAEE synthesis have been identified: FAEE synthase (FAEES) esterifies ethanol with free FAs whereas acyl-CoA-ethanol-*O*-acyltransferase (AEAT) transfers acyl moieties from acyl-CoA to ethanol (Fig. 3) (69,78,79). In addition, FAEE may also form upon “ethanolysis” (instead of hydrolysis) of ester bonds in glycerolipids such as triacylglycerol or phospholipids catalyzed by (phospho)lipases (80,81). FAEES activity has been found in soluble and microsomal fractions of multiple tissues with highest activities in pancreas and liver (69,79,82,83). Inhibitor studies suggest that FAEES enzymes belong to the serine hydrolase family (83–85). Accordingly, several serine hydrolases with FAEES activity have been identified including carboxylesterases from heart, liver and adipose tissue, cholesterol ester hydrolase and triacylglycerol lipase from pancreas and lipoprotein lipase (81,86–91). Estimates on the contribution of these enzymes to tissue FAEES activities mainly rely on tissue expression patterns and enzyme inhibition studies. Tsujita and Okuda (80) removed FAEES activity from extracts of adipose tissue, testis, liver, small intestine and lung by immunoprecipitation with an antiserum generated against purified adipose tissue carboxylesterase indicating a possible function of this enzyme as FAEES. Interestingly, substantial residual FAEES activity was observed in liver, small intestine and kidney suggesting tissue-specific contributions of different FAEES

isoforms. The differential susceptibility of hepatic and pancreatic FAEEs to organophosphates further supports this notion (92). However, as none of these *in vitro* studies has been verified in mutant animal models, the contribution of each FAEEs enzyme to FAEE formation *in vivo* remains unknown. AEAT activity has been measured in homogenates and microsomal fractions of multiple tissues with highest levels in liver and small intestine (83,93). Notably, AEAT activity was membrane-associated and oriented mainly toward the lumen of isolated microsomes (68,79,93). Differential inhibition of AEAT and FAEEs by several reagents indicates that these activities are mediated by distinct enzymes (83,84). The cysteine-reactive compound *p*-chloromercuribenzenesulfonate inhibited both AEAT and acyl-CoA hydrolase activities suggesting a relationship between both enzyme classes (79). However, unlike FAEEs AEAT enzymes have not been purified from tissues and the molecular identity of AEAT remains elusive. FAEE hydrolyase (FAEEH) catalyzes the cleavage of the ester bond within the FAEE molecule resulting in the release of FA and ethanol. This reaction may counteract FAEE formation or remove FAEE from cellular deposits and therefore likely determines the tissue half-life of FAEE (Fig. 3). FAEEH activities have been measured in a multitude of tissues, and highest activities have been observed in pancreas and liver (83). Several serine hydrolases with FAEEH activity have been identified including hepatic carboxylesterase isoforms, hormone-sensitive lipase and monoacylglycerol lipase (MGL) (84,94,95). Based on the abundance of carboxylesterase isoforms Diczfalusy et al. concluded that Es-4 (also known as Ces1f) may be responsible for a considerable portion of rat hepatic FAEEH activity. However, the same group failed to detect expression of this isoform in human liver suggesting species-specific differences in the molecular identity of hepatic FAEEH (83,84). Recently, overexpression of MGL, a rate-limiting enzyme for monoacylglycerol hydrolysis in multiple tissues, has been shown to attenuate FAEE accumulation in cultured cells incubated with ethanol. Conversely, pharmacological inhibition of MGL augmented intracellular FAEE suggesting that FAEE hydrolysis by MGL is a major determinant of cellular FAEE levels. Interestingly, MGL as well as FAEE were found to associate with lipid droplets (LDs), which are cellular storage organelles of hydrophobic esters such as triacylglycerol or steryl esters. This suggests that FAEE are transiently stored in LDs and hydrolyzed by LD-associated MGL (95).

FAEE as Biomarker of Ethanol Intake

FAEE depositions in hair have been proposed as suitable markers for the retrospective detection of alcohol abuse and the monitoring of abstinence (96). Hair FAEEs are most likely incorporated from sebum, which is produced by the sebaceous glands and regularly coats the hair shaft (97). FAEE can persist for months in hair of alcoholics even after a time of abstinence (97). Lower levels of FAEE are also found in hair of social drinkers, and traces are occasionally also detected in

teetotalers likely reflecting FAEE formation from ectopically applied ethanol-containing cosmetics (97). Typical for hair analysis, there is no clear relationship between consumed ethanol and hair FAEE levels. Besides differences in hair growth and ethanol metabolism also hair care and the use of cosmetics may affect hair FAEE concentrations. Nevertheless, suitable cutoff values have been established to discriminate heavy alcohol consumers from social drinkers (10,97). FAEE have also been detected in meconium of newborns thus permitting determination of prenatal ethanol exposure (98). It has been shown that FAEE are unable to cross the placenta and hence derive from ethanol metabolism in the fetus (99). 75% of the FAEE detected in meconium reflect ethanol intake during the last 8 weeks of pregnancy suggesting that fetal ethanol exposure during early pregnancy may not be adequately detected by this approach (55). Other matrices for the detection of FAEE are blood and sebum (55,77).

Biological Significance of FAEE

Since the identification of FAEE in organs commonly damaged by ethanol such as heart, brain, pancreas and liver, an increasing number of studies linked FAEE formation to ethanol toxicity. Toxic cellular effects ascribed to FAEE include inhibition of cell proliferation, destabilization of lysosomes, mitochondrial depolarization and induction of apoptosis (100–104). Of note, infusion of FAEE or a combination of ethanol and FA into rodents elicited pancreatic edema, inflammation and necrosis resembling the characteristics of alcoholic pancreatitis (103,105). Incubation of isolated pancreatic acinar cells with FAEE induced mitochondrial depolarization, depletion of cellular ATP and sustained elevations of intracellular Ca^{2+} levels ultimately associated with cellular dysfunction and cell death (106). Similar effects were observed when endogenous FAEE formation was promoted by incubating acinar cells with FA and ethanol in the presence of pharmacological inhibitors of ethanol oxidation. Of note, pharmacological inhibition of FAEE formation ameliorated pancreatic toxicity induced by FA and ethanol both *in vitro* and *in vivo* (103). At present it is unclear if FAEE itself or FAEE metabolites such as FA mediate FAEE-associated cellular toxicity. It has been speculated that FAEE hydrolysis and the subsequent liberation of FA may be a critical molecular event through which FAEE induces cellular dysfunction (107,108). This hypothesis is supported by a recent study showing that infusion of free FAs causes more severe pancreatic damage than infusion of the corresponding FAEE species (109). In addition to a possible role in mediating ethanol toxicity FAEEs have also been characterized as odor active substances that contribute to the flavor of food and beverages. Moreover, specific FAEE species act as pheromones in insects indicating that FAEEs can activate olfactory/pheromone receptors (110). It remains to be investigated whether FAEEs produced in response to alcohol consumption also act as receptor agonist contributing to psychoactive (or other) effects of ethanol.

Conclusions

Although the quantitative contribution of nonoxidative pathways to human ethanol metabolism is low, the resultant metabolites have important analytical and biological implications. The detection of nonoxidative ethanol metabolites in body fluids, hair and neonatal matrices provides a valuable tool for the monitoring and retrospective assessment of ethanol intake. Different elimination rates of nonoxidative ethanol metabolites permit a wide range of analytical time frames for the verification of ethanol intake ranging from hours to months after termination of ethanol consumption. The enzymatic reaction of ethanol with cellular lipids generates bioactive metabolites such as FAEE and PEth, which have been shown to interfere with cellular signaling pathways and organelle function and may therefore contribute to specific manifestations of ethanol toxicity. Although considerable research has been performed regarding the enzymology of nonoxidative ethanol metabolism our current knowledge is limited mainly to *in vitro* studies. The characterization of enzymes and signaling pathways mediating effects of nonoxidative ethanol metabolites *in vivo* is thus inevitable to improve our understanding of nonoxidative ethanol metabolites as biomarkers and bioactive molecules.

References

- [1] Shield, K. D., Parry, C., and Rehm, J. (2013) Chronic diseases and conditions related to alcohol use. *Alcohol Res.* 35, 155 – 173.
- [2] Dinis-Oliveira, R. J. (2016) Oxidative and non-oxidative metabolomics of ethanol. *Curr. Drug Metab.* 17, 327–335.
- [3] Rowland, A., Miners, J. O., and Mackenzie, P. I. (2013) The UDP-glucuronosyltransferases: their role in drug metabolism and detoxification. *Int. J. Biochem. Cell Biol.* 45, 1121–1132.
- [4] Kamil, I. A., Smith, J. N., and Williams, R. T. (1951) The metabolism of aliphatic alcohols; glucuronide formation. *Biochem. J.* 49, xxxviii.
- [5] Jaakonmaki, P. I., Knox, K. L., Horning, E. C., and Horning, M. G. (1967) The characterization by gas-liquid chromatography of ethyl beta-d-glucosiduronic acid as a metabolite of ethanol in rat and man. *Eur. J. Pharmacol.* 1, 63–70.
- [6] Halter, C. C., Dresen, S., Auwaerter, V., Wurst, F. M., and Weinmann, W. (2008) Kinetics in serum and urinary excretion of ethyl sulfate and ethyl glucuronide after medium dose ethanol intake. *Int. J. Legal Med.* 122, 123–128.
- [7] Hoiseth, G., Bernard, J. P., Karinen, R., Johnsen, L., Helander, A., et al. (2007) A pharmacokinetic study of ethyl glucuronide in blood and urine: applications to forensic toxicology. *Forensic Sci. Int.* 172, 119–124.
- [8] Wurst, F. M., Schuttler, R., Kempter, C., Seidl, S., Gilg, T., et al. (1999) Can ethyl glucuronide be determined in post-mortem body fluids and tissues? *Alcohol Alcohol* 34, 262–263.
- [9] Schloegl, H., Rost, T., Schmidt, W., Wurst, F. M., and Weinmann, W. (2006) Distribution of ethyl glucuronide in rib bone marrow, other tissues and body liquids as proof of alcohol consumption before death. *Forensic Sci. Int.* 156, 213–218.
- [10] Wurst, F. M., Thon, N., Yegles, M., Schruck, A., Preuss, U. W., et al. (2015) Ethanol metabolites: their role in the assessment of alcohol intake. *Alcohol Clin. Exp. Res.* 39, 2060–2072.
- [11] Vestermark, A. and Bostrom, H. (1959) Studies on ester sulfates. V. On the enzymatic formation of ester sulfates of primary aliphatic alcohols. *Exp. Cell Res.* 18, 174–177.
- [12] Helander, A. and Beck, O. (2004) Mass spectrometric identification of ethyl sulfate as an ethanol metabolite in humans. *Clin. Chem.* 50, 936–937.
- [13] Politi, L., Morini, L., Mari, F., Groppi, A., and Bertol, E. (2008) Ethyl glucuronide and ethyl sulfate in autopsy samples 27 years after death. *Int. J. Legal Med.* 122, 507–509.
- [14] Morini, L., Falcon, M., Pichini, S., Garcia-Algar, O., Danesino, P., et al. (2011) Ethyl-glucuronide and ethyl-sulfate in placental and fetal tissues by liquid chromatography coupled with tandem mass spectrometry. *Anal. Biochem.* 418, 30–36.
- [15] Skopp, G., Schmitt, G., Potsch, L., Dronner, P., Aderjan, R., et al. (2000) Ethyl glucuronide in human hair. *Alcohol Alcohol* 35, 283–285.
- [16] Al Saabi, A., Allorge, D., Sauvage, F. L., Tournel, G., Gaulier, J. M., et al. (2013) Involvement of UDP-glucuronosyltransferases UGT1A9 and UGT2B7 in ethanol glucuronidation, and interactions with common drugs of abuse. *Drug Metab. Dispos.* 41, 568–574.
- [17] Schwab, N. and Skopp, G. (2014) Identification and preliminary characterization of UDP-glucuronosyltransferases catalyzing formation of ethyl glucuronide. *Anal. Bioanal. Chem.* 406, 2325–2332.
- [18] Foti, R. S. and Fisher, M. B. (2005) Assessment of UDP-glucuronosyltransferase catalyzed formation of ethyl glucuronide in human liver microsomes and recombinant UGTs. *Forensic Sci. Int.* 153, 109–116.
- [19] Fu, J., Liu, H., Xing, H., Sun, H., Ma, Z., and Wu, B. (2014) Comparative analysis of glucuronidation of ethanol in treeshrews, rats and humans. *Xenobiotica* 44, 1067–1073.
- [20] Kurogi, K., Davidson, G., Mohammed, Y. I., Williams, F. E., Liu, M. Y., et al. (2012) Ethanol sulfation by the human cytosolic sulfotransferases: a systematic analysis. *Biol. Pharm. Bull.* 35, 2180–2185.
- [21] Tibbs, Z. E., Rohn-Glowacki, K. J., Crittenden, F., Guidry, A. L., and Falany, C. N. (2015) Structural plasticity in the human cytosolic sulfotransferase dimer and its role in substrate selectivity and catalysis. *Drug Metab. Pharmacokinet.* 30, 3–20.
- [22] Stachel, N. and Skopp, G. (2016) Identification and characterization of sulfotransferases catalyzing ethyl sulfate formation and their inhibition by polyphenols. *Int. J. Legal Med.* 130, 139–146.
- [23] Dahl, H., Stephanson, N., Beck, O., and Helander, A. (2002) Comparison of urinary excretion characteristics of ethanol and ethyl glucuronide. *J. Anal. Toxicol.* 26, 201–204.
- [24] Helander, A., Bottcher, M., Fehr, C., Dahmen, N., and Beck, O. (2009) Detection times for urinary ethyl glucuronide and ethyl sulfate in heavy drinkers during alcohol detoxification. *Alcohol Alcohol* 44, 55–61.
- [25] Schummer, C., Appenzeller, B. M., and Wennig, R. (2008) Quantitative determination of ethyl glucuronide in sweat. *Ther. Drug Monit.* 30, 536–539.
- [26] Hegstad, S., Johnsen, L., Morland, J., and Christophersen, A. S. (2009) Determination of ethylglucuronide in oral fluid by ultra-performance liquid chromatography-tandem mass spectrometry. *J. Anal. Toxicol.* 33, 204–207.
- [27] Pragst, F. and Yegles, M. (2008) Determination of fatty acid ethyl esters (FAEE) and ethyl glucuronide (EtG) in hair: a promising way for retrospective detection of alcohol abuse during pregnancy? *Ther. Drug Monit.* 30, 255–263.
- [28] Himes, S. K., Dukes, K. A., Tripp, T., Petersen, J. M., Raffo, C., et al. (2015) Clinical sensitivity and specificity of meconium fatty acid ethyl ester, ethyl glucuronide, and ethyl sulfate for detecting maternal drinking during pregnancy. *Clin. Chem.* 61, 523–532.
- [29] Appenzeller, B. M., Agirman, R., Neuberger, P., Yegles, M., and Wennig, R. (2007) Segmental determination of ethyl glucuronide in hair: a pilot study. *Forensic Sci. Int.* 173, 87–92.
- [30] Kharbouche, H., Steiner, N., Morelato, M., Staub, C., Boutrel, B., et al. (2010) Influence of ethanol dose and pigmentation on the incorporation of ethyl glucuronide into rat hair. *Alcohol* 44, 507–514.
- [31] Kintz, P. (2015) 2014 consensus for the use of alcohol markers in hair for assessment of both abstinence and chronic excessive alcohol consumption. *Forensic Sci. Int.* 249, A1–A2.
- [32] Lewis, S. S., Hutchinson, M. R., Zhang, Y., Hund, D. K., Maier, S. F., et al. (2013) Glucuronic acid and the ethanol metabolite ethyl-glucuronide cause toll-like receptor 4 activation and enhanced pain. *Brain Behav. Immun.* 30, 24–32.
- [33] Alling, C., Gustavsson, L., and Anggard, E. (1983) An abnormal phospholipid in rat organs after ethanol treatment. *FEBS Lett.* 152, 24–28.
- [34] Aradottir, S., Lundqvist, C., and Alling, C. (2002) Phosphatidylethanol in rat organs after ethanol exposure. *Alcohol Clin. Exp. Res.* 26, 514–518.

- [35] Aradottir, S., Seidl, S., Wurst, F. M., Jonsson, B. A., and Alling, C. (2004) Phosphatidylethanol in human organs and blood: a study on autopsy material and influences by storage conditions. *Alcohol Clin. Exp. Res.* 28, 1718–1723.
- [36] Viel, G., Boscolo-Berto, R., Cecchetto, G., Fais, P., Nalesso, A., et al. (2012) Phosphatidylethanol in blood as a marker of chronic alcohol use: a systematic review and meta-analysis. *Int. J. Mol. Sci.* 13, 14788–14812.
- [37] Javors, M. A., Hill-Kapturczak, N., Roache, J. D., Karns-Wright, T. E., and Dougherty, D. M. (2016) Characterization of the pharmacokinetics of phosphatidylethanol 16:0/18:1 and 16:0/18:2 in human whole blood after alcohol consumption in a clinical laboratory study. *Alcohol Clin. Exp. Res.* 40, 1228–1234.
- [38] Metz, S. A. and Dunlop, M. (1991) Inhibition of the metabolism of phosphatidylethanol and phosphatidic acid, and stimulation of insulin release, by propranolol in intact pancreatic islets. *Biochem. Pharmacol.* 41, R1–R4.
- [39] Lundqvist, C., Aradottir, S., Alling, C., Boyano-Adanez, M. C., and Gustavsson, L. (1994) Phosphatidylethanol formation and degradation in brains of acutely and repeatedly ethanol-treated rats. *Neurosci. Lett.* 179, 127–131.
- [40] Bruhl, A., Faldum, A., and Loffelholz, K. (2003) Degradation of phosphatidylethanol counteracts the apparent phospholipase D-mediated formation in heart and other organs. *Biochim. Biophys. Acta* 1633, 84–89.
- [41] Gustavsson, L. and Alling, C. (1987) Formation of phosphatidylethanol in rat brain by phospholipase D. *Biochem. Biophys. Res. Commun.* 142, 958–963.
- [42] Kobayashi, M. and Kanfer, J. N. (1987) Phosphatidylethanol formation via transphosphatidylation by rat brain synaptosomal phospholipase D. *J. Neurochem.* 48, 1597–1603.
- [43] Frohman, M. A. (2015) The phospholipase D superfamily as therapeutic targets. *Trends Pharmacol. Sci.* 36, 137–144.
- [44] Bocchino, S. B., Wilson, P. B., and Exton, J. H. (1987) Ca²⁺-mobilizing hormones elicit phosphatidylethanol accumulation via phospholipase D activation. *FEBS Lett.* 225, 201–204.
- [45] Pai, J. K., Siegel, M. I., Egan, R. W., and Billah, M. M. (1988) Activation of phospholipase D by chemotactic peptide in HL-60 granulocytes. *Biochem. Biophys. Res. Commun.* 150, 355–364.
- [46] Kotter, K. and Klein, J. (1999) Ethanol inhibits astroglial cell proliferation by disruption of phospholipase D-mediated signaling. *J. Neurochem.* 73, 2517–2523.
- [47] Holbrook, P. G., Pannell, L. K., Murata, Y., and Daly, J. W. (1992) Molecular species analysis of a product of phospholipase D activation. Phosphatidylethanol is formed from phosphatidylcholine in phorbol ester- and bradykinin-stimulated PC12 cells. *J. Biol. Chem.* 267, 16834–16840.
- [48] Huang, C. F. and Cabot, M. C. (1990) Phorbol diesters stimulate the accumulation of phosphatidate, phosphatidylethanol, and diacylglycerol in three cell types. Evidence for the indirect formation of phosphatidylcholine-derived diacylglycerol by a phospholipase D pathway and direct formation of diacylglycerol by a phospholipase C pathway. *J. Biol. Chem.* 265, 14858–14863.
- [49] Siddiqi, A. R., Srajer, G. E., and Leslie, C. C. (2000) Regulation of human PLD1 and PLD2 by calcium and protein kinase C. *Biochim. Biophys. Acta* 1497, 103–114.
- [50] Metz, S. A. and Dunlop, M. (1990) Production of phosphatidylethanol by phospholipase D phosphatidyl transferase in intact or dispersed pancreatic islets: evidence for the in situ metabolism of phosphatidylethanol. *Arch. Biochem. Biophys.* 283, 417–428.
- [51] Pai, J. K., Liebl, E. C., Tettenborn, C. S., Ikegwuonu, F. I., and Mueller, G. C. (1987) 12-O-tetradecanoylphorbol-13-acetate activates the synthesis of phosphatidylethanol in animal cells exposed to ethanol. *Carcinogenesis* 8, 173–178.
- [52] Aradottir, S., Moller, K., and Alling, C. (2004) Phosphatidylethanol formation and degradation in human and rat blood. *Alcohol Alcohol* 39, 8–13.
- [53] Tomaszewski, M. and Buchowicz, J. (1972) Alcoholysis of the endogenous phosphate esters in rats treated with large doses of ethanol. *Biochem. J.* 129, 183–186.
- [54] Varga, A., Hansson, P., Johnson, G., and Alling, C. (2000) Normalization rate and cellular localization of phosphatidylethanol in whole blood from chronic alcoholics. *Clin. Chim. Acta* 299, 141–150.
- [55] Kummer, N., Lambert, W. E., Samyn, N., and Stove, C. P. (2016) Alternative sampling strategies for the assessment of alcohol intake of living persons. *Clin. Biochem.* 49, 1078–1091.
- [56] Helander, A., Ullah, S., and Beck, O. (2015) Phosphatidylethanol in breath: a possible noninvasive screening test for heavy alcohol consumption. *Clin. Chem.* 61, 991–993.
- [57] Guizzetti, M., Thompson, B. D., Kim, Y., VanDeMark, K., and Costa, L. G. (2004) Role of phospholipase D signaling in ethanol-induced inhibition of carbachol-stimulated DNA synthesis of 1321N1 astrocytoma cells. *J. Neurochem.* 90, 646–653.
- [58] Pannequin, J., Delaunay, N., Darido, C., Maurice, T., Crespy, P., et al. (2007) Phosphatidylethanol accumulation promotes intestinal hyperplasia by inducing ZONAB-mediated cell density increase in response to chronic ethanol exposure. *Mol. Cancer Res.* 5, 1147–1157.
- [59] Lundqvist, C., Rodriguez, F. D., Simonsson, P., Alling, C., and Gustavsson, L. (1993) Phosphatidylethanol affects inositol 1,4,5-trisphosphate levels in NG108-15 neuroblastoma × glioma hybrid cells. *J. Neurochem.* 60, 738–744.
- [60] Omodeo-Sale, F., Lindi, C., Palestini, P., and Masserini, M. (1991) Role of phosphatidylethanol in membranes. Effects on membrane fluidity, tolerance to ethanol, and activity of membrane-bound enzymes. *Biochemistry* 30, 2477–2482.
- [61] Bondeson, J. and Sundler, R. (1987) Phosphatidylethanol counteracts calcium-induced membrane fusion but promotes proton-induced fusion. *Biochim. Biophys. Acta* 899, 258–264.
- [62] Suju, M., Davila, M., Poleo, G., Docampo, R., and Benaim, G. (1996) Phosphatidylethanol stimulates the plasma-membrane calcium pump from human erythrocytes. *Biochem. J.* 317 (Pt 3), 933–938.
- [63] Asaoka, Y., Kikkawa, U., Sekiguchi, K., Shearman, M. S., Kosaka, Y., et al. (1988) Activation of a brain-specific protein kinase C subspecies in the presence of phosphatidylethanol. *FEBS Lett.* 231, 221–224.
- [64] Nishida, A., Saito, H., Shimizu, M., Zensho, H., and Yamawaki, S. (1997) Phosphatidylethanol inhibits phosphatidylinositol-phospholipase C activity in a competitive manner with phosphatidylinositol-4,5-bisphosphate. *Drug Alcohol Depend.* 44, 117–122.
- [65] Chang, C. Y., Farrell, K. R., and Baker, R. C. (2000) Phosphatidylethanol stimulates calcium-dependent cytosolic phospholipase A(2) activity of a macrophage cell line (RAW 264.7). *J. Biomed. Sci.* 7, 311–316.
- [66] Goodman, D. S. and Deykin, D. (1963) Fatty acid ethyl ester formation during ethanol metabolism in vivo. *Proc. Soc. Exp. Biol. Med.* 113, 65–67.
- [67] Hungund, B. L., Goldstein, D. B., Villegas, F., and Cooper, T. B. (1988) Formation of fatty acid ethyl esters during chronic ethanol treatment in mice. *Biochem. Pharmacol.* 37, 3001–3004.
- [68] Manautou, J. E. and Carlson, G. P. (1991) Ethanol-induced fatty acid ethyl ester formation in vivo and in vitro in rat lung. *Toxicology* 70, 303–312.
- [69] Laposata, E. A. and Lange, L. G. (1986) Presence of nonoxidative ethanol metabolism in human organs commonly damaged by ethanol abuse. *Science* 231, 497–499.
- [70] Laposata, E. A., Harrison, E. H., and Hedberg, E. B. (1990) Synthesis and degradation of fatty acid ethyl esters by cultured hepatoma cells exposed to ethanol. *J. Biol. Chem.* 265, 9688–9693.
- [71] Chang, W., Waltenbaugh, C., and Borensztajn, J. (1997) Fatty acid ethyl ester synthesis by the isolated perfused rat heart. *Metabolism* 46, 926–929.
- [72] Yoerger, D. M., Best, C. A., McQuillan, B. M., Supple, G. E., Guerro, J. L., et al. (2006) Rapid fatty acid ethyl ester synthesis by porcine myocardium upon ethanol infusion into the left anterior descending coronary artery. *Am. J. Pathol.* 168, 1435–1442.
- [73] Doyle, K. M., Cluette-Brown, J. E., Dube, D. M., Bernhardt, T. G., Morse, C. R., et al. (1996) Fatty acid ethyl esters in the blood as markers for ethanol intake. *JAMA* 276, 1152–1156.
- [74] Bearer, C. F., Gould, S., Emerson, R., Kinnunen, P., and Cook, C. S. (1992) Fetal alcohol syndrome and fatty acid ethyl esters. *Pediatr. Res.* 31, 492–495.
- [75] Hungund, B. L. and Gokhale, V. S. (1994) Reduction of fatty acid ethyl ester accumulation by ganglioside GM1 in rat fetus exposed to ethanol. *Biochem. Pharmacol.* 48, 2103–2108.

- [76] Villaro, A. C., Garayoa, M., Lezaun, M. J., and Sesma, P. (1999) Light and electron microscopic study of the hindgut of the ant (*Formica nigricans*, hymenoptera): I. Structure of the ileum. *J. Morphol.* 242, 189–204.
- [77] Borucki, K., Dierkes, J., Wartberg, J., Westphal, S., Genz, A., et al. (2007) In heavy drinkers, fatty acid ethyl esters remain elevated for up to 99 hours. *Alcohol Clin. Exp. Res.* 31, 423–427.
- [78] Grigor, M. R. and Bell, I. C. Jr. (1973) Synthesis of fatty acid esters of short-chain alcohols by an acyltransferase in rat liver microsomes. *Biochim. Biophys. Acta* 306, 26–30.
- [79] Treloar, T., Madden, L. J., Winter, J. S., Smith, J. L., and de Jersey, J. (1996) Fatty acid ethyl ester synthesis by human liver microsomes. *Biochim. Biophys. Acta* 1299, 160–166.
- [80] Tsujita, T. and Okuda, H. (1992) Fatty acid ethyl ester synthase in rat adipose tissue and its relationship to carboxylesterase. *J. Biol. Chem.* 267, 23489–23494.
- [81] Tsujita, T. and Okuda, H. (1994) Fatty acid ethyl ester-synthesizing activity of lipoprotein lipase from rat postheparin plasma. *J. Biol. Chem.* 269, 5884–5889.
- [82] Laposata, E. A., Scherrer, D. E., Mazow, C., and Lange, L. G. (1987) Metabolism of ethanol by human brain to fatty acid ethyl esters. *J. Biol. Chem.* 262, 4653–4657.
- [83] Diczfalusy, M. A., Bjorkhem, I., Einarsson, C., Hillebrant, C. G., and Alexson, S. E. (2001) Characterization of enzymes involved in formation of ethyl esters of long-chain fatty acids in humans. *J. Lipid. Res.* 42, 1025–1032.
- [84] Diczfalusy, M. A., Bjorkhem, I., Einarsson, C., and Alexson, S. E. (1999) Formation of fatty acid ethyl esters in rat liver microsomes. Evidence for a key role for acyl-CoA: ethanol O-acyltransferase. *Eur. J. Biochem.* 259, 404–411.
- [85] Haber, P. S., Apte, M. V., Moran, C., Applegate, T. L., Pirola, R. C., et al. (2004) Non-oxidative metabolism of ethanol by rat pancreatic acini. *Pancreatology* 4, 82–89.
- [86] Bora, P. S., Guruge, B. L., Miller, D. D., Chaitman, B. R., and Ruyle, M. S. (1996) Purification and characterization of human heart fatty acid ethyl ester synthase/carboxylesterase. *J. Mol. Cell. Cardiol.* 28, 2027–2032.
- [87] Kaphalia, B. S., Fritz, R. R., and Ansari, G. A. (1997) Purification and characterization of rat liver microsomal fatty acid ethyl and 2-chloroethyl ester synthase and their relationship with carboxylesterase (pl 6.1). *Chem. Res. Toxicol.* 10, 211–218.
- [88] Kaphalia, B. S. and Ansari, G. A. (2001) Purification and characterization of rat hepatic microsomal low molecular weight fatty acid ethyl ester synthase and its relationship to carboxylesterases. *J. Biochem. Mol. Toxicol.* 15, 165–171.
- [89] Lange, L. G. (1982) Nonoxidative ethanol metabolism: formation of fatty acid ethyl esters by cholesterol esterase. *Proc. Natl. Acad. Sci. USA* 79, 3954–3957.
- [90] Tsujita, T. and Okuda, H. (1994) The synthesis of fatty acid ethyl ester by carboxylester lipase. *Eur. J. Biochem.* 224, 57–62.
- [91] Riley, D. J., Kyger, E. M., Spilburg, C. A., and Lange, L. G. (1990) Pancreatic cholesterol esterases. 2. Purification and characterization of human pancreatic fatty acid ethyl ester synthase. *Biochemistry* 29, 3848–3852.
- [92] Kaphalia, B. S., Mericle, K. A., and Ansari, G. A. (2004) Mechanism of differential inhibition of hepatic and pancreatic fatty acid ethyl ester synthase by inhibitors of serine-esterases: in vitro and cell culture studies. *Toxicol. Appl. Pharmacol.* 200, 7–15.
- [93] Polokoff, M. A. and Bell, R. M. (1978) Limited palmitoyl-CoA penetration into microsomal vesicles as evidenced by a highly latent ethanol acyltransferase activity. *J. Biol. Chem.* 253, 7173–7178.
- [94] DePergola, G., Kjellstrom, C., Holm, C., Conradi, N., Pettersson, P., et al. (1991) The metabolism of ethyl esters of fatty acids in adipose tissue of rats chronically exposed to ethanol. *Alcohol Clin. Exp. Res.* 15, 184–189.
- [95] Heier, C., Taschler, U., Radulovic, M., Aschauer, P., Eichmann, T. O., et al. (2016) Monoacylglycerol lipases act as evolutionarily conserved regulators of non-oxidative ethanol metabolism. *J. Biol. Chem.* 291, 11865–11875.
- [96] Schrock, A., Thierauf, A., Wurst, F. M., Thon, N., and Weinmann, W. (2014) Progress in monitoring alcohol consumption and alcohol abuse by phosphatidylethanol. *Bioanalysis* 6, 2285–2294.
- [97] Pragst, F., Rothe, M., Moench, B., Hastedt, M., Herre, S., et al. (2010) Combined use of fatty acid ethyl esters and ethyl glucuronide in hair for diagnosis of alcohol abuse: interpretation and advantages. *Forensic Sci. Int.* 196, 101–110.
- [98] Bearer, C. F., Lee, S., Salvator, A. E., Minnes, S., Swick, A., et al. (1999) Ethyl linoleate in meconium: a biomarker for prenatal ethanol exposure. *Alcohol Clin. Exp. Res.* 23, 487–493.
- [99] Chan, D., Knie, B., Boskovic, R., and Koren, G. (2004) Placental handling of fatty acid ethyl esters: perfusion and subcellular studies. *J. Pharmacol. Exp. Ther.* 310, 75–82.
- [100] Szczepiorkowski, Z. M., Dickersin, G. R., and Laposata, M. (1995) Fatty acid ethyl esters decrease human hepatoblastoma cell proliferation and protein synthesis. *Gastroenterology* 108, 515–522.
- [101] Haber, P. S., Wilson, J. S., Apte, M. V., and Pirola, R. C. (1993) Fatty acid ethyl esters increase rat pancreatic lysosomal fragility. *J. Lab. Clin. Med.* 121, 759–764.
- [102] Mohan, S. S., Ping, X. D., Harris, F. L., Ronda, N. J., Brown, L. A., et al. (2015) Fatty acid ethyl esters disrupt neonatal alveolar macrophage mitochondria and derange cellular functioning. *Alcohol Clin. Exp. Res.* 39, 434–444.
- [103] Huang, W., Booth, D. M., Cane, M. C., Chvanov, M., Javed, M. A., et al. (2014) Fatty acid ethyl ester synthase inhibition ameliorates ethanol-induced Ca²⁺-dependent mitochondrial dysfunction and acute pancreatitis. *Gut* 63, 1313–1324.
- [104] Alhomsy, K., Selig, M., Sustic, T., Katrangi, E., Weissig, V., et al. (2008) Induction of apoptosis and necrosis in human peripheral blood mononuclear cells by fatty acid ethyl esters. *Alcohol Clin. Exp. Res.* 32, 534–543.
- [105] Werner, J., Laposata, M., Fernandez-del Castillo, C., Saghir, M., Iozzo, R. V., et al. (1997) Pancreatic injury in rats induced by fatty acid ethyl ester, a nonoxidative metabolite of alcohol. *Gastroenterology* 113, 286–294.
- [106] Criddle, D. N., Raraty, M. G., Neoptolemos, J. P., Tepikin, A. V., Petersen, O. H., et al. (2004) Ethanol toxicity in pancreatic acinar cells: mediation by nonoxidative fatty acid metabolites. *Proc. Natl. Acad. Sci. USA* 101, 10738–10743.
- [107] Criddle, D. N., Murphy, J., Fistetto, G., Barrow, S., Tepikin, A. V., et al. (2006) Fatty acid ethyl esters cause pancreatic calcium toxicity via inositol trisphosphate receptors and loss of ATP synthesis. *Gastroenterology* 130, 781–793.
- [108] Lange, L. G. and Sobel, B. E. (1983) Mitochondrial dysfunction induced by fatty acid ethyl esters, myocardial metabolites of ethanol. *J. Clin. Invest.* 72, 724–731.
- [109] Patel, K., Durgampudi, C., Noel, P., Trivedi, R. N., de Oliveira, C., et al. (2016) Fatty acid ethyl esters are less toxic than their parent fatty acids generated during acute pancreatitis. *Am. J. Pathol.* 186, 874–884.
- [110] Leoncini, I., Le Conte, Y., Costagliola, G., Plettner, E., Toth, A. L., et al. (2004) Regulation of behavioral maturation by a primer pheromone produced by adult worker honey bees. *Proc. Natl. Acad. Sci. USA* 101, 17559–17564.