Alcohol produces distinct hepatic lipidome and eicosanoid signature in lean and obese

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Abstract Alcohol- and obesity-related liver diseases often coexist. The hepatic lipidomics due to alcohol and obesity interaction is unknown. We characterized the hepatic lipidome due to 1) alcohol consumption in lean and obese mice and 2) obesity and alcohol interactions. In the French-Tsukamoto mouse model, intragastric alcohol or isocaloric dextrose were fed with either chow (lean) or high-fat, high-cholesterol diet (obese). Four groups (lean, lean alcohol, obese, and obese alcohol) were studied. MS was performed for hepatic lipidomics, and data were analyzed. Alcohol significantly increased hepatic cholesteryl esters and diacylglycerol in lean and obese but was more pronounced in obese. Alcohol produced contrasting changes in hepatic phospholipids with significant enrichment in lean mice versus significant decrease in obese mice, except phosphatidylglycerol, which was increased in both lean and obese alcohol groups. Most lysophospholipids were increased in lean alcohol and obese mice without alcohol use only. Prostaglandin E2; 5-, 8-, and 11-hydroxyeicosatetraenoic acids; and 9- and 13-hydroxyoctadecadienoic acids were considerably increased in obese mice with alcohol use. Alcohol consumption produced distinct changes in lean and obese with profound effects of obesity and alcohol interaction on proinflammatory and oxidative stress-related eicosanoids.—Puneet, P. J. Xu, T. Vihervaara, R. Katainen, K. Ekroos, K. Daita, H-K. Min, A. Joyce, F. Mirshahi, H. Tsukamoto, and A. J. Sanyal. Alcohol produces distinct hepatic lipidome and eicosanoid signature in lean and obese. J. Lipid Res. 2016. 57: 1017–1028.

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Alcoholic and nonalcoholic fatty liver diseases (NAFLDs) are two of the most common causes of chronic liver disease worldwide. While alcohol consumption causes alcoholic liver disease (ALD), obesity and insulin resistance underlie the development of NAFLD. ALD remains a leading cause of liver-related mortality in most parts of the world (1, 2), while NAFLD is a rapidly rising cause of end-stage liver disease and hepatocellular carcinoma in North America (3). Historically, NAFLD was defined as a condition with a spectrum of liver histology similar to that seen in ALD but occurred in the absence of alcohol consumption in amounts that was considered harmful (4). Consequently, in studies in NAFLD, an alcohol consumption threshold below 20–30 g/day has been used (5–7). Conversely, the development of ALD is considered to require sustained consumption of >60 g of alcohol daily over many years (8–10). ALD and NAFLD are thus considered to represent distinct conditions separated by the amount of alcohol consumed despite having similar liver histology. Implicit in this concept is that these conditions have a distinct molecular and physiological basis for disease development.

Over the past decade, these apparently clean distinctions have become increasingly blurred. About two-thirds of the adult American population is obese and overweight (11). At the same time, alcohol use is widely prevalent in the United States with 87.6% of people ages 18 or older reporting alcohol consumption at some point in their lifetime; of these 71% consumed an alcoholic drink in the past year, and 56.3% consumed alcohol within the past month (12). It is therefore no surprise that alcohol use...
and obesity often coexist. While clinical data indicate that obese individuals who consume alcohol are more likely to develop fatty liver disease (13), there is a paucity of literature on how these risk factors interact to contribute to the pathogenesis of the underlying liver disease.

Both ALD and NAFLD are characterized by steatosis, and lipotoxicity is believed to contribute to their pathogenesis (14, 15). Biologically active lipids are important mediators of multiple physiological and pathologic processes. Perturbed lipid homeostasis can lead to steatosis, inflammation, and fibrotic processes that are significant pathophysiological determinants of disease progression and severity in both NAFLD and ALD (16–22). Most studies have, however, focused on either NAFLD or ALD, and the interactions between obesity and alcohol consumption on lipid metabolism have not been previously described. Advances in analytical methodology now permit simultaneous quantitative measurement of individual lipid species across all major lipid classes from biological samples. This allows an unbiased analysis of the changes in lipid metabolism from the profile of such lipid species measured by these high-throughput techniques.

The objective of the current studies was to evaluate the effects of obesity and alcohol consumption on the hepatic lipidome in C57B/6 mice. The specific aims of the study were to characterize: 1) the effects of alcohol consumption on hepatic lipidome in lean and obese mice and 2) the changes in hepatic lipidome due to obesity and alcohol interactions. This was accomplished by comparison of the hepatic lipidome in 1) lean mice fed chow diet or an isocaloric chow diet and alcohol with alcohol administration via intragastric (iG) gavage (French-Tsumakamoto model) (23); 2) obese mice fed a high-fat, high-cholesterol diet (HFCD) or isocaloric diet and alcohol; 3) obese mice versus lean mice fed an HFCD or chow diet, respectively; and 4) obese versus lean mice fed alcohol-containing diets.

MATERIALS AND METHODS

Mouse model

The studies were performed in C57B/6 mice. These mice have been used for studies in both diet-induced obesity and alcohol previously (24). Initially, 8-week-old male C57B/6 mice were fed an HFCD containing 20% calories from lard and 1% cholesterol (Dyets Inc., #180724) or chow diet ad libitum for 2 weeks as described previously (23). The iG catheter was then implanted, and iG feeding was initiated for 8 weeks that provided 60% of total daily caloric intake while the mice consumed remaining 40% calories via ad libitum intake of chow or HFCD (23). The iG feeding delivered a high-fat liquid diet (36% calories from corn oil) plus ethanol (26–27 g/kg/day) (CHOW+Alc, HFCD+Alc) or isocaloric dextrose (CHOW+Cont, HFCD+Cont). Thus, four groups of mice were studied: 1) lean mice (i.e., mice fed a chow diet alone and iG administration of isocaloric diet); 2) lean-alcohol (LA) mice (i.e., mice fed a chow diet and iG administration of alcohol); 3) obese mice (i.e., mice fed an HFCD ad libitum with isocaloric diet); and 4) obese-alcohol (OA) mice (i.e., mice fed HFCD ad libitum with iG alcohol).

On the last day of the experiments, the animals were anesthetized, venous blood collected, and then the animals were euthanized. The entire liver and spleen were removed and weighed, and livers were processed for analyses including lipidomic analysis and hematoxylin and eosin and reticulin staining. Plasma aspartate aminotransferase and alanine aminotransferase were analyzed by a kinetic assay, and albumin and bilirubin levels by autoanalyzer. The animal research was conducted in conformity with the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals, incorporated in the Institute for Laboratory Animal Research (ILAR) Guide for Care and Use of Laboratory Animals. The animal protocol for this study was approved by the Institutional Animal Care and Use Committee at the University of Southern California.

Lipid sample preparation and extraction

Lipid extraction, MS-based lipid detection, and data analysis were performed by Zora Biosciences Oy (25). The liver tissue samples (≈50–150 μg) were weighed, pulverized with CP02 CryoPrep Dry Pulverization System (Covaris Inc., Woburn, MA), and resuspended in ice-cold methanol (LC/MS grade; Sigma-Aldrich GmbH, Steinheim, Germany) containing 0.1% butyl-hydroxytoluene (≥99.0%; Sigma-Aldrich) in a concentration of 100 mg/ml.

Eicosanoids were extracted from 50 μl of liver homogenate using solid-phase extraction (SPE; Strata-X 35u Polymeric RP 96-well Plate, 60 mg/well; Phenomenex, Torrance, CA) as described in Ref. (26). Prior to extraction, known amounts of isotope-labeled standards (arachidonic acid (AA)-d8, docosahexaenoic acid-d5, eicosapentaenoic acid-d5, prostaglandin D2 (PGD2)-d4, thromboxane B2-d4, leukotriene B4-d4, lipoxin A4-d5, 8,9-dihydroxyicosatetraenoic acid (DHET)-d11, 5-HETE-d8, 12-HETE-d8, 13-hydroxy-9,11-octadecadienoic acid (13-HODE)-d4, and 9-hydroxy-10,12-octadecadienoic acid (9-HODE)-d4; Cayman Chemicals, Ann Arbor, MI) were included as synthetic internal standards. Samples were loaded to SPE plate after conditioning the plate with methanol and ultrapure water (both LC/MS grade; Sigma Aldrich). The SPE wells were washed with 35% methanol prior to elution with acetonitrile (LC/MS grade; Sigma Aldrich). The samples were then evaporated under nitrogen until dry and constituted in methanol followed by addition of external standard mixture [15(S)-HETE-d8 and PGD2-d9; Cayman Chemicals].

In shotgun lipidomics, lipids were extracted from 10 μl of liver homogenate using a modified Folch lipid extraction, using chloroform (HPLC grade), methanol, and acetic acid (both LC/MS grade) for liquid-liquid extraction (27) performed on 96-well plates using a Hamilton Microlab Star system (Hamilton Robotics AB, Kista, Sweden). All solvents were purchased from Sigma-Aldrich. Samples were spiked with known amounts of lipid-class specific, nonendogeneous synthetic internal standards. After lipid extraction, samples were reconstituted in chloroform-methanol (1:2, v/v), and synthetic external standards were postextract spiked to the extracts (28).

Quality controls (QCs) were prepared along with the samples for both eicosanoid and shotgun lipidomic analyses to monitor the extraction and MS performance. In addition, calibration lines were prepared to determine the linear dynamic range of the MS analyses. QCs and calibration lines were prepared in reference liver matrix.

MS analyses and data processing

Eicosanoids were analyzed on a hybrid triple quadrupole/linear ion trap mass spectrometer (5500 QTTRAP; AB Sciex, Concord, Canada) equipped with an ultra-high-pressure liquid chromatography system (CTC HTC PAL autosampler, CTC
Analyses of hepatic lipid depots by lipidomic methods revealed distinct changes in different lipid classes in lean mice compared with obese mice. The hepatic lipidome was compared in lean mice with or without alcohol using multiplex reaction monitoring (MRM)-based method in negative ion mode as in Ref. (26). Eicosanoids were identified based on their retention times and compound-specific MRM ion pairs, and quantified by normalizing to the respective isotope-labeled internal standard and the sample amount. Data were processed using Multiquant (AB Sciex) and SAS (SAS Institute Inc., Cary, NC) software.

In shotgun lipidomics, lipid extracts were analyzed on a hybrid triple quadrupole/linear ion trap mass spectrometer (QTRAP 5500) equipped with a robotic nanoflow ion source (NanoMate; Advion Biosciences Inc., Ithaca, NY) as described (28). Molecular lipids were analyzed in both positive and negative ion modes using multiple precursor ion scanning-based methods (29, 30). The molecular lipid species were identified and quantified in absolute [cholesterol ester (CE), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidyserine, lyso-PC (LPC), lyso-PE (LPE), diacylglycerol (DAG), SM] or semiabsolute [phosphatidylinositol (PI), PC-O, PC-P, PE-O, PE-P] amounts (31) by normalizing to their respective synthetic internal standard and the sample amount. Data processing was performed by LipidView (AB Sciex) and SAS. The concentrations of all lipids are presented as picomoles per microgram wet tissue weight.

Statistical analysis

Missing concentrations that were below the detection limit were substituted with a value that is half of the lowest observed concentration within the group. Groups that had only substituted values (variance = 0) were excluded from the testing. Unpaired Student’s t-test was performed to log-transformed lipid concentrations. Testing was performed only if study groups had three or more observations. The results of the analysis included group averages, relative change between the group averages, and P value (from log scale). Changes are expressed as percentage changes of average concentrations of a group and calculated as follows: %Change = 100 × [(average (Group 2) – average (Group 1))/average (Group 1)]. Data were analyzed using Tableau 9 and GraphPad Prism 6 software.

RESULTS

A total of six to nine mice were studied in each of the four groups noted above. The mice on chow diet had no significant change in weight without and with alcohol use (lean and LA groups). In contrast, compared with lean mice, HFCD-fed mice gained weight compared with baseline values: 21.5% without alcohol (obese, P = 0.01) and 7.5% with alcohol (OA, P = 0.03). This combination of diet and alcohol use mimicked the common human phenotype and resulted in biochemical and histological changes similar to human ALD as previously reported (24). A detailed lipidomic data for all four groups (mean ± SD) are provided in supplementary Tables 1–7.

Differential effects of alcohol in lean versus obese mice

To determine whether alcohol feeding led to similar or differential changes in the context of background obesity, the hepatic lipidome was compared in lean mice with or without alcohol feeding and in mice fed an HFCD with or without alcohol. The key hepatic lipidome findings are described below.

**Alcohol use increased hepatic CEs and DAG.** Alcohol significantly increased total hepatic CE content in lean mice (LA vs. lean, +214% difference; P = 0.009) (Fig. 1A). This increase was even more pronounced in obese mice (OA vs. obese, +505% difference; P = 0.003). Interestingly, the CE increase in obese mice was driven by a marked increase in several saturated and mono- and polyunsaturated fatty acid species in CE. Alcohol use also produced significantly increased total hepatic DAG concentration in both lean (LA vs. lean, mean relative change +96%; P = 0.009) and obese mice (OA vs. obese, mean relative change +35%; P = 0.02). The relative effect was, however, more pronounced in lean mice. Further, hepatic DAGs were enriched in mono- and polyunsaturated fatty acids at the C2 position, more prominently in the lean mice (Fig. 1B).

**Alcohol differentially altered phospholipids in lean and obese.** Alcohol intake significantly increased several classes of phospholipids in lean mice (Fig. 1C). Specifically, total hepatic PC content was higher in LA versus lean mice (+24.5% difference, P = 0.04). Similarly, total hepatic PE (+38% difference, P = 0.001) and total hepatic PI (+32.5% difference, P = 0.01) were also significantly increased in LA versus lean mice. In contrast, alcohol significantly decreased total hepatic PC (27% decrease, P = 0.002) in OA versus obese mice. While phosphatidylglycerol (PG) increased with alcohol consumption in both lean and obese mice, the effect size was substantially greater (P < 0.05) in OA mice. Alcohol consumption also had opposing effects on the levels of lysophospholipids in lean versus obese mice (Fig. 1D). LA mice demonstrated significantly elevated total hepatic LPE (+90% difference, P = 0.0004), lyso-PC (LPC; +119% difference, P = 0.002), and lyso-PI (LPI; +165% difference, P = 0.002) compared with lean mice. On the other hand, total hepatic LPC (−52% difference, P = 0.002) and LPE (−37% difference, P = 0.01) were reduced in OA versus obese mice. The directionality of these hepatic lysosphospholipid changes indicates that alcohol produces distinct effects in the setting of background obesity versus a lean state.

**Alcohol use increased hepatic cyclooxygenase-derived eicosanoids.** Several proinflammatory cyclooxygenase (COX) metabolites were increased in both lean and obese mice fed alcohol (Fig. 2A). Specifically, total hepatic prostaglandin E2 (PGE2) content was markedly elevated in LA versus lean mice (+241% difference, P = 0.01). These changes were further greatly enhanced in the setting of obesity with OA mice having a markedly greater increase in PGE2 versus obese mice (+569% difference, P = 0.0001). Also, alcohol use in obese mice resulted in pronounced increase in total hepatic PGD2 and prostaglandin F2α (PGF2α) compared with lean mice.

**Alcohol induced differential effect on lipoygenase products in lean versus obese mice.** Interestingly, alcohol consumption produced varying effects in lean mice versus mice with obesity with respect to lipooxygenase (LOX) metabolites (Fig. 2B). Specifically, OA versus obese mice had significantly increased hepatic 5-HEPE (+125% difference,
Alcohol increased cytochrome P450 and nonenzymatic oxidative eicosanoid metabolites. Among the cytochrome P450 (CYP450) metabolites (Fig. 2D), alcohol use resulted in concordant changes for obese and lean groups with the exception of 5,6-EET and 11,12-EET, which were markedly increased in OA versus obese mice but not in LA versus lean mice. A similar divergent effect of alcohol was also noticeable for total EPA with significantly increased hepatic content (+90% difference, $P < 0.05$) in OA versus obese mice (Fig. 2C).

**Fig. 1.** Distinct effects of alcohol consumption in lean and obese mice. Total and lipid species within CE (A) and DAG (B). C: Alcohol increased hepatic phospholipids (PLs) in lean and depleted PLs in obese, most notably in PC. Total PG was significantly higher in OA versus obese compared with LA versus lean. D: Hepatic lyso-PLs in lean and obese. L, lean; O, obese. Red bar indicates increase; blue bar indicates decrease. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

$P = 0.02$), 12-HEPE (+531.7% increase, $P = 0.0003$), and 15-HEPE (+95.6% increase, $P = 0.01$). Notably, alcohol use in obese mice produced markedly increased 5-HETE, 12-HETE, and 15-HETE in contrast to lean mice where a modest decrease in these LOX metabolites was noted. Also, alcohol use in obese mice remarkably increased total hepatic 8-HETE (OA vs. obese, +273% difference; $P = 0.003$) without any significant change in the lean mice (Fig. 2C).
LA versus lean mice but not in OA versus obese mice. Further, DHETs also had concordant decrease with alcohol use in both obese and lean mice. Specifically, both 11,12-DHETs (LA vs. lean, −52.5% difference, \( P < 0.001 \); OA vs. obese, −55% difference, \( P < 0.001 \)) and 14,15-DHETs (LA vs. lean, −59% difference, \( P = 0.001 \); OA vs. obese, −76% difference, \( P = 0.00001 \)) were significantly decreased. Additionally, markedly decreased 5,6-DHET (−48% difference, \( P = 0.01 \)) and 8,9-DHET (−46% difference, \( P = 0.008 \)) were observed in OA versus obese mice only.

Alcohol use also induced marked hepatic changes in the nonenzymatic oxidative metabolites (Fig. 2E). The total hepatic 9-HODE (+95% difference, \( P = 0.04 \)) and 13-HODE (+83% difference, \( P = 0.02 \)) levels were markedly elevated in LA versus lean mice. Similar changes were also noted in OA versus obese mice.

**Interactions between alcohol consumption and obesity**

The interactions between obesity on the hepatic lipidomic response to alcohol consumption were evaluated by comparison of the hepatic lipidome in OA versus LA mice and relating them to changes seen in obese versus lean mice.

**Obesity increased hepatic CEs and DAG.** Hepatic CE (Fig. 3A) and DAG (Fig. 3B) were both increased by
obesity, and these changes were sustained with alcohol consumption. Together with the data shown in “Alcohol Use Increased Hepatic CEs and DAG,” these data demonstrate that both obesity and alcohol consumption increased hepatic CE accumulation as well as DAGs. The HFCD-induced obesity with and without alcohol use and resulted in prominent increase in MUFA and PUFA in both CE and DAGs.

Fig. 2. Distinct alcohol effects on eicosanoids in lean and obese mice. COX (PGE2, PGD2, and PGF2α) (A), LOX (HEPE and HETE) (B), 8-HETE (C), and CYP450 pathway (EETs and DHETs) and EPA changes with alcohol (D). E: Nonenzymatic oxidative stress markers 9- and 13-HODEs, and 11-HETE. EET, epoxyeicosatrienoic acid; HEPE, hydroxyeicosapentaenoic acid. Red bar indicates increase; blue bar indicates decrease. * P < 0.05; ** P < 0.01; *** P < 0.001.
Alcohol and obesity interaction resulted in distinct changes in hepatic glycerophospholipids. Contrasting changes were noted in most glycerophospholipids in obese versus lean with alcohol use. Specifically, total hepatic PC (+21% difference, \( P = 0.04 \)) and PE (+20%, \( P < 0.05 \)) contents were significantly higher in obese versus lean mice (Fig. 3C). In contrast, OA mice had significantly depleted total hepatic PC (-29% difference, \( P = 0.003 \)) and PE (-19.3% difference, \( P < 0.01 \)) compared with LA mice (Fig. 3C). Similarly, while all lysophospholipids (LPC, LPE, LPG, and LPI) were markedly increased in obese versus lean mice, no significant changes were noted in OA versus LA mice (Fig. 3D). On the other hand, OA mice had substantially elevated hepatic PG content compared with LA mice (+203.6% difference, \( P = 0.007 \)), whereas obese mice had a smaller increment compared with lean mice (+55% difference, \( P = 0.001 \)) (Fig. 3C).

Obesity enhances alcohol-induced increments in proinflammatory COX metabolites. The effects of obesity on hepatic COX metabolite content were qualitatively similar to that seen with alcohol. Regardless of whether alcohol was consumed, proinflammatory PGD\(_2\) and PGE\(_2\) were increased. Specifically, OA mice had a marked increase in total hepatic PGE\(_2\) (+147% difference, \( P = 0.03 \)) compared with LA mice. Together with the effects of alcohol consumption in lean and obese mice presented above, these results indicate that obesity not only increases these eicosanoids but further exaggerates the alcohol related production of these proinflammatory compounds in the liver.

Obesity promotes proinflammatory hepatic LOX metabolites regardless of alcohol consumption. Compared with lean mice, obese mice had significantly increased 5-HETE and decreased 5-HEPE levels (Fig. 4B). However, with alcohol consumption, the relative changes were substantially exaggerated [e.g., total 5-HETE was increased by 120.5% in obese vs. lean mice (\( P < 0.008 \)) and by 277% (\( P < 0.02 \)) in OA vs. LA mice]. Both 12- and 15-HETE were also notably increased in OA versus LA mice. Similarly, OA mice had strikingly high total hepatic 8-HETE content (+391% difference, \( P = 0.001 \)) compared with LA mice (Fig. 2C).

Obesity has differential effects on CYP450 and nonenzymatic oxidative metabolites of eicosanoids in the presence or absence of alcohol. The hepatic 11,12-EET content did not increase significantly in obese versus lean mice. However, there was a substantial increase in this metabolite in OA versus LA mice (+220% difference, \( P = 0.02 \)) indicating a specific interaction between obesity and alcohol consumption on
this metabolite. Similarly, total hepatic 11-HETE level was markedly higher in OA versus LA mice (+273% difference, \( P = 0.03 \)) (Fig. 4C). The total hepatic 13-HODE, however, increased only in obese versus lean mice (+86% difference, \( P = 0.04 \)) (Fig. 4C).

**DISCUSSION**

Both ALD and NAFLD are associated with increased steatosis, cell death, and inflammation. Lipids are important mediators of cell viability versus death, inflammation,
and other biological processes commonly associated with chronic inflammatory disorders. The study of hepatic lipid metabolism in these conditions is therefore of importance in fully elucidating the role of lipids in the genesis of these diseases. Lipidomics allows an unbiased approach to evaluating the perturbation in lipid metabolism and can be used for hypothesis generation and identification of potential therapeutic targets based on the known effects of specific lipid metabolites on relevant disease pathways.

The current study provides novel data demonstrating complex and sometimes divergent effects of alcohol consumption in the lean versus obese state. Conversely, the development of obesity with an HFCD produced varying effects on the lipidome in the abstinent versus alcohol-fed state. The four principal effects of alcohol consumption were (1) an increase in CEs and DAGs, which were more pronounced in mice with obesity; (2) increases in several hepatic phospholipids in lean mice but a remarkable depletion of PC in obesity; (3) an increase in lysophospholipids mainly in lean mice; and (4) an increase in proinflammatory COX, LOX, and oxidative stress-related eicosanoids (HODEs and 11-HETE), which were considerably exaggerated in obese mice compared with lean mice with a more pronounced magnitude of change due to interaction of obesity and alcohol (Fig. 5A, B).

The changes in hepatic PC content following alcohol consumption are noteworthy due to the opposite effects noted in lean versus obese states. In the former, there was...
an increase in total PC in alcohol-fed mice compared with lean mice that did not receive alcohol. On the other hand, alcohol feeding led to a marked depletion in hepatic PC in mice with obesity. PC is a critical component of cell membranes and a central player in lipid metabolism (32–35). It thus plays an essential role in ensuring cell viability and normal organelle function (36). In the liver, specific PC with lauryl groups at the 12 position have been shown to be endogenous ligands for LRH-1, an orphan nuclear receptor that regulates bile acid synthesis (37).

Depletion of PC has multiple deleterious effects on cellular physiology and promotes cell death and activation of inflammatory signaling (38–42). PC depletion has been implicated in the pathogenesis of NAFLD (17, 18, 43, 44). The current studies demonstrate that alcohol consumption in the background of obesity can lead to depletion of PC. We hypothesize that this may be one of several key metabolic changes that trigger accelerated cell injury and inflammation and may contribute to disease acceleration when both risk factors for fatty liver disease are present.

Another novel finding is an eicosanoid signature implicating the proinflammatory COX metabolite PGE₂ as a common target of the interactions between alcohol consumption and obesity. Alcohol consumption, even in lean mice, resulted in a +241% increase in PGE₂. Furthermore, in obese mice, alcohol consumption produced a +569% increment in PGE₂ compared with mice fed a similar diet but without alcohol. While the current studies do not provide information on the cell of origin, both activated macrophages and other cell types can synthesize PGE₂ by hydrolysis of membrane phospholipids followed by modification of AA by COX (45). PGE₂ can promote inflammation by activation of TH17 cells, a subset of CD4⁺ helper T cells that produces interleukin-17, which can facilitate monocytes and neutrophils recruitment (46). On the other hand, PGE₂ may also aid in inflammation resolution through inhibition of interleukin-2 synthesis thereby suppressing effector T-cell proliferation and activation (47, 48). It is thus likely that PGE₂ has complex effects both promoting and suppressing inflammation. Future studies targeting PGE₂ are needed to clarify whether specific suppression of PGE₂ could ameliorate liver disease where both obesity and alcohol consumption are present as risk factors.

The current study also demonstrated differential changes in several LOX metabolites that are likely to be pathophysiologically relevant. HETE metabolites are largely proinflammatory and derived from (20:4 n6) AA by the actions of LOX, while anti-inflammatory hydroxyeicosapentanoic derivatives (HEPEs) are derived from the n3 eicosapentaenoic acid (49). Whereas alcohol consumption in lean mice led to a decrease in proinflammatory HETEs, there was a marked increase in different HETEs in obese mice that also received alcohol. We have previously demonstrated an increase in various HETEs in the

![Fig. 4. Hepatic eicosanoids due to alcohol and obesity interaction. Changes in COX (PGE₂, PGD₂, and PGF₂α) (A) and LOX (HEPE and HETE) (B) mediators. C: Nonenzymatic oxidative stress markers: 11-HETE, 9- and 13-HODEs. Red bar indicates increase; blue bar indicates decrease. * P < 0.05; ** P < 0.01; *** P < 0.001.](http://www.jlr.org/content/suppl/2016/03/28/jlr.M066175.DC1.html)
circulation of humans with NAFLD (18). In this murine study, these data are extended to show an increase in various HETEs in obesity with a marked further upregulation with alcohol feeding. While alcohol feeding also increased levels of the anti-inflammatory HEPEs in obese mice, the relative effects on HETEs was much greater suggesting that the eicosanoid profile is shifted toward a more proinflammatory state.

Several other metabolites were also altered with the use of alcohol in lean mice versus mice with obesity. These included a number of DHETs, which are important regulators of endothelial function and microcirculation (50). Also, both anti-inflammatory CYP450-derived EETs (51) and 11-HETE, a marker of oxidative stress (18, 52), were increased in obese mice fed alcohol compared with lean mice fed alcohol. These demonstrate the complex nature of the pro- and anti-inflammatory responses generated following alcohol consumption in lean versus obese states.

In interpreting these data, one must, however, be cognizant that mice models of both ALD and NAFLD do not precisely model human disease. However, this caveat notwithstanding, the data from this study provide novel insights on the complex effects on the hepatic lipidome with alcohol consumption and the differential effects seen in the lean versus obese state. The data also permit specific hypothesis generation regarding specific metabolites that can be tested in more focused mechanistic studies in the future. It further confirms the diversity of the effects of alcohol consumption on hepatic lipid metabolism in the background of obesity.

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